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Paper Poster Session
Non-tuberculous mycobacteria

Inhibition of Mycobacterium abscessus biofilms by Methylobacterium sp.

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Background: *Methylobacterium sp.* is usually isolated from water distribution systems and buildings including hospitals. Its isolation in biofilms has been linked with a lower presence of *Mycobacterium avium*. We aimed to determine the *in vitro* activity of *Methylobacterium sp.* in the structure of *Mycobacterium abscessus* biofilm.

Material/methods: *M. abscessus* DSM 44196 biofilm was developed using 2x4-well plates with an uncoated hydrophobic surface, incubated at 37º (80 rpm) for 96h. *Methylobacterium sp.* CECT 7805 was added in different forms (suspension of live bacteria (LB), autoclaved (AB) and an extract obtained after sonication (ES)) at different times (24, 48 and 72 hours), leaving one well as a control (96 hours). The medium was replaced daily. The experiment was performed using the protocol previously described by Muñoz-Egea et al (BMC Microbiol 4 February 2015; 15:18). The statistical data were analyzed by pairwise comparisons using the nonparametric Mann-Whitney test with a level of statistical significance of $p<0.05$.

Results: The values of maximal inhibition of the percentage of covered surface and thickness of *M. abscessus* biofilm after exposure to all forms of *Methylobacterium sp.* were obtained at 72 hours, while the highest emission of autofluorescence was observed at 48 hours.

After 72 hours of exposure, there were no statistical differences in thickness between LB and AB, but a statistical difference between LB and ES was detected, being higher the reduction obtained with LB. There were no differences between AB and ES.

As for the percentage of covered surface, there was a significant difference at 72 hours between AB and ES, being higher the effect of AB in reducing this parameter. Similarly, it was shown a significant reduction in this parameter when comparing ES and LB, being higher the effect of LB. There were differences between AB and LB at 24 and 48 hours of exposure, being higher the effect produced by the live bacteria, although they were not statistically significant at 72 hours.

Meanwhile, the percentage of autofluorescence was not significantly affected by the use of any form of *Methylobacterium sp.*

Conclusions: *Methylobacterium sp.* is able to inhibit *M. abscessus* biofilm formation, affecting both the thickness and the covered surface. *Methylobacterium sp.* alive is not necessary to inhibit a preformed biofilm of *M. abscessus* due to the addition of autoclaved *Methylobacterium sp.* and an extract of it showed an inhibition of *M. abscessus* biofilm. However, the inhibition of the covered surface and thickness of *M. abscessus* biofilm was significantly higher when it was exposed to the live *Methylobacterium sp.*, especially after 24-48 hours. An increase in the autofluorescence emission by *M.abscessus* biofilms and extracellular fluorescence were observed after adding *Methylobacterium sp.*