Objectives. Macrolides are fundamental in the therapy of *Mycobacterium avium* complex (MAC) infections. In this work, we intended to understand the mechanism by which clarithromycin resistance develops during the course of the infection caused by *M. avium* and *M. intracellulare* strains.

Methods. Eight MAC strains (four *M. avium* and four *M. intracellulare*), were characterized by quantitative drug susceptibility testing (qDST) for clarithromycin, rifabutin, amikacin, ethambutol, moxifloxacin and clofazimine, using the MGIT960/TB eXIST. qDST for clarithromycin was performed in presence of the efflux inhibitors (EIs) verapamil, thioridazine, chlorpromazine, flupenthixol, haloperidol, arylpiperazine and sodium orthovanadate. The expression level of MAC efflux pump genes was assessed by RT-qPCR and efflux activity evaluated by a semi-automated fluorometric method. Partial sequence of 23 rDNA gene was used to search for mutations. MAC strains susceptible to clarithromycin were subject to serial passages in medium containing this antibiotic in order to mimic the development of resistance.

Results. Exposure of *M. avium* strains to clarithromycin induced the activity of pumps that made the bacteria clinically resistant to this antibiotic. qDST of clarithromycin changed from 2 to $\geq 512$ µg/ml, depending on the strain. The susceptibility values for the other antibiotics remained unchanged. This increased resistance to clarithromycin could be reduced by the EIs tested. Efflux activity was demonstrated for all strains and was accompanied by a general and marked overexpression of all efflux genes upon exposure to clarithromycin. Adaptation of *M. intracellulare* strains to clarithromycin does not induce the activity of efflux pumps. The susceptibility level changed from 0.25 to 1 µg/ml which is below the susceptibility breakpoint. No overexpression of efflux genes was detected in the adapted strains upon exposure to clarithromycin. Analysis of gene expression in these strains reveals the overexpression of all efflux genes. These results indicate that the induction of efflux pumps it's a late stress response in *M. intracellulare* and also indicate that the emergence of resistance to clarithromycin at the beginning is due to a different mechanism.

Conclusions. The results indicate that *M. avium* and *M. intracellulare* show distinct behaviours towards the development of resistance to clarithromycin, with efflux pumps playing an important role in the emergence of resistance to clarithromycin in *M. avium* but not in *M. intracellulare*. These results have laboratory and clinical implications and understanding the mechanisms of the emergence of drug resistance in both species will aid the development of better preventive strategies.