

P1958

Abstract (poster session)

The emergence of clarithromycin resistance in *Mycobacterium avium* complex

D. Machado*, L. Rodrigues, I. Couto, L. Amaral, M. Viveiros (Lisboa, PT)

Objectives: Emergence of resistance to macrolides in *Mycobacterium avium* complex (MAC) is problematic. Treatment options are a dilemma since MAC is usually resistant to conventional antituberculosis drugs. Clarithromycin (CLA) is crucial for MAC therapy; but resistance emerges promptly. Previously, we demonstrate that efflux pumps (EP) play a significant role in MAC resistance to macrolides. In this work, we evaluated the ability of efflux inhibitors (EIs) to reduce CLA acquired resistance in MAC strains. **Methods:** Three reference strains CLAs and four clinical strains CLAr were characterized by CLA antibiotic susceptibility testing and minimum inhibitory concentration (MIC) determination of CLA (presence/absence of EIs verapamil, thioridazine, and chlorpromazine), rifambutin, moxifloxacin, and clofazimine, using the MGIT960/TB eXIST. Partial sequence of 23 rDNA gene was used to search for mutations. MAC CLAs were subjected to constant concentrations of CLA (4 µg/ml), in order to induce a phenotypic response through EPs. Detection of efflux activity was performed with a semi-automated fluorometric method using ethidium bromide as EP substrate. **Results:** Exposure of CLAs strains to constant concentrations of CLA induced the activity of pumps that made the bacteria more resistant to this antibiotic. MICs of CLA for the adapted strains changed from 1 to 32 µg/ml, an intermediate level of resistance. The EIs used led to a significant reduction of resistance to CLA and efflux activity was demonstrated in all the adapted strains. In spite of the presence of a mutation in some CLAr strains, efflux activity was also detected. The MIC values for the other three antibiotics remained practically unchanged. **Conclusion:** This work demonstrates that CLA is substrate for efflux in MAC, and phenotypic resistance can emerge in a susceptible isolate, preceding the genotypic resistance, via active drug-efflux. A period of intermediate resistance, sustained by the activity of EPs, after which the cells have an enhanced probability to mutate and acquire high level resistance, has been noted. The final resistance level of the CLAr strains is a balance between the resistance due to the mutation and due to the efflux-activity of this antibiotic. The latest can be reversed by EIs. In conclusion, we demonstrate that emergence of resistance to CLA in MAC is acquired by efflux during therapy and can be reduced by EIs as adjuncts of conventional therapeutic regimen.