JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2005, p. 1928–1929 Vol. 43, No. 4
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.4.1928–1929.2005

Misidentification of *Mycobacterium leprae* as *Mycobacterium intracellulare* by the COBAS AMPLICOR *M. intracellulare* Test

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Received 2 August 2004/Returned for modification 10 September 2004/Accepted 5 December 2004

Commercially available nucleic acid probe- and amplification-based systems for detection and differentiation of mycobacteria are widely used in clinical microbiology laboratories. Here we report two cases of human leprosy in which the COBAS AMPLICOR *Mycobacterium intracellulare* test led to false- positive results. Correct identification of *Mycobacterium leprae* was possible only by amplification and comparative sequence analysis of the 16S rRNA gene.

Conventional diagnosis of mycobacterial infection relies on acid-fast staining, culture, and phenotypic characterization. Nucleic acid probe- and amplification-based methods have been introduced for identification of cultured mycobacteria or direct detection in clinical samples, thus significantly reducing the time to diagnosis (5). Fast and accurate differentiation between *Mycobacterium tuberculosis* complex (MTBC) members and nontuberculous mycobacteria in clinical specimens that tested positive for acid-fast bacilli is important for adequate patient management and for transmission control. In infections with nontuberculous mycobacteria, accurate identification is crucial for specific therapy. The use of the COBAS AMPLICOR system for detection of MTBC, *Mycobacterium avium*, and *Mycobacterium intracellulare* DNA in clinical specimens or identification of culture-grown mycobacteria has been evaluated extensively over the past years (1, 2, 6, 7). Here for the first time we report false-positive results with the COBAS AMPLICOR *M. intracellulare* test for two cases of human leprosy.

Case 1. A 36-year-old man from Ghana presented with fever, respiratory insufficiency, and diarrhea. He was diagnosed positive for human immunodeficiency virus type 1 for the first time with a high viral load (>100,000 copies of RNA/ml). One of three respiratory specimens was microscopically positive for acid-fast bacilli, and all three specimens were examined for MTBC DNA by use of the COBAS AMPLICOR test for MTBC. As the test for MTBC was negative, additional tests for *M. avium* and *M. intracellulare* were performed. All three specimens tested positive repeatedly for *M. intracellulare* DNA with the COBAS AMPLICOR system, with A_{660} values of 0.499 to 0.810 (cutoff, 0.350). Specimens were cultivated in parallel using MGIT liquid medium (Becton Dickinson, Le Pont-de-Claix, France) and Stonebrink and Löwenstein-Jensen medium slants according to a standard procedure (4). No growth was detected after 8 weeks of incubation.

Case 2. After a long history of stable cutaneous lesions on his back and left thigh, a 60-year-old male Turkish patient presented with progression and alteration of his old lesions and new lesions on his left arm. Biopsy specimens showed acid-fast bacilli in noncaseating granulomas. We first tested for MTBC and then for *M. avium* and *M. intracellulare. M. intracellulare* test results were repeatedly positive for four different specimens, with acid-fast bacilli showing A_{660} values between 0.417 and 1.356 (cutoff, 0.350). Cultivation for mycobacteria according to standard procedures (see case 1) revealed no growth after 8 weeks.

Unfortunately, it is not possible to determine the sequence of amplicons generated with the COBAS AMPLICOR *M. intracellulare* test by cycle sequencing using fluorescently labeled dideoxynucleotides. Instead, we utilized 16S rRNA gene primers in close proximity to COBAS primers (KY 18 and KY 75) and generated amplicons of approximately 600 bases containing the region amplified by the COBAS AMPLICOR *M. intracellulare* test. The amplicons were identified by comparative sequence analysis (EMBL and RIDOM databases). The highest scores (≥99.7% identity over a minimum of 450 bases) were found for *Mycobacterium leprae.* In contrast, multiple mismatches were found for all *M. intracellulare* sequence entries deposited in the EMBL and RIDOM databases (e.g., 92.9% identity over 450 bases as the highest score in the RIDOM database). Sequencing of 16S rRNA gene amplification products gave no evidence for mixed amplicons. Identity was confirmed by PCR amplification and sequencing of an *M. leprae*-specific proline-rich-antigen gene (3). The absence of mycobacterial growth following standard cultivation procedures without prior antimycobacterial chemotherapy also indicated the absence of *M. intracellulare* in the samples examined. These results indicate an unspecific hybridization of the COBAS AMPLICOR *M. intracellulare* probe to *M. leprae* 16S rRNA genes.

Discrepancies between culture results and the COBAS AMPLICOR *M. intracellulare* test results have been described previously by Katila and colleagues (2). Of 393 liquid media showing mycobacterial growth, 19 tested positive for *M. intracellulare* by the COBAS AMPLICOR test. Of those, only 15 were re-

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garded as true positives since the results were confirmed by conventional identification. Unfortunately, no information was given regarding any other species leading to positive results with the *M. intracellulare* test. A critical interpretation of positive COBAS AMPLICOR *M. intracellulare* test results is mandatory. Especially in cases of leprosy, a very rare disease in developed countries, misleading results from a commercially available nucleic acid probe- and amplification-based test combined with a lack of clinical experience may lead to an erroneous diagnosis. Any discrepancies between a positive result for *M. intracellulare* DNA with the COBAS AMPLICOR test and clinical evaluation of the patient should suggest the need for alternative identification techniques, such as conventional methods or PCR amplification and sequencing of 16S rRNA genes.

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