

University of Minho, Campus de Gualtar, Braga, Portugal

(2) Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal

Bacterial vaginosis (BV) and urinary tract infections (UTIs) are among the most common disorders in women affecting hundreds of millions each year. UTIs occur when pathogenic bacteria ascend from the vagina and replicate on/or within the bladder uroepithelium. *Gardnerella vaginalis* (Gv) is the most frequent microorganism found in BV, while other bacterial species, namely, *Escherichia coli* (Ec), *Enterococcus faecalis* (Ef) and *Staphylococcus saprophyticus* (Ss) are the most frequent pathogens found in UTIs. This study aimed to characterize the interactions between Gv and Ec, Ef, or Ss using a dual-species biofilm assembly, consisting in combination of species: Gv/Ec, Gv/Ef and Gv/Ss. Biofilms were grown in microtiter plates for 48 hours, and quantified using the crystal violet assay and fluorescence in situ hybridization (FISH) method. Our results showed that *G. vaginalis* enhanced biofilm formation in the presence of urogenital pathogens. However, the relative abundance of the species varied remarkably in different combinations of dual species. Moreover, visualization of dual-populations species in the biofilm, using the epifluorescence microscopy, revealed that all of the urogenital pathogens co-existed with *G. vaginalis*. In conclusion, our work suggest that different species can co-operatively form mature biofilms in vagina, but that the behavior of the species within the biofilm may vary due to interspecies interactions. Thus, this study contribute to our understanding the progression of the urogenital disorders.

This work was co-funded by FCT project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462), FCT Strategic Project PEst-C/SAU/UI0709/2011 and by QREN, FEDER, ON2 project (NORTE-07-0124-FEDER-000027).