Introduction: Mixed bacterial-fungal colonization of the endotracheal tubes is now evident, with microbial interplay withstanding common antimicrobial therapy and paying for persistent and severe VAP infections. While alternative therapeutic strategies effectively targeting inter-kingdom biofilms are required, the role of each microorganism need to be appraised to deliver effective treatments.

Hypothesis and aims: We earlier reported the combination therapy involving polymyxin B (PMB) and amphotericin B (AMB) as holding an attractive therapeutic option to treat dual-species biofilms. This study aimed to determine the “post-antimicrobial” phenomenon of PMB/AMB combined action in P. aeruginosa (PA) + Candida albicans (CA) biofilms, and to ascertain the events underlying biofilm growth restoration.

Methodology: Post-antimicrobial effect of PMB combined with AMB was assessed in 24-h dual-species biofilms. Cell culturability and viability were evaluated by CFU and Live/Dead staining, respectively. The gene expression profile was assessed by qPCR.

Results: Results showed that PA+CA biofilms lost their culturability straightaway being exposed to PMB/AMB combined solution. However, 24h was enough to both species recover their growth onto agar medium, with microbial counts approximating those observed for pre-treated biofilms. Following the subsequent treatment cycle, CFU estimation was only slightly disturbed. L/D results revealed that PA and CA populations displayed a compromised status at the end of the first PMB/AMB treatment cycle. Finishing the 24-h-regrowth cycle, most biofilm-encased species exhibited viability, which endured after the second treatment period. Transcriptional analysis of dual-species biofilms exposed to PMB/AMB combined action showed a high expression level in all PA resistance-encoded genes – anrB, galU, mexA and algD – and in ERG3 and ALS2 CA genes.

Conclusion: Our findings showed that PA+CA biofilms were able to escape to the combined action of PMB/AMB, and both species had a preeminent role while retaining adaptive resistance mechanisms that likely contributed for their recovery and adaptation on the ensuing treatments.