Molecular identification by random amplified polymorphic DNA analysis of a pseudo-outbreak of *Mycobacterium fortuitum* due to cross-contamination of clinical samples

Non-pigmented rapidly growing mycobacteria (NPRGM) are environmental organisms that can be found in several sources. They can cause contamination of samples and cultures (Wallace *et al*., 1998; LaBombardi *et al*., 2002) and can also be the cause of human infections. Outbreaks and pseudo-outbreaks due to NPRGM have increasingly been reported in recent times (Wallace *et al*., 1998), and in many cases, infections caused by these organisms have been associated with contaminated surgical devices and solutions. The main source of contamination is water collected from taps, faucets, fountains, ice, ice machines and plumbing parts in hospitals, *Mycobacterium chelonae* and *Mycobacterium fortuitum* being the most frequent NPRGM isolated (Ashford *et al*., 1997; Cox *et al*., 1997; Kauppinen *et al*., 1999; Gillespie *et al*., 2000). Here we describe a pseudo-outbreak of *M. fortuitum*, probably due to cross-contamination from a true positive urine specimen, detected in our laboratory.

All specimens sent to the mycobacteriology laboratory were decontaminated with N-acetylcyesteine-NaOH according to commonly accepted protocols (Esteban *et al*., 2006b). After decontamination, all samples were inoculated into MGIT liquid culture medium (BD) and onto Coletsos slants (bioMérieux). Identification of the isolates was performed using common biochemical tests and PCR-RFLP analysis of the *hsp65* gene according to the technique reported by Telenti *et al.* (1993) with minor modifications (Esteban *et al*., 2006a). Susceptibility testing of the strains was done using amikacin, clarithromycin, ciprofloxacin and imipenem E-test strips on Müller–Hinton agar plates enriched with 5% sheep blood incubated at 37 °C in a 5% CO₂ atmosphere for 3 days. The molecular epidemiology analysis was done by the random amplified polymorphic DNA (RAPD) technique using the primers OPA-2, OPA-18, INS-2 and IS-986FP according to the protocol described previously (Esteban *et al*., 2006a). Strains were considered to be identical when the electrophoretic pattern was the same with at least three primers. Other unrelated *M. fortuitum* strains isolated from clinical samples from our collection were used as controls.

A NPRGM was recovered in five samples processed on the same day. During the inoculation in the culture media, samples were located near to each other, and even next to each other in some cases. In three samples, the mycobacterial strain was isolated in the MGIT medium, and in another one, only one colony grew on a Coletsos slant (bioMérieux). All the other media inoculated with the four samples gave negative results. The last sample showed abundant growth on solid media and the same strain grew also in MGIT liquid medium. The sample was urine from a patient with a urinary tract infection. However, cultures performed several days later to confirm the results were negative and the patient was asymptomatic when the second culture was performed, so the isolate was considered to be not clinically significant. The strain was identified as *M. fortuitum* by phenotypic characteristics and the PRA electrophoretic pattern. All the strains were susceptible to ciprofloxacin and amikacin, and resistant to clarithromycin and imipenem; the same MIC results were obtained for all the strains. Molecular analysis of the isolates showed that the RAPD patterns were identical for the pseudo-outbreak strains and different for the other *M. fortuitum* strains with the primers OPA-2, INS-2 and IS-986FP. However, different patterns appeared with the primer OPA-18 with the pseudo-outbreak strains. According to these data, we think that the four strains with scanty growth were in fact contaminants, probably due to aerosolization from the true positive sample during the inoculation of the cultures.

The growth of NPRGM in culture media is not always the result of the presence of mycobacteria in the patient. The isolation of nontuberculous mycobacteria in a clinical sample may be the result of colonization or even contamination of the sample (Cox *et al*., 1997; Wallace *et al*., 1998; LaBombardi *et al*., 2002). It is very important to detect such pseudo-outbreaks because infections could be misdiagnosed and patients could be treated for non-existing infections. The rapid identification of the strain that produces the pseudo-outbreak can help to avoid such problems, achieving a better management of patients and saving costs for the hospital (Wurtz *et al*., 1996; Wallace *et al*., 1998).

To evaluate the identity of the isolates, the RAPD technique gave a similar band pattern for the pseudo-outbreak isolates with three of four primers used, the profiles being different with the other primer. This result, together with the identity of the phenotypic tests and the susceptibility to several antibiotics, prompted us to consider the isolates as a pseudo-outbreak without clinical relevance. Because the samples were from patients located in several units, and even in different hospitals, we discarded the hypothesis of contamination of the samples during their obtainment, and suspect contamination during the processing of the samples in the mycobacteria laboratory.

These contaminations are very difficult to avoid in a laboratory and may lead the clinician to misinterpret the results, so a...
rapid detection of such contamination is needed. RAPD analysis is a rapid and useful technique for the molecular analysis of pseudo-outbreaks (Wallace et al., 1998; Esteban et al., 2006b) and in our experience the results obtained are very useful for epidemiological characterization if the strains are characterized with at least three primers and all the isolates are processed together, to avoid reproducibility problems (Esteban et al., 2006a).

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