Natural Occurrence of Horizontal Transfer of *Mycobacterium avium*-Specific Insertion Sequence IS1245 to *Mycobacterium kansasii*

Michelle Christiane da Silva Rabello,† Rosangela Siqueira de Oliveira,‡ Rosa Maria Silva,§ and Sylvia Cardoso Leao†*  
Departamento de Microbiologia, Immunologia e Parasitologia, Universidade Federal de Sao Paulo, Rua Botucatu, 862 3° Andar, Sao Paulo, SP, Brazil,† and Setor de Micobacterias, Instituto Adolfo Lutz, Avenida Dr. Arnaldo, 355, Sao Paulo, SP, Brazil‡

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*Mycobacterium kansasii* carrying IS1245, a highly prevalent insertion sequence among *Mycobacterium avium* isolates, was detected in a mixed culture of *M. avium* and *M. kansasii*. The insertion sequence was stable and able to transpose by a replicative mechanism in *M. kansasii*. These findings may have significant implications for molecular diagnosis and treatment outcome.

*Mycobacterium avium* and *Mycobacterium kansasii* are major human mycobacterial pathogens, and both can cause pulmonary infections in immunocompetent individuals, as well as disseminated infections in the immunocompromised (6). Coinfection with *M. avium* and *M. kansasii* has also been documented in HIV-positive patients (8, 15). Current treatment of *M. avium* and *M. kansasii* infections is based on long-term antibiotic therapy and relies on different drug combinations, justifying all efforts directed to the correct identification of clinical isolates. Insertion sequences (IS) are mobile genetic elements within mycobacteria that are often species specific, a property that can be exploited for diagnosis and epidemiological studies (4). The host range of the IS1245 element was considered to be limited to *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *silvaticum* (3, 9, 11–13, 17).

Detection of IS1245 by PCR was used for confirmation of the presence of *M. avium* in a mixed culture from a bone marrow specimen from an HIV-positive patient. Fifteen slow-growing colonies were recovered from the bone marrow primary culture by plating dilutions on Middlebrook 7H10 solid medium supplemented with oleic acid, albumin, catalase, and dextrose (7H10-OADC). Four colonies (88.1 to 88.4) were nonpigmented, slow-growing mycobacteria identified as *M. avium* by PCR-restriction enzyme analysis (PRA) using the 16S-23S rRNA internal transcribed spacer (ITS) sequence as the target (14). Eleven colonies (88.5 to 88.15) produced yellow pigment after exposure to light and were identified as *M. kansasii* by PRA-ITS (Fig. 1A). For further characterization of these colonies, a 427-bp fragment from IS1245 was amplified by PCR (3). Unexpectedly, IS1245 amplicons were detected not only in the nonpigmented *M. avium* colonies but also in 8 out of 11 *M. kansasii* colonies (Fig. 1B). Amplicons generated with *M. kansasii* DNA were sequenced and showed 100% identity with the deposited IS1245 sequence (accession number L33879) (data not shown). Final identification of the 15 colonies to the species level was obtained by sequencing a 440-bp fragment of the 5′ 16S rRNA gene (*Escherichia coli* positions 54 to 510) (16). Colonies 88.1 through 88.4 showed 100% sequence similarity to the corresponding sequence of *M. avium* type strain ATCC 25291 (accession number EF521895). Colonies 88.5 through 88.15 showed 100% similarity to the corresponding sequence of *M. kansasii* type strain CIP 104589 (accession number AF547940). The two sequences differed at 12 positions (97.3% similarity).

To confirm the presence of IS1245 copies in the eight colonies of *M. kansasii*, restriction fragment length polymorphism (RFLP) experiments were performed by using IS1245 as a probe (17). While the *M. avium* colonies (88.1 to 88.4) produced multiband RFLP patterns characteristic of *M. avium* subsp. *hominissuis*, the eight *M. kansasii* colonies that had produced amplicons by PCR-IS1245 (88.8 to 88.15) showed a single IS1245-hybridizing band of approximately 4,750 bp. Moreover, a second IS1245-hybridizing band was detected in two of these colonies (88.11 and 88.14). Furthermore, the three PCR-IS1245-negative colonies of *M. kansasii* (88.5 to 88.7) lacked IS1245 hybridization bands (Fig. 1C). Except for the presence of IS1245, pulsed-field gel electrophoresis patterns indicated that all of the 11 *M. kansasii* colonies were, in fact, the same strain (Fig. 1D).

The results obtained strongly suggest that *M. kansasii* acquired the IS1245 element from the *M. avium* strain in a horizontal DNA transfer event which occurred either within the patient or during specimen or culture storage. This hypothesis was reinforced by the fact that both species were isolated from a unique specimen collected from one patient and by the finding of several *M. kansasii* colonies (88.5 to 88.7) of the same strain devoid of this insertion sequence element in that specimen (Fig. 1B, C, and D). The presence of the IS1245 element in *M. kansasii* was not detected by the analysis of five additional *M. avium-M. kansasii* mixed cultures from different patients (data not shown), which agrees with the hypothesis that the event described here was the result of a particular horizontal DNA transfer episode.

Antimicrobial susceptibility testing was performed with three isolates using a microdilution method previously de-
sualized in 11 out of 19 M. kansasii colonies analyzed, pointing to the occurrence of insertion sequence transposition by a replicative mechanism (Fig. 2).

Colonies of both species showed variations in RFLP-IS1245 patterns during this study. Besides the IS1245 transposition detected in M. kansasii, two or three RFLP-IS1245 band polymorphisms were also detected in the M. avium colonies (Fig. 1C). Recent IS1245 transposition events in M. avium have been observed in other studies with single colonies of the same isolate or isolates collected from individual patients over time (1, 3, 11, 12).

The IS1245 insertion sequence was initially considered to be species specific for M. avium, but additional studies have shown that this element is sporadically present in M. intracellulare, M. malmoense, M. scrofulaceum, and M. nonchromogenieicum (2, 7, 9). To our knowledge, the results obtained in this study confirm, for the first time, the presence of one or more copies of IS1245 in a strain of M. kansasii by PCR, RFLP, and DNA sequencing.

As a consequence, the utilization of IS1245 as a genetic marker for the identification of M. avium and its distinction from M. kansasii should be done with caution and combined with other genetic markers. The absence of IS1245 copies in some M. avium strains must also be taken into account (5, 11, 13).

IS1245 transposition events such as those described here can produce genome plasticity by the interruption or deletion of genes, affecting biological properties, which deserves further investigation.

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