Tuberculosis 93 S1 (2013) S15–S20

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Recent progress towards understanding genetic variation in the *Mycobacterium abscessus* complex

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KEYWORDS

Mycobacterium abscessus
Subspecies
Variants
Strain typing
Genomics
Infection models

ABSTRACT

Mycobacterium abscessus is an emerging cause of respiratory disease and soft tissue infections. Whole genome sequencing and other molecular approaches are enhancing our understanding of outbreaks, antibiotic resistance mechanisms, and virulence properties, and of the phylogeny of the *M. abscessus* complex. Infection models are providing further insights into factors such as colony phenotype that impact host-pathogen interactions. This paper reviews recent developments in our understanding of genetic variation in *M. abscessus* and the potential relevance for disease and treatment.

1. Introduction

Mycobacterium abscessus was first isolated from a knee abscess,[1] and is now increasingly identified as a cause of skin, ocular, and other soft tissue infections associated with injury, cosmetic or medical procedures.[2-5] However, it is arguably of greatest concern as an emerging respiratory pathogen, particularly in cystic fibrosis (CF) patients.[6-8] Risk factors for *M. abscessus* respiratory disease include CF, bronchiectasis, tuberculosis or other mycobacterial respiratory disease,[2,6,9-12] and symptoms include cough and fatigue, with cavitation occurring in about 15% of cases.[2,13] *M. abscessus* is the leading of cause of respiratory disease cases due to rapidly-growing mycobacteria (RGM).[13]

Recently described as an "antibiotic nightmare",[14] *M. abscessus* is naturally resistant to most antibiotics in clinical use, including first-line antitubercular drugs.[2] Unlike *M. tuberculosis*, respiratory infection with *M. abscessus* is believed to occur from environmental sources,[15] with only limited evidence suggesting patient-to-patient transmission.[10,11,16] Isolation from water, a high level of resistance to chlorine, and the ability of *M. abscessus* to form biofilms in household plumbing materials are consistent with recent studies pointing to municipal water systems as a key source of infection, especially with respiratory disease.[17-19]

Genetic studies on *M. abscessus* have lagged far behind those on *M. tuberculosis* and the organism can be difficult to mutate,[20] hindering our understanding of the relevance of strain differences and the genetic factors that may influence disease type and progression, antibiotic resistance, environmental distribution, and other properties. Indeed, the first full genome sequence of an *M. abscessus* strain[21] was published over 10 years after the sequence of *M. tuberculosis* H37Rv.[22] Much remains to be learned regarding genotypic diversity in *M. abscessus* and its clinical significance. This review highlights some of the recent progress towards understanding the genetic differences, and the relevance of these differences, in the *M. abscessus* complex.

2. The *M. abscessus* complex

M. abscessus is a complex of subspecies, but these subspecies are less well-defined than in the *M. tuberculosis* complex, and the nomenclature is undergoing revisions.[23,24] Three subspecies of *M. abscessus* have been described in the literature, based on differentiation by PCR and multilocus sequencing of housekeeping genes: *M. abscessus* subsp. *abscessus* (*M. abscessus* sensu stricto), *M. abscessus* subsp. *bolletii* (*M. bolletii*), and *M. abscessus* subsp. *massiliense* (*M. massiliense*).[23-26] The three subspecies have identical sequence in the portion of the 16S rRNA gene often used to discriminate between species of RGM.[27-29] Nucleotide differences in *rpoB*, *hsp65*, *recA*, and *sodA* indicated that the type strains of *M. bolletii* and *M. massiliense* differed from *M. abscessus* ATCC 19977, which was originally the type strain for the species and is now the type strain for *M. abscessus* sensu stricto (see Table 1).

Although sequence analysis of housekeeping genes was instrumental for subdividing *M. abscessus* into subspecies, some clinical isolates have a composite genetic pattern with housekeeping genes corresponding to more than one subspecies, and therefore assessment of multiple genetic regions is recommended.[25,26,30,31] For some strains with ambiguous identities, assignment to a subspecies has been assisted by sequence analysis of additional

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1472-9792/$ – see front matter

Table 1
Featured strains of the *M. abscessus* complex

Strain	Alternate designations [a]	Notes	Genbank accession #	References
M. abscessus sensu stricto ATCC19977	TMC1543, Hauduroy L948, CIP104536	Type strain, first sequenced genome, isolated from knee abscess, inducible resistance to macrolides	CU458896	(1, 21, 43, 45)
M. bolletii BD	CCUG 50184, CIP 108541	Type strain, sputum isolate, mutational resistance to macrolides	AHAS00000000	(27, 58)
M. massiliense CCUG 48898 [b]	CIP108297	Type strain, respiratory isolate, macrolide-susceptible	AKVF00000000	(28, 43, 59)
M. massiliense BRA100 [b]		Epidemic strain originally identified in Brazil; highly resistant to glutaraldehyde	CP003699 (isolate GO-06); ATFQ00000000 (isolate CRM-0020)	(32, 66, 87, 88)
M. massiliense TPE101		Epidemic strain from Taiwan, associated with extrapulmonary infections		(68)

[a]Designations obtained from www.straininfo.net and www.dsmz.de; abbrevations, ATCC, American Type Culture Collection; CIP, Collection Institut Pasteur; CCUG, Culture Collection of the University of Goteborg
[b]Due to the ongoing revisions of names within the *M. abscessus* complex, these strains are referred to as *M. bolletii* in some publications

housekeeping genes,[25,26] and by repetitive-sequence-based PCR and pulsed-field gel electrophoresis (PFGE). The latter methods reveal that strains of a given subspecies often have similar molecular typing patterns.[25] The frequency with which strains with hybrid genomes are recovered suggests that horizontal gene transfer (HGT) occurs readily within the *M. abscessus* complex.[24,26] Of course, if extensive genetic transfer has occurred in a strain, assignment to a specific subspecies based on selected genes may not be very meaningful and as yet, there is no consensus on how many genes or regions should be analyzed. The increasing application of whole-genome sequencing for strains of the *M. abscessus* complex[11,32] may help to address this issue by identifying the most suitable regions for separating the taxa. Interestingly, a new report by Shallom et al. used comparative genome hybridization to provide a more extensive comparison of genomes of the type strains and clinical isolates.[24] They identified four indel regions that could assign most isolates to one of the three subspecies based on the size of PCR amplicons. Their results further revealed subgroups within *M. abscessus* sensu stricto and *M. massiliense*, but they noted that some isolates appeared to fall between taxa, again possibly due to HGT.[24] Assigning isolates to specific taxa within the complex has become increasingly important in view of the evidence for subspecies differences in antibiotic susceptibility and potentially in virulence,[33,34] as described further below.

3. Macrolide susceptibility

The macrolides clarithromycin and azithromycin are important therapeutic agents for the treatment of *M. abscessus* respiratory infections.[2] Mutational resistance to these macrolides results from point mutations at positions 2058 or 2059 in the 23S rRNA gene, and is detected as growth in three days in the presence of clarithromycin.[35,36] Mutational resistance to macrolides has been detected in all three subspecies of the complex,[34,37] and the type strain *M. bolletii* BD (see Table 1) was reported to have this form of resistance.[27] More recently, inducible resistance to macrolides was discovered in the *M. abscessus* complex and this is detected as growth following extended incubations in clarithromycin.[9,38] Inducible resistance is conferred by the *erm*(41) gene,[38] one of the few antibiotic resistance genes to have been characterized in the *M. abscessus* complex. Similar to *erm*(37) of *M. tuberculosis*,[39] *erm*(41) encodes a methyltransferase that methylates 23S rRNA, resulting in resistance.

Laboratories in Korea and France reported that specific deletions inactivate *erm*(41) gene in *M. massiliense*, rendering this subspecies macrolide susceptible.[33,34] Consistent with a nonfunctional *erm*(41) gene, clinical studies have reported higher rates of radiological improvement, conversion to negative sputum smears, and maintenance of negative cultures with respiratory infections from *M. massiliense* compared to *M. abscessus* sensu stricto, after combination therapy that included clarithromycin.[40,41] These findings indicate that inducible resistance impacts the effectiveness of standard treatment regimens containing clarithromycin, and therefore that patients infected with strains lacking inducible resistance are more likely to have a better treatment outcome. Interestingly, distribution of the subspecies appears to vary by geographic region,[30,41,42] and although the basis for these geographic differences is unknown, regions with higher rates of respiratory infections due to *M. massiliense* should see higher response rates to macrolide-based treatment. However, an intriguing new paper indicates that azithromycin has a greater efficacy than clarithromycin against strains with inducible resistance in experimental models, and this correlated with a reduced ability of azithromycin to induce *erm*(41).[43] In strains in which erm(41) was not functional, the two macrolides had similar efficacy.[43] Azithromycin may therefore be the more suitable macrolide for treatment of infections by the *M. abscessus* complex,[44] especially when it is unknown if the infecting strain has inducible macrolide resistance.

It is important to note that there is no absolute correlation between subspecies and inducible resistance, and that macrolide susceptibility testing is required to confirm whether a strain has inducible resistance. Although it was previously proposed that *M. massiliense* be defined by a truncated *erm*(41) gene,[33,34] Shallom and colleagues discovered two isolates of *M. massiliense* with full-length, functional *erm*(41) genes when using indels to differentiate between the subspecies.[24] Additionally, some strains of *M. abscessus* sensu stricto were reported to be macrolide-susceptible due to a T28C transition in *erm*(41) that reduces the activity of the enzyme.[33,34,38] However, there is some evidence that strains with this mutation are capable of induced macrolide resistance if they are pre-exposed to low levels of clarithromycin.[43]

4. Genomic analyses

M. abscessus strain ATCC19977 (CIP 104536T) was designated the type strain for *M. abscessus*[45] before separation of the complex into subspecies. It is now the type strain for *M. abscessus* sensu stricto, and became the first strain of the *M. abscessus* complex to have a fully sequenced genome.[21,46] Prior to completion of the full genome sequence of 5.1 Mb, the most extensive sequence data available for ATCC19977 was of a 25.2 kb region that is missing from some strains[47] and that corresponds to ORFs MAB_2066c to