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**Paper Poster Session**

**STI due to *Chlamydia trachomatis* and *Neisseria gonorrhoeae***

### **Anti-bacterial activity of 17 strains of lactobacilli against elementary bodies of *Chlamydia trachomatis***

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**Background:** *Chlamydia trachomatis* (CT) is the most frequent bacterial sexually transmitted infection (STI) worldwide. The human vaginal microbioma plays a key-role in preventing a large number of urogenital diseases, including STIs. These protective functions are mainly attributed to *Lactobacillus spp.* dominating the vaginal niche of healthy women. Up to now only few studies have investigated the possible interaction between lactobacilli and CT.

The aim of our study was to investigate anti-bacterial activity of 17 vaginal lactobacilli isolates against elementary bodies (EBs) of CT in relation to their metabolic profiles.

**Material/methods:** Seventeen *Lactobacillus* strains, isolated from vaginal swabs of healthy pre-menopausal women and cultured in MRS broth were used: in particular, 8 strains of *L. crispatus* (BC1-8), 6 of *L. gasseri* (BC9-14) and 3 of *L. vaginalis* (BC15-17). After turbidimetric determination of cell concentration, cultures were centrifuged to separate cell pellets (CP) from cell-free supernatants (CFS). All CP and CFS corresponding to  $2.5 \times 10^8$ ,  $2.5 \times 10^7$  and  $2.5 \times 10^5$  CFU were mixed with  $5 \times 10^3$  IFU of EBs of CT serotype D. pH were measured in the final volume; EBs diluted in PBS were used as controls. Tubes were incubated for 7, 15 and 60 minutes and then centrifuged.

Supernatants were used to infect confluent Hela cells in single tubes containing sterile coverslips. After 48h of incubation at 37°C we evaluated CT infectivity by direct immunofluorescence (Meridian). IFUs were counted in 30 randomly chosen 200× microscopic fields and compared with controls. Statistical analyses were performed using GraphPad Prism software, applying Wilcoxon test. <sup>1</sup>H-NMR analysis of metabolic profiles of CFS was conducted on AVANCE spectrometer (Bruker); signals were assigned comparing their chemical shifts and multiplicity with Chenomx software data bank (Chenomx, ver8.02). Statistical analysis of <sup>1</sup>H-NMR data was performed using R computational software, applying Wilcoxon test.

**Results:** CT infectivity was significantly reduced only by CFS (p=0.038), in contrast to CP (p=0.425). CSF at the highest concentration showed the strongest anti-chlamydial activity. Under these conditions CFS were found to have pH<4.5, differently from the less concentrated ones.

We classified lactobacilli into 3 groups on the basis of p value of each CFS compared with control: "active" (BC1, BC2, BC4, BC6, BC7, BC8, BC13; p<0.2), "partially active" (BC5, BC9, BC11, BC15, BC16; 0.2<p<0.6) and "non active" (BC3, BC10, BC12, BC14, BC17 p>0.6). Comparing metabolic

profiles of the 3 groups we identified one metabolite produced in significantly higher concentration in active group: orotic acid ( $p=0.005$ ). Anyway, when tested alone at the concentration of 0,03mM, the highest concentration found among active CFS, it showed no anti-chlamydial activity.

**Conclusions:** Our results suggest a possible role of vaginal lactobacilli in the inhibition of CT infectivity, exercised by their metabolites with synergic functions. Anti-chlamydial activity is highly dependent on acid microenvironment.