

Morphologic characterization of *Mycobacterium tuberculosis* circulating strains in a Lisbon hospital.

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Tuberculosis (TB) is one of the major causes of mortality and morbidity worldwide accounting for 3.1 million deaths per year. This disease, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) made a deadly comeback, during the 1990's, triggered mainly by the emergence of acquired immunodeficiency syndrome (AIDS). More recently, the emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) *M. tuberculosis* strains, uncovered the most frightening face of this disease an incurable infection with the currently available therapeutic tools [1]. Although Portugal is considered a medium incidence setting, annually are reported MDR and even XDR TB cases. The majority of these cases occur in the Lisbon area and the strains involved are genetically related being known as *Lisboa* family [2].

In the present work a group of 283 *M. tuberculosis* isolates collected in a Lisbon hospital during a two years period (2008-2009) were studied. The morphology of colonies grown on Lowenstein-Jensen slants was studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) using previously described procedures [3,4]. The aim of the study was the establishment of a link between mycobacteria drug susceptibility and structure. In the first part of the study approximately 20 isolates, with different drug susceptibility profiles ranging from pan-susceptible to XDR, were grown on Lowenstein-Jensen slants and their morphology was compared. Although all mycobacteria originated rough colonies their size differ with the drug susceptibility profile. The pan-susceptible strains generated larger colonies than drug resistant strains as shown in figure 1. These colonies were then processed for SEM analysis. The results obtained show that mycobacteria surface are distinct in susceptible and drug resistant strains as shown in figure 2.A and B. While drug susceptible mycobacteria have a homogenous surface (Figure 1A), drug resistant bacteria present a heterogeneous surface (Figure 2B) with small protrusions (Fig. 2B inset). In order to evaluate the existence of differences in the ultrastructure of circulating *M. tuberculosis* strains the colonies were processed and analysed by TEM. For this approach were selected only two isolates: the pan-susceptible R188/09 and the XDR HPV108/09.

The results obtained by the analysis of at least 300 bacteria present in non consecutive sections show that mycobacteria cell width (\cong 350 nm) is similar for both bacteria (Table 1). Nevertheless, their cell length and cell envelope width are significantly different. The XDR strain is shorter ($p=0.009$) and has a thicker cell envelope ($p=0.004$) than the pan-susceptible strain. These results are in agreement with those published in the literature [5,6].

Altogether our data clearly shows the existence of a link between mycobacteria ultrastructure and drug susceptibility. In order to better evaluate these differences a larger number of isolates must be studied. The use of other electron microscopy techniques, such as CEMOVIS, will avoid the formation of undesirable artefacts (e.g. mesosome) produced by dehydration and room temperature sectioning allowing a better characterization of mycobacteria ultrastructure [3].

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Figure 1. *Mycobacterium tuberculosis* colonies grown on Lowenstein-Jensen slants. The colonies shown are representative of those obtained during the study. A is a pan susceptible *M. tuberculosis* strain (R188/09) and B is a XDR (HPV108/09).

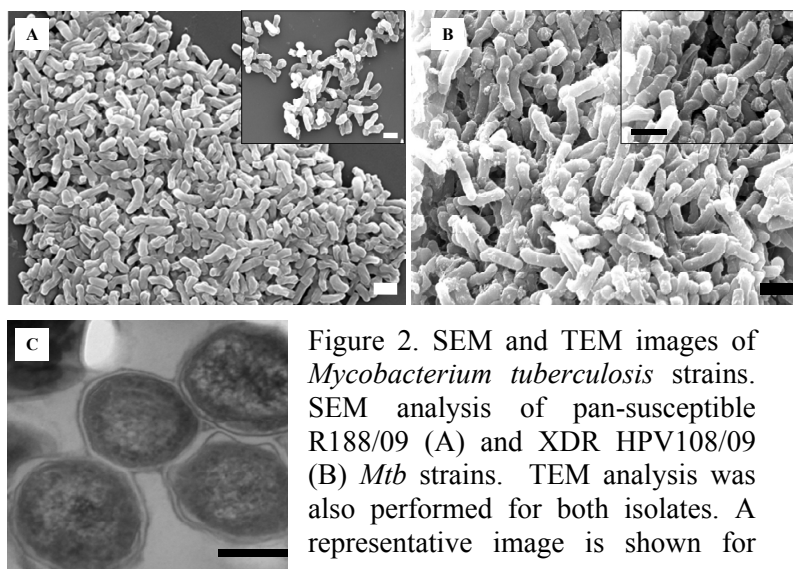


Figure 2. SEM and TEM images of *Mycobacterium tuberculosis* strains. SEM analysis of pan-susceptible R188/09 (A) and XDR HPV108/09 (B) *Mtb* strains. TEM analysis was also performed for both isolates. A representative image is shown for

Table 1. Measurement of mycobacteria cell envelope, cell width and length.

<i>M. tuberculosis</i> strain	Cell width (nm)		Cell length (nm)		Cell envelope (nm)	
	average	SD	average	SD	average	SD
R 188/09	350.6	37.3	919.6	12.9	15.3	2.58
HPV108/09	335.3	41.9	855.7	22.9	21.8	1.39