Clinically relevant polyculture biofilm produces protective environments for the development of resistance

*Philip Skipper* (1), *A Gillet* (1), *R Dixon* (1)
(1) University of Lincoln, UK

Antimicrobial resistance in clinical practice has become a substantial challenge for infection control. Although it is now established that most pathogens exist in monoculture or polyculture biofilms in infection situations, most studies still rely on traditional MICs with planktonic organisms in broth cultures. The factors surrounding eradication by antibiotics of polyculture biofilms modelled in vitro have been poorly studied. In this present study, antimicrobial effects of various antibiotics against either monoculture or polyculture biofilms and planktonic cultures of *Acinetobacter baumannii, E. coli* and *Klebsiella pneumoniae* - three clinically relevant pathogens - were determined. Biofilms were grown using the well-established Calgary 96 peg method. Comparison of the polyculture biofilms to monoculture ones showed a decrease in sensitivity to selected antimicrobial agents in 45% of combinations, with 4, 8 and even 16 fold decreases in susceptibility. Surprisingly, antibacterial resistant colonies isolated from mixed biofilms continued to show decreased susceptibility to the antibiotics up to 120 hours after the initial isolation. The reduced susceptibility of bacteria from polyculture biofilms to antibiotics was maintained in cells sub-cultured into drug-free medium, demonstrating that the polyculture biofilm provides an enhanced protective environment to antibacterial action in which antimicrobial resistance has been seen to develop. A greater understanding of the polyculture biofilm environment will be needed to ensure better clinical targeting of infection in patients with antimicrobials in order to extend their usefulness until the next generation becomes available.

Isolation of *Gardnerella vaginalis* from BV patients and healthy women: analysis of virulence through adherence, biofilm formation and cytotoxicity assays

(1) University Of Minho, Portugal

*Bacterial vaginosis* (BV) is one of the most common gynaecological disorder affecting women in the reproductive age. Microbiological analysis of BV has shown *Gardnerella vaginalis* to be the most frequent organism in BV. However, *G. vaginalis* colonization do not always lead to BV. This raised the question whether there are pathogenic and commensal lineages within this species. In an effort to understand the differences between *G. vaginalis* strains, we performed in vitro assays to compare virulence properties of recently isolated 14 *G. vaginalis* strains from Portuguese women with and without BV. *G. vaginalis* strains were characterised for their initial adhesion ability to a monolayer of HeLa cells by incubating the bacteria with this monolayer and quantifying the adhesion by staining with DAPI and fluorescence microscopy. These assays revealed that the BV isolates of *G. vaginalis* had a stronger initial adhesion capability than non-BV isolates. The biofilm-forming capacity was then assessed by allowing each of the strains to form biofilms under anaerobic conditions for 48 hours and using different growth media. It was possible to observe that BV isolates tend to grow preferentially as biofilms while non-BV isolates had a lower intrinsic tendency towards biofilm formation. In addition, BV isolates of *G. vaginalis* displayed robust cytotoxicity in the epithelial cells after 3 hours in the contact with a monolayer of HeLa cells. Thus, this study outlines two distinct variants of *G. vaginalis*, one apparently commensal and one pathogenic, and presents evidence for disparate virulence potentials.