

**Methods** MtrR was ectopically expressed in HO41 (named as SC4) and confirmed to be functional by western blot and qRT-PCR analyses. HO41 and SC4 were compared for their susceptibility to antibiotics in laboratory media and in the presence of ME180 cervical epithelial with or without IPTG induction.

**Results** In both laboratory media and in ME180 cell culture, we found that expression of MtrR in SC4 (HO41 *mtrR*+) decreased *mtrCDE* gene expression and increased gonococcal susceptibility to beta-lactam antibiotics. Importantly, MtrR-mediated repression of *mtrCDE* decreased the MIC of penicillin to a level below the MIC breakpoint recommended clinical treatment dose.

**Conclusion** We demonstrate the MtrR-mediated dampening of *mtrCDE* can greatly increase gonococcal susceptibility to penicillin. Thus, novel adjunctive therapeutics that decrease levels of MtrCDE may allow for the return of penicillin as an option for treating otherwise resistant strains of gonococci.

#### P1.12 ROLE OF VAGINAL LACTOBACILLI IN COUNTERACTING *CHLAMYDIA TRACHOMATIS* INFECTIVITY IN AN IN VITRO MODEL

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**Introduction** Lactobacilli play a fundamental role in maintaining the ecological equilibrium of the vaginal niche, preventing the overgrowth of endogenous microorganisms and impeding the colonisation of pathogens. Although many studies have focused on the mechanisms displayed by lactobacilli in counteracting several urogenital pathogens, a few data are available on the interaction between lactobacilli and *Chlamydia trachomatis* (CT). The aim of this study was therefore to assess the *in vitro* activity of different vaginal *Lactobacillus* strains against CT infectivity, investigating two different fractions of bacteria (cell pellets and cell-free supernatants), by three different mechanisms of action (competition, exclusion and displacement).

**Methods** A total of 17 *Lactobacillus* strains, isolated from vaginal swabs of healthy premenopausal women and belonging to *L. crispatus* (BC1; BC3-BC8), *L. gasseri* (BC9-BC14), *L. vaginalis* (BC16-BC17) and *L. plantarum* (BC18-19) species, were included in the study. The capacity of lactobacilli cell pellets (CP) and cell-free supernatants (CFS) to interfere with CT adhesion and entry in HeLa epithelial cells was evaluated, by means of competition, exclusion and displacement mechanisms. In particular, lactobacilli fractions corresponding to  $5 \times 10^7$  colony forming units (CFU) were incubated with  $5 \times 10^3$  CT elementary bodies (EBs) of strain GO/86, (serotype D), following different timelines. CT infection was evaluated by counting chlamydia inclusion forming units (IFUs) by direct immunofluorescence. Moreover, on the basis of CT infectivity interference results, 5 lactobacilli were selected for dose-effect assays and the same experiments were repeated, using CP or CFS fractions with  $5 \times 10^6$  and  $5 \times 10^5$  lactobacilli cells, in order to verify if a dose-dependent activity was present. Finally, the capacity of *Lactobacillus* strains to adhere to HeLa cells was assessed as well: results were read at light-microscopy and HeLa cells were scored for the presence and number of lactobacilli attached.

**Results** We found that lactobacilli cell pellets were the most active fraction in counteracting CT infectivity, particularly by means of an exclusion strategy, and that *L. crispatus* was the most effective species, even though a strain-specific activity was detected. Moreover, the anti-chlamydial activity was not correlated with the level of lactobacilli adhesion on epithelial cells and it was significantly maintained with low numbers of lactobacilli, although in presence of a dose-response effect.

**Conclusion** We identified specific vaginal *Lactobacillus* strains (*L. crispatus* BC4, *L. crispatus* BC5, *L. crispatus* BC7, *L. gasseri* BC14 and *L. plantarum* BC19) able to interfere with CT EBs adhesion and entry in epithelial cells and we were able to shed light on the mechanisms displayed by lactobacilli in counteracting CT infectivity. A major potential application lies on the use of these *Lactobacillus* strains as probiotics for the prophylaxis and/or adjuvant therapy of CT infections.

#### P1.13 VAGINAL MICROBIOME SIGNATURES IN *CHLAMYDIA TRACHOMATIS* INFECTED WOMEN

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**Introduction** In healthy women, lactobacilli play a crucial role in maintaining the microbial homeostasis of the vaginal niche. In case of bacterial vaginosis (BV), a condition characterised by a depletion of lactobacilli and an increasing number of anaerobes, a higher risk of urogenital and sexually transmitted infections (STIs) is reported. The vaginal environment of healthy and BV-positive women have been extensively studied, leading to the identification of the microbial species dominating these opposite conditions and to the description of specific metabolic profiles. Besides that, less is known about the vaginal microbiome in case of STIs, as *Chlamydia trachomatis* (CT) infections. The aim of this study was to analyse the composition of the endogenous microbiota and the metabolic signatures of the vaginal niche in 3 different conditions: healthy, BV and CT infections.

**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 vaginal symptoms or presence of risk factors for CT infection. Patients with vaginal candidiasis were excluded. For all the patients, 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 and centrifuged. Cell pellets were examined with a DNA-microarray platform including 17 probe sets specific for the most representative vaginal bacterial groups and with a quantitative real-time PCR targeting 16s rRNA gene of *Gardnerella vaginalis* (GV). Cell-free supernatants were used for metabolomic analysis by means of <sup>1</sup>H-NMR spectroscopy. NMR spectra were recorded with an AVANCE spectrometer (Bruker). Similarities among microbial and metabolic profiles of samples were investigated by means of a principal component analysis (PCA). Differences in GV DNA loads and metabolites concentrations were