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Multilocus sequence typing scheme for the *Mycobacterium abscessus* complex

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Abstract

We developed a multilocus sequence typing (MLST) scheme for *Mycobacterium abscessus* sensu lato, based on the partial sequencing of seven housekeeping genes: *argH*, *cya*, *glpK*, *gnd*, *murC*, *pta* and *purH*. This scheme was used to characterize a collection of 227 isolates recovered between 1994 and 2010 in France, Germany, Switzerland and Brazil. We identified 100 different sequence types (STs), which were distributed into three groups on the tree obtained by concatenating the sequences of the seven housekeeping gene fragments (3576 bp): the *M. abscessus* sensu stricto group (44 STs), the "*M. massiliense*" group (31 STs) and the "*M. bolletii*" group (25 STs). SplitTree analysis showed a degree of intergroup lateral transfers. There was also evidence of lateral transfer events involving *rpoB*. The most prevalent STs in our collection were ST1 (CC5; 20 isolates) and ST23 (CC3; 31 isolates). Both STs were found in Europe and Brazil, and the latter was implicated in a large

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post-surgical procedure outbreak in Brazil. Respiratory isolates from patients with cystic fibrosis belonged to a large variety of STs; however, ST2 was predominant in this group of patients.

Our MLST scheme, publicly available at www.pasteur.fr/mlst, offers investigators a valuable typing tool for *M. abscessus* sensu lato in future epidemiological studies throughout the world.

Keywords: *Mycobacterium abscessus* complex; Molecular typing; Multilocus sequence typing; Phylogenetic analysis; Molecular identification; *rpoB*; Cystic fibrosis; Opportunistic mycobacteria; Non-tuberculous mycobacteria

1. Introduction

Mycobacterium abscessus sensu lato (i.e., *Mycobacterium abscessus* sensu stricto, *Mycobacterium massiliense* and *Mycobacterium bolletii*) is a rapidly growing mycobacterium (RGM) that has emerged as an important pathogen following its recognition as an entity distinct from *Mycobacterium chelonae* in 1992 [28]. It is now recognized as the most frequent RGM causing lung disease in humans, far ahead of *M. chelonae* and *Mycobacterium fortuitum* [2]. *M. abscessus* sensu lato lung disease most often, but not exclusively, develops in subjects with underlying lung disorders [20,21]. The disease is particularly prevalent in patients with cystic fibrosis (CF), including young children, and is becoming a major issue in most CF centers in Europe, Israel and North America [21,25,33,40,46,51]. It has recently been shown that, likewise *M. tuberculosis*, the rough morphotype of *M. abscessus*, is more virulent and tends to persist. The molecular mechanisms of this finding are related to genetic modifications within the peptide gene cluster *mps1-mps2-gap* or *mmpl4b* implicated in the synthesis and transport of glyco-peptido-lipids [42,54]. *M. abscessus* sensu lato is also a leading cause of sporadic and epidemic cases of skin and soft-tissue (SST) infections following local trauma, the use of contaminated syringes or needles or after surgery [4,16,26]. Several large outbreaks of skin and soft-tissue infection have been reported following injection of adrenal cortex extract, mesotherapy, abdominoplasty, tattooing and piercing [2,7,30]. *M. abscessus* sensu lato may be responsible for disseminated, sometimes fatal, infections in immunodeficient patients, especially following organ transplantation [9].

Several methods have been developed for typing *M. abscessus* sensu lato isolates. These have been used to investigate outbreaks or pseudo-outbreaks of respiratory and skin and soft-tissue infections and to determine if *M. abscessus* sensu lato could be transmitted between patients in CF populations [10,25,39,52,58]. The typing methods described include multilocus enzyme electrophoresis (MLEE) [17,61,63], pulsed-field gel electrophoresis (PFGE); [62,67], random amplified polymorphic DNA (RAPD) [29,66], enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) [48], repetitive sequence-based PCR (rep-PCR) [65] and multispacer sequence typing [49]. The reference typing method is PFGE, which is technically demanding and fastidious [67].

MLST is an unambiguous sequence-based typing method that has been used to investigate the population structure of different bacterial pathogens belonging to various genera including *Neisseria*, *Escherichia*, *Burkholderia*, *Listeria*, *Streptococcus* and *Staphylococcus* [8,11,12,36,37,43]. MLST involves sequencing of 350- to 600-bp internal fragments of several housekeeping genes (typically seven). Data are stored in a central database and are freely available to laboratories worldwide (http://www.mlst.net/). MLST is thus particularly suitable for local and global epidemiological studies because it provides data that can be easily compared between laboratories via the Internet without exchanging strains.

Infections with *M. abscessus* sensu lato are widespread as they are found in all continents and associated with a large panel of pathologies. Nevertheless, nothing is known as to the association of particular isolates of this species to epidemics or the tropism of this species for different anatomic sites or for CF patients. The MLST method constitutes an ideal tool for approaching these questions, but no MLST scheme is thus far available for typing of mycobacterial species, including isolates of the *M. abscessus* sensu lato species.

In the present study, we developed a MLST scheme and applied it to a large collection of *M. abscessus* sensu lato isolates of various clinical and geographical origins. The use of this MLST scheme, which is publicly available (www.pasteur.fr/mlst), will lead to a better understanding of the epidemiology of *M. abscessus* sensu lato throughout the world.

2. Materials and methods

2.1. Bacterial strains and culture conditions

We studied 224 isolates of *M. abscessus* sensu lato, including 223 clinical isolates from 197 patients and one environmental isolate (Table S1); 120 isolates were from our recent multilocus sequence analysis (MLSA) study [35]. The isolates were recovered between 1994 and 2010 and originated in France ($N = 181$), Germany ($N = 6$), Switzerland ($N = 7$) and Brazil ($N = 30$). Twenty-two of the Brazilian isolates were related to an outbreak and had been previously typed by pulsed-field electrophoresis (Fig. S1) [30]. Among the 181 "French" isolates, 4 were from the Reunion Island (Indian Ocean, southeast Africa) and 12 from Guadeloupe (Caribbean Ocean, Central America). Only one isolate was taken per subject ($N = 181$), except for 43 serial isolates taken from 16 CF patients (2 isolates from 9 patients, 3 isolates from 4 patients, 4 isolates from 2 and 5 isolates from 1 patient) (Table S1). The type strains *M. abscessus* (sensu stricto) CIP 104536^T (=ATCC