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Paper 2.2

Presence of mycobacteria in Portuguese swimming pools

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Synopsis

Mycobacteria have emerged as a major cause of opportunistic infections. In our study, 50 swimming pools were analysed for the presence of *Mycobacterium* species. Samples were analysed by culture and molecular biology methods. Our results indicate a 32% of positive results with mainly saprophytic mycobacteria. Around sixty three percent of these results belong to strains of *M. gordonae* indicating a generalized proliferation of this specie in the lisbon water distribution system. A total of 21.05% of the isolates are from *M. kansasii, M. intracellulare* and *M. chelonae.*

Background

Most non-tuberculous Mycobacteria (NTM) are ubiquitous environmental organisms (Collins *et al.*, 1984; Covert *et al.*, 1999; Dailloux *et al.*, 1999) that rarely cause disease in humans. There are, however, increasing reports of NTM causing infections in humans, even in immunocompetent patients (Von Reyn *et al.*, 1994; Peters *et al.*, 1995; Mangione *et al.*, 2001; Takahara *et al.* 2002). Drinking water is also a source of disseminated *M. avium* infection in hospitalized AIDS patients (Von Reyn *et al.*, 1994; Le Dantec *et al.*, 2002).These reports also suggest that water is the vehicle from which NTM can infect and colonize the human body. Usually, NTM infections are transmited by ingestion and inhalation from environmental sources and not from person to person transmission (Wolinsky E., 1979; Falkinham J.O., 1996).

The resistance of Mycobacteria to chlorine as been demonstrated in water systems and in *in vitro* studies (Stedham, 1980; du Moulin *et al.*, 1988; Le Dantec *et al.*, 2002). To this resistance also contributes the mycobacteria capacity to colonize and replicate in biofilms. This solid-liquid interface may be considered as selective enrichment site for these organisms (Falkinham III *et al.*, 2001; Dailloux *et al.*, 2003).

Until the present, Mycobacteria have been isolated from numerous water locations, including distribution systems, ice machines, heated nebulizers (Laussucq *et al.*, 1988; Schulze-Robbecke *et al.*, 1995; Neuman *et al.*, 1997; Chang *et al.*, 2002; Le Dantec *et al.*, 2002; Winthorp *et al.*, 2002) among others. Some household infections caused by mycobacteria have been reported usually related with showers and water baths (Mangione *et al.*, 2001; Takahara *et al.*, 2003). It is, therefore, of the utmost importance to conduct further studies in order to evaluate the current situation regarding mycobacteria contamination on water systems

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Samples were collected in 50 swimming pools located in the Lisbon area. At each collection site, two litter water samples were collected in plastic bottles, containing sodium thiosulfate in order to neutralize residual chlorine. All samples were processed within two days after collection.
Water samples were filtered through a 0,22 μm pore size nylon membrane (Millipore, USA) and ressuspended in 5 mL of water sample. The membrane was agitated for 30 minutes at room temperature and then decontaminated by Petroff's method (Petroff, 1915): briefly, sodium hydroxide (NaOH) was added in a 1:1 proportion to the water sample (5 mL), incubated at room temperature for 15 minutes, and neutralized with hydrochloric acid (HCl). The samples were then inoculated in the Bactec® system (Becton Dickinson, USA); three mL of treated sample were added to 2 mL of a mixture of antibiotics (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin - PANTA mixture) which is routinely used in the Bactec® system. The Bactec® vials were inoculated and incubated in the Bactec apparatus for 42 days. Additionally, 3 Lowenstein-Jansen (LJ) tubes were inoculated with 50 μL of the decontaminated sample and incubated at 22ºC, 37ºC and 44ºC for 60 days until considered negative.

Whenever a positive result was obtained in the Bactec® system or when suspected colonies were found in LJ, Auramine-Rhodamine coloration was performed.
Identification of the isolated Mycobacteria species was performed by the Genotype® Mycobacterium (Hain Life sciences, Germany), according to the manufacture's instructions.

Our results indicate a significant level of positive results in swimming pools of the Lisbon area (32%), being most of the positives from the species Mycobacterium gordonae, avium and Chelonae.

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