

# Proposal that *Mycobacterium massiliense* and *Mycobacterium bolletii* be united and reclassified as *Mycobacterium abscessus* subsp. *bolletii* comb. nov., designation of *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. and emended description of *Mycobacterium abscessus*

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The names '*Mycobacterium abscessus* subsp. *abscessus*' and '*Mycobacterium abscessus* subsp. *massiliense*', proposed by Leao *et al.* (2009, *J Clin Microbiol* **47**, 2691–2698), cannot be validly published. The purpose of this report is to provide a description in accordance with the Rules of the *Bacteriological Code* (1990 Revision). Moreover, the proposal of the name '*Mycobacterium abscessus* subsp. *massiliense*' is contrary to Rule 38 and the correct name of this taxon, at the rank of subspecies, is *Mycobacterium abscessus* subsp. *bolletii* comb. nov. A description of *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. and an emended description of *Mycobacterium abscessus* are also given.

The species *Mycobacterium bolletii* (Adékambi *et al.*, 2006a) and *Mycobacterium massiliense* (Adékambi *et al.*, 2004, 2006b) cannot be separated from *Mycobacterium abscessus* (Moore & Frerichs, 1953; Kusunoki & Ezaki, 1992) by phenotypic tests and results derived from genotypic studies, such as DNA–DNA hybridization and RFLP-16S rRNA, supporting the proposition that the three taxa actually represent a single species with internal variability. Consequently, Leao *et al.* (2009) proposed the union of *M. bolletii* and *M. massiliense*, and the recognition of two subspecies within *M. abscessus*: '*Mycobacterium abscessus* subsp. *abscessus*' and '*Mycobacterium abscessus* subsp. *massiliense*'.

In contradiction to the *Bacteriological Code* (1990 Revision) (Lapage *et al.*, 1992; De Vos & Trüper, 2000), Leao *et al.* (2009) do not provide protologues for the new subspecies (see Rule 27) and therefore the names '*Mycobacterium abscessus* subsp. *abscessus*' and '*Mycobacterium abscessus*

subsp. *massiliense*' cannot be validly published by citation in a Validation List.

Moreover, the proposal of '*Mycobacterium abscessus* subsp. *massiliense*' is not in accordance with the Rules:

- The name *Mycobacterium bolletii* was validly published on page 140 of the January 2006 issue of the *International Journal of Systematic and Evolutionary Microbiology*.
- The name *Mycobacterium massiliense* was validly published on page 2025 of the September 2006 issue of the *International Journal of Systematic and Evolutionary Microbiology*, by citation in Validation List 111.
- According to Rule 24b(2), the name *Mycobacterium bolletii* therefore has priority over the name *Mycobacterium massiliense*. Data presented by Leao *et al.* (2009) indicate that these taxa are heterotypic synonyms. According to Rule 38, if these taxa are considered to be one species, they must be united under the name *Mycobacterium bolletii*, and, according to Rule 50b, if that species is considered to be a subspecies of

A supplementary table is available with the online version of this paper.

*M. abscessus*, then the name of the subspecies must be *Mycobacterium abscessus* subsp. *bolletii*.

According to Rule 27(2) (Lapage *et al.*, 1992; De Vos & Trüper, 2000), a reference to a previous effectively published description is in accordance with the Rules. So, we propose the valid publication of the names *Mycobacterium abscessus* subsp. *bolletii* comb. nov. and *Mycobacterium abscessus* subsp. *abscessus* subsp. nov. with reference to the study by Leao *et al.* (2009) and the correction included in this report. The species description of *M. abscessus* is also emended to cover both *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *abscessus*.

The data from Leao *et al.* (2009), as well as other publications (Kim *et al.*, 2008; Macheras *et al.*, 2009; Zelazny *et al.*, 2009; Leao *et al.*, 2010), showed a clear internal variability at the phenotypic and genotypic levels in the species *M. abscessus*. Such a characteristic suggests strongly that possible future descriptions of other subspecies in the species *M. abscessus* cannot be discounted.

#### Emended description of *Mycobacterium abscessus* (Moore and Frerichs 1953) Kusunoki and Ezaki 1992

*Mycobacterium abscessus* (abs.ces'sus. L. gen. n. *abscessus* of an abscess).

The following properties are displayed in addition to those listed in the species description by Kusunoki & Ezaki (1992): alkaline phosphatase, pyrrolidonyl arylamidase and tolerance to 5% (w/v) NaCl are present. Colonies can be smooth and grow on 5% sheep blood agar. Several enzymic activities show within-species variability, such as urease, catalase at 68 °C,  $\beta$ -glucosidase and  $\beta$ -galactosidase. More stable phenotypic characteristics are absence of growth at  $\geq 42$  °C, negative result for nitrate reduction and negative result for utilization of sodium citrate. The mycolic acid pattern, displayed by using HPLC, is very similar to those of other related species, showing only minor differences and low discriminatory power (Leao *et al.*, 2009). *M. abscessus* has a single ribosomal operon (*rrn* operon) per genome (Domenech *et al.*, 1994; Leao *et al.*, 2009). This species has two characteristic PRA-*hsp65* patterns: type 1, showing *Bst*EII (bp) (235, 210) and *Hae*II (bp) (145, 70, 60, 50), and type 2 with *Bst*EII (bp) (235, 210) and *Hae*III (bp) (200, 70, 60, 50). The trait of 711 bp of the *rpoB* and the trait of 401 bp of the *hsp65* nucleotide sequences show up to 96.6 and 98.7% within-species similarity, respectively.

*Mycobacterium abscessus* (Moore and Frerichs 1953) Kusunoki and Ezaki 1992 emend. Leao *et al.* (authorship reads Leao, Tortoli and Garcia) encompasses two subspecies: *Mycobacterium abscessus* subsp. *bolletii* (Adékambi *et al.* 2006a) Leao, Tortoli and Garcia, comb. nov., and *Mycobacterium abscessus* subsp. *abscessus* (Moore and Frerichs 1953) Kusunoki and Ezaki 1992, subsp. nov.

The type strain is Hauduroy L948<sup>T</sup> (=TMC 1543<sup>T</sup> = ATCC 19977<sup>T</sup> =CCUG 20993<sup>T</sup> =CIP 104536<sup>T</sup> =DSM 44196<sup>T</sup> =JCM 13569<sup>T</sup> =NCTC 13031<sup>T</sup>).

#### Description of *Mycobacterium abscessus* subsp. *bolletii* (Adékambi *et al.* 2006a) Leao, Tortoli and Garcia, comb. nov.

*Mycobacterium abscessus* subsp. *bolletii* (bol.le'ti.i. N.L. gen. masc. n. *bolletii* of Bollet, to honour Claude Bollet, a famous clinical microbiologist and taxonomist).

Basonym: *Mycobacterium bolletii* Adékambi *et al.* 2006a.

The description is based on that given for *Mycobacterium bolletii* by Adékambi *et al.* (2006a), with the addition of the description given by Adékambi *et al.* (2006b) for *Mycobacterium massiliense*. As demonstrated by Leao *et al.* (2009), the characterization of a wide set of isolates clearly showed further within-subspecies variability in this subspecies. Thus, the phenotypic characteristics of this subspecies show variation between strains in relation to the characteristics indicated in the first description given by Adékambi *et al.* (2006a). For example, several strains are able to grow in 5% (w/v) NaCl and others reveal a positive result for urease. The variability is even stronger when comparing antimicrobial susceptibility: several strains are susceptible to clarithromycin, in contrast to the resistance described in the initial report. It is important to mention that discrepancies in susceptibility tests could be also ascribed to the method used. *Mycobacterium abscessus* subsp. *bolletii* has the properties given in the emended description of *Mycobacterium abscessus*. Additional features that distinguish this subspecies are the PRA-*hsp65* pattern and the *rpoB* and *hsp65* gene sequence differences. The PRA-*hsp65* pattern that characterizes *Mycobacterium abscessus* subsp. *bolletii* is *Mycobacterium abscessus* type 2. The trait of 711 bp of the *rpoB* and the trait of 401 bp of the *hsp65* nucleotide sequences from *Mycobacterium abscessus* subsp. *bolletii* show up to 95.6 and 98.5% similarity, respectively, to *Mycobacterium abscessus* subsp. *abscessus*. This subspecies includes strains of the species *Mycobacterium massiliense* described previously (Adékambi *et al.*, 2004, 2006b).

The type strain is BD<sup>T</sup> (=CCUG 50184<sup>T</sup> =CIP 108541<sup>T</sup> =JCM 15297<sup>T</sup>).

#### Description of *Mycobacterium abscessus* subsp. *abscessus* (Moore and Frerichs 1953) Kusunoki and Ezaki 1992, subsp. nov.

*Mycobacterium abscessus* subsp. *abscessus* (abs.ces'sus. L. gen. n. *abscessus* of an abscess).

Rule 40d (formerly Rule 46) of the *Bacteriological Code* (1990 Revision) (Lapage *et al.*, 1992; De Vos & Trüper, 2000) states that 'the valid publication of a subspecific name which excludes the type of the species automatically creates another subspecies which includes the type and whose name bears the same specific and subspecific epithets as the name of the type'. As *Mycobacterium bolletii* is transferred to the species *Mycobacterium abscessus* (*Mycobacterium abscessus* subsp. *bolletii* comb. nov.), an automatic consequence of this rule is that the subspecies *Mycobacterium abscessus* subsp. *abscessus*

(Moore and Frerichs 1953) Kusunoki and Ezaki 1992 must be created.

At the time of writing, the Judicial Opinion about the request by Tindall & Garrity (2008) has not been published. Thus, the authorship of the subspecies *Mycobacterium abscessus* subsp. *abscessus* follows the current Rule 40d.

The description is as that given for *Mycobacterium abscessus* by Kubica *et al.* (1972) and Kusunoki & Ezaki (1992). *Mycobacterium abscessus* subsp. *abscessus* has the properties given in the emended description of *Mycobacterium abscessus*. Additional features that distinguish this subspecies are the PRA-*hsp65* pattern and the *rpoB* and *hsp65* gene sequence differences. *Mycobacterium abscessus* subsp. *abscessus* shows the characteristic PRA-*hsp65* pattern *Mycobacterium abscessus* type 1. The trait of 711 bp of the *rpoB* and the trait of 401 bp of the *hsp65* nucleotide sequences from *Mycobacterium abscessus* subsp. *abscessus* show up to 95.6 and 98.5% similarity, respectively, to *Mycobacterium abscessus* subsp. *bolletii*.

The type strain is Hauduroy L948<sup>T</sup> (=TMC 1543<sup>T</sup> =ATCC 19977<sup>T</sup> =CCUG 20993<sup>T</sup> =CIP 104536<sup>T</sup> =DSM 44196<sup>T</sup> =JCM 13569<sup>T</sup> =NCTC 13031<sup>T</sup>).

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