

P1431 **Effect of *Methylobacterium* sp. combined with clarithromycin on *Mycobacterium abscessus* biofilms**

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Background: *Methylobacterium* sp. is usually isolated from water distribution systems and buildings. Its isolation in biofilms has been related with a lower presence of *Mycobacterium avium*. In addition, it has been demonstrated that *Methylobacterium* sp. in different forms is able to inhibit *Mycobacterium abscessus* biofilm formation.

The aim of this study is to determine the effect of clarithromycin combined with *Methylobacterium* sp. on the development of *M.abscessus* biofilms.

Materials/methods: *M.abscessus* DSM 44196 biofilms were developed using 2x4-well plates with an uncoated hydrophobic surface, incubated at 37° for 96h. *Methylobacterium* sp. CECT 7805 was added in two forms (autoclaved bacteria (AB) and a crude extract (EB)) combined with clarithromycin (2mg/l) at 24, 48 and 72 hours, leaving one well as a control (96 hours). The experiment was performed in triplicate using the protocol previously described (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: Covered surface, thickness and autofluorescence were significantly lower in *M.abscessus* biofilms after exposure to AB and EB combined with clarithromycin with respect to the control.

The inhibition of biofilm is higher with AB at 24, 48 and 72 h, being reduced covered surface and thickness. However, the difference in percentage of autofluorescence is statistically significant only at 48 and 72 h. The percentage of dead bacteria after exposure to AB or EB with clarithromycin, was statistically higher at 72 hours, being higher with AB.

AB combined with clarithromycin is the most effective combination that decreases thickness. However, the percentage of covered surface is higher when AB is present, regardless it is combined with clarithromycin or not. The addition of clarithromycin to EB reduces significantly the covered surface at 24 and 72 hours. Regarding to autofluorescence, it is reduced with the addition of antibiotic at all hours.

Conclusions: The combination of *Methylobacterium* sp. with clarithromycin has a synergic effect on the decrease of *M.abscessus* biofilms thickness. Covered surface is also reduced when AB is present. Autofluorescence is significantly reduced by the incorporation of clarithromycin.