

P0927

Poster Session III

Mycobacterial susceptibility and molecular epidemiology

EFFECT OF CIPROFLOXACIN IN THE ULTRASTRUCTURE AND DEVELOPMENT OF BIOFILMS FORMED BY RAPIDLY GROWING MYCOBACTERIA

M.C. Muñoz Egea¹, M. García-Pedrazuela¹, J. Esteban¹

¹Department of Microbiology, IIS-Fundación Jiménez Díaz, Madrid, Spain

Objectives: To study the influence of ciprofloxacin in the development and the behavior of autofluorescence covered area, thickness and amount of dead bacteria in a biofilm formed by rapidly growing mycobacteria.

Methods: *Mycobacterium fortuitum* ATCC 6841, *Mycobacterium abscessus* DSM 44196 and *Mycobacterium chelonae* ATCC 19235 were used in the experiments. The biofilm development test was made using a previously described protocol (Muñoz-Egea et al. Appl Environ Microbiol. 2013. 79(3): 1065-7). Incubation with Ciprofloxacin (CIP) 1µg/ml was started at 24, 48 and 72 hour (named series 1, 2 and 3 respectively). A well with no antibiotics was used as a control (C). All experiments were performed in triplicates for each strain. Statistical analysis was performed using Epi-Info version 3.5.1.

Results: There was a statistical significance in the thickness between species related to the exposure to CIP at all times ($P \leq 0.0001$, ANOVA test). *M. fortuitum* biofilm C was the thickest one ($31.31 \pm 7.58 \mu\text{m}$), followed by *M. chelonae* ($24.86 \pm 5.58 \mu\text{m}$), and *M. abscessus* ($22.37 \pm 5.09 \mu\text{m}$). Biofilms of *M. fortuitum* series 1 ($15.08 \pm 3.59 \mu\text{m}$) and 2 ($24.63 \pm 5.40 \mu\text{m}$) showed also an important reduction in this parameter ($P \leq 0.0001$), as happened with biofilms of *M. abscessus* series 1 ($13.77 \pm 4.74 \mu\text{m}$; $P \leq 0.0001$), 2 ($14.84 \pm 5.45 \mu\text{m}$; $P \leq 0.0001$) and 3 ($18.75 \pm 5.81 \mu\text{m}$; $P = 0.016$). Biofilms of *M. chelonae* series 1 ($18.42 \pm 5.49 \mu\text{m}$; $P \leq 0.0001$) and 2 ($21.56 \pm 4.70 \mu\text{m}$; $P = 0.0023$) presented also an important reduction.

There were no statistical differences in the % of autofluorescence and % of covered surface between species. However a statistical significance was found for the percentage of dead bacteria at series 2 ($P = 0.0382$) and 1 ($P = 0.0086$) between all strains. The highest percentage of dead bacteria inside the biofilm was detected at series 1.

Autofluorescence was not affected by the antibiotic. ANOVA revealed that both time and species were not significant factors to explain the minimal differences found in the autofluorescence.

The percentage of covered surface was not affected by CIP for *M. fortuitum* and *M. abscessus*, although there was a statistical significance for *M. chelonae* between series 3 and 1 ($P = 0.0407$).

Conclusions: Ciprofloxacin has an important effect in the thickness of biofilms formed by rapidly growing mycobacteria, but not in other parameters, including the presence of autofluorescence.