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Inaccuracy of Single-Target Sequencing for Discriminating Species of the *Mycobacterium abscessus* Group▽

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We determined nucleotide sequences of *rpoB*, *hsp65*, and *sodA* in 59 clinical isolates (from 58 patients) of the *Mycobacterium abscessus* group. Identification to the species level, based on three target genes, was concordant for 44 isolates (25 *M. abscessus*, 13 *Mycobacterium massiliense*, and 6 *Mycobacterium bolletii* isolates) and discordant for 15 isolates which had "interspecific composite patterns." Sequence analysis of five housekeeping genes also showed composite patterns in 8 of these 15 isolates.

Mycobacterium abscessus is a rapidly growing mycobacterium (RGM) causing a wide spectrum of disease in humans, including pulmonary disease, skin and soft tissue disease, and disseminated disease (8). It is a major pathogen in patients with cystic fibrosis (CF) (15, 23, 25, 29), in which case it is responsible for severe lung disease and may cause disseminated infection following transplantation (10). *M. abscessus* has undergone many taxonomic changes since its first description by Moore and Frerichs in 1953, in which this organism was reported to be morphologically and biochemically different from the hitherto known RGM and was named *Mycobacterium abscessus* (*M. abscessus* strain ATCC 19977^T) (22). In 1972, Stanford et al. reported another RGM differing from *M. abscessus* by only a few characters. The two RGMs were then classified as one species (*Mycobacterium chelonei*) with two subspecies—*M. chelonei* subsp. *chelonei* and *M. chelonei* subsp. *abscessus* (formerly *M. abscessus*) (19, 32). In 1984, the species name was changed from *M. chelonei* to *Mycobacterium chelonae* (16). This taxonomy remained valid until the beginning of the 1990s, after which DNA/DNA hybridization showed that *M. chelonae* subsp. *abscessus* and *M. chelonae* subsp. *chelonae* constituted two different species (*M. abscessus* and *M. chelonae*) (20). Further development of molecular identification methods in the late 1990s led to a new classification (34). Using different targets, mainly *hsp65* and *rpoB*, it soon became obvious that isolates of *M. abscessus* were relatively heterogeneous (2, 26, 31). On the basis of *rpoB* sequence data, a new species, *Mycobacterium massiliense*, was identified within the *M. abscessus* group in 2004, followed by another new species, *Mycobacterium bolletii*, in 2006 (1, 5). Thus, *M. abscessus* (*M. abscessus* sensu lato) is now divided into three species, *M. abscessus* sensu stricto, *M. massiliense*, and *M. bolletii*.

We recently isolated an RGM strain which was identified as *M. abscessus* sensu stricto (for reasons of simplicity, *M. abscessus* sensu stricto will hereinafter be referred to as *M. abscessus*) on the basis of the *rpoB* sequence but as *M. massiliense* on the basis of the *hsp65* sequence (strain AP3). This suggested the existence of isolates with "interspecific composite patterns" (e.g., isolates with an *M. abscessus rpoB* sequence and an *M. massiliense hsp65* sequence) within the *M. abscessus* group, potentially leading to inaccuracy for diagnostic approaches based on single-target sequencing. We investigated this issue by determining the sequences of *rpoB*, *hsp65*, and another widely used molecular target, *sodA* (3, 18), in a large panel of *M. abscessus* sensu lato strains.

The studied panel included 59 clinical isolates of *M. abscessus* sensu lato obtained from 58 CF patients in France between 1997 and 2007. Strains were grown on sheep blood agar at 37°C for 4 days to obtain visible colonies. Smooth (S) and rough (R) phenotypes were determined as described previously (9). A loopful of colonies was used for DNA extraction by using Tris-EDTA, lysozyme, and proteinase K, in the presence of thiourea to avoid DNA degradation (37). The strains *M. abscessus* CIP 104536^T (same as ATCC 19977^T), *M. massiliense* CIP 108297^T, and *M. bolletii* CIP 108541^T were included for control purposes. In all strains, *hsp65* (441 bp [26]), *rpoB* (723 bp [3]), and *sodA* (541 bp [3]) were amplified by PCR using AmpliTaq Gold polymerase (Applied Biosystems, Courtaboeuf, France). Dideoxy sequencing was carried out on both strands with the BigDye Terminator cycle sequencing kit (Applied Biosystems). Sequencing products were purified by gel filtration (Bio-Gel P-100; Bio-Rad, Marnes-la-Coquette, France) and were run on a 3700 DNA analyzer (Applied Biosystems).

Species identification based on *rpoB*, *hsp65*, and *sodA* sequencing was concordant for 44 isolates (25 *M. abscessus* [16 S and 9 R], 13 *M. massiliense* [8 S and 5 R], and 6 *M. bolletii* [4 S and 2 R] isolates) and discordant for 15 isolates (8 S and 7 R). In 8 of these 15 isolates (see Table 2), both *rpoB* and *hsp65* were 100% identical to the *M. abscessus* type strain sequence, whereas *sodA* shared the highest identity with the *M. bolletii*

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TABLE 1. Primers used for PCR and sequencing

Gene	Primer	Sequence	Amplified fragment size (bp)	Temp (°C)	Reference
rpoB	MYCOF	5′-GGCAAGGTCACCCCGAAGGG-3′	723	68	3
	MYCOR	5′-AGCGGCTGCTGGGTGATCATC-3′		68	
hsp65	TB11	5′-ACCAACGATGGTGTGTCCAT-3′	441	60	33
	TB12	5′-CTTGTCGAACCGCATACCCT-3′		62	
sodA	SODLGF	5′-GAAGGAATCTCGTGGCTGAATAC-3′	541	68	3
	SODLGR	5′-AGTCGGCCTTGACGTTCTTGTAC-3′		70	
argH	ARGHF	5′-GACGAGGGCGACAGCTTC-3′	629	60	This study
	ARGHSR1	5′-GTGCGCGAGCAGATGATG-3′		58	
glpK	GLPKSF1	5′-AATCTCACCGGCGGTGTC-3′	609	58	This study
	GLPKSR2	5′-GGACAGACCCACGATGGC-3′		60	
murC	MURCSF1	5′-CGGACGAAAGCGACGGCT-3′	607	60	This study
	MURCSR2	5′-CCAAAACCCTGCTGAGCC-3′		58	
gnd	GNDF	5′-GTGACGTCGGAGTGGTTGG-3′	634	62	This study
	GNDSR1	5′-CTTCGCCTCAGGTCAGCTC-3′		62	
cya	ACF	5′-GTGAAGCGGGCCAAGAAG-3′	647	58	This study
	ACSR1	5′-AACTGGGAGGCCAGGAGC-3′		60	

(isolates 7, 48, 59, 70, AP2, AP6, and AP11) or *M. massiliense* type strain sequence (isolate 31). In three other isolates, *rpoB* was most similar to that of *M. abscessus* (isolates R3, AP3, and 63), whereas both *hsp65* and *sodA* were 100% identical to those of *M. massiliense*. For isolates 6, 47, 19, and 38, comparisons of *rpoB*, *hsp65*, and *sodA* with the control sequences yielded contradictory data, resulting in the identification of *M. massiliense* (99.7 to 100% identity) or *M. bolletii* (99.8 to 100% identity), depending on the target gene.

The 15 isolates showing "interspecific composite patterns" were further studied by partially sequencing the housekeeping genes *argH* (argininosuccinate lyase), *glpK* (glycerol kinase), *murC* (UDP-*N*-acetylmuramate-L-Ala ligase), *gnd* (6-phosphogluconate dehydrogenase), and *cya* (adenylate cyclase). The nucleotide sequences of these five genes were obtained from GenBank (27) for *M. abscessus* CIP 104536^T and were determined using the primers presented in Table 1 for *M. massiliense* CIP 108297^T and *M. bolletii* CIP 108541^T; these sequences were submitted to GenBank (accession numbers FJ609780, FJ609781, FJ609782, FJ609783, and FJ609784 for *argH*, *glpK*, *murC*, *gnd*, and *cya* of *M. bolletii* and FJ609785, FJ609786, FJ609787, FJ609788, and FJ609789 for *argH*, *glpK*, *murC*, *gnd*, and *cya* of *M. massiliense*, respectively). The proteins encoded by *argH*, *gnd*, and *cya* have been used for multilocus enzyme electrophoresis analysis of *Mycobacterium tuberculosis* and the *Mycobacterium avium-Mycobacterium intracellulare* complex (14, 30), whereas *murC* and *glpK* are known housekeeping genes of *M. tuberculosis* and *Mycobacterium leprae* (21, 28). Dideoxy sequencing was carried out on both strands as described above.

Sequence data obtained with *argH*, *glpK*, *murC*, *gnd*, and *cya* were concordant in seven isolates (three S and four R; all five genes had the highest identity to the same species) and were discordant (four or more genes had the highest identity to the same species) in eight isolates (five S and three R) (Table 2). Among the seven isolates with concordant data, four shared the strongest identity with *M. abscessus* (isolates 48, AP2, AP6, and AP11) and three with *M. massiliense* (isolates R3, 6, and 47). These results were consistent with sequence data obtained with *hsp65* in all seven isolates, with *rpoB* in five isolates (48, AP2, AP6, AP11, and 6), and with *sodA* in only two isolates (R3 and 47). Among the eight isolates showing composite patterns, six could be presumptively identified to the species level (same species assignment provided by two genes among *rpoB*, *hsp65*, and *sodA* and by four genes among *argH*, *glpK*, *murC*, *gnd*, and *cya*)—three as *M. abscessus* (isolates 7, 59, and 70), two as *M. massiliense* (isolates AP3 and 63), and one as *M. bolletii* (isolate 38). Finally, two isolates had a highly complex pattern (isolates 19 and 31) that did not allow species identification.

All isolates were tested for their susceptibility to clarithromycin and minocycline, using the broth microdilution method (Sensititre RGMYCO; Biocentric, Bandol, France) (24). All but 4 of the 44 isolates with concordant *rpoB*, *hsp65*, and *sodA* sequences had MICs to clarithromycin of ≤2 mg/liter. The remaining four isolates (two *M. abscessus*, one *M. massiliense*, and one *M. bolletii*) had MICs of >32 mg/liter. Thus, in contrast to previous reports (4), most of the *M. bolletii* isolates studied (five of six) were susceptible to clarithromycin. All but one of the *M. abscessus* and *M. bolletii* isolates with concordant *rpoB*, *hsp65*, and *sodA* sequences had MICs to minocycline of ≥16 mg/liter, while 7 of the 13 *M. massiliense* isolates had MICs of ≤8 mg/liter, including four isolates with MICs of ≤1 mg/liter. The MICs to clarithromycin and minocycline of the 15 strains with "interspecific composite patterns" are shown in Table 2. The MICs to clarithromycin were ≤2 mg/liter for nine isolates and ≥16 mg/liter for five isolates (one was presumptively identified as *M. abscessus*, two as *M. massiliense*, one as *M. bolletii*, and one was unknown). All but one of the isolates (isolate 47, which had a MIC of 8 mg/liter and was presumptively identified as *M. massiliense*) had MICs to minocycline of ≥32 mg/liter.

Today, the main method for identifying an RGM to the species level is based on the sequencing of one gene in particular, *rpoB*, which has been widely studied (1, 2). Our study shows that this strategy is not suitable for identifying species within the *M. abscessus* group, due to the significant number of isolates displaying a composite genetic structure—more than a quarter of the isolates in our series. This relatively high number does not seem to reflect a bias in selection; indeed, we

TABLE 2. Isolates with "composite" gene patterns identified in this study

Isolate[a]	S/R morphotype	MIC (mg/liter)[b]		Species[c]	% Identity[d]								Presumptive identification[e]
		CLA	MNO		*rpoB*	*hsp65*	*sodA*	*argH*	*glpK*	*murC*	*gnd*	*cya*	
7 (Nantes, 2004)	R	>32	>32	*M. abscessus*	**100**	**100**	97.9	**98.4**	**100**	**99.4**	**99.4**	97.9	*M. abscessus*
				M. massiliense	96.6	98.3	98.1	95.2	98.1	98.2	97.7	**99.8**	
				M. bolletii	95.9	97.9	**100**	96.2	98.6	96.7	95.9	97.1	
48 (Pessac, 2004)	S	4	>32	*M. abscessus*	**100**	**100**	97.6	**98.8**	**99.8**	**99.3**	**98.9**	**99.6**	*M. abscessus*
				M. massiliense	96.2	98.3	97.8	96.2	97.9	97.9	97.4	98.1	
				M. bolletii	95.4	97.9	**99.2**	97.0	98.4	96.3	95.6	97.7	
59 (Paris, 2000)	R	1	>32	*M. abscessus*	100	**100**	97.7	**98.2**	**100**	**99.6**	**99.6**	97.3	*M. abscessus*
70 (Vannes, 2002)	S	2	32	*M. massiliense*	96.3	98.2	97.9	95.0	98.1	98.3	97.9	97.3	
				M. bolletii	95.5	97.9	**99.8**	96.0	98.6	96.9	96.4	**99.2**	
AP2 (Paris, 2005)	R	≤0.12	>32	*M. abscessus*	**100**	**100**	97.8	**98.2**	**100**	**99.6**	**99.6**	**100**	*M. abscessus*
AP6 (Paris, 2007)	R	≤0.12	32	*M. massiliense*	96.5	98.5	98.0	95.0	98.1	98.3	97.9	98.1	
AP11 (Paris, 2005)	S	0.25	32	*M. bolletii*	95.9	98.2	**99.8**	96.0	98.6	96.9	96.2	98.1	
31 (Montpellier, 2004)	S	2	>32	*M. abscessus*	**100**	**100**	99.4	95.8	98.0	98.0	97.3	**99.6**	?
				M. massiliense	96.5	98.5	**100**	**99.8**	**100**	**100**	**99.4**	98.1	
				M. bolletii	95.7	98.2	98.1	97.4	98.9	96.7	96.7	97.7	
R3 (Roscoff, 2002)	R	≤0.12	>32	*M. abscessus*	**100**	98.5	99.5	95.6	98.0	**99.6**	97.6	98.1	*M. massiliense*
				M. massiliense	96.6	**100**	**100**	**99.8**	**100**	98.3	**100**	**100**	
				M. bolletii	95.8	99.1	98.2	97.0	98.9	97.0	96.9	97.3	
AP3 (Boulogne, 2007)	S	≤0.12	>32	*M. abscessus*	**99.4**	98.2	99.5	95.6	98.0	97.8	97.7	97.9	*M. massiliense*
				M. massiliense	97.2	**100**	**100**	**100**	**100**	**99.8**	**99.8**	97.9	
63 (Tours, 2004)	S	≤0.12	>32	*M. bolletii*	96.2	98.8	98.2	97.2	98.9	96.5	97.2	**99.4**	
6 (Nantes, 2003)	R	>32	32	*M. abscessus*	96.3	98.5	97.8	95.6	97.9	97.8	97.6	97.7	*M. massiliense*
				M. massiliense	**100**	**100**	98.0	**99.6**	**99.8**	**99.1**	**99.6**	**99.6**	
				M. bolletii	98.2	99.1	**99.8**	97.2	98.8	96.9	96.9	96.9	
47 (Grenoble, 2004)	S	>32	8	*M. abscessus*	99.7	98.2	99.4	95.6	97.7	98.0	97.3	97.7	*M. massiliense*
				M. massiliense	98.4	**100**	**100**	**99.6**	**99.6**	**100**	**99.4**	**99.6**	
				M. bolletii	**100**	99.1	98.2	97.2	98.6	96.7	96.7	96.9	
19 (Paris, 2004)	R	16	>32	*M. abscessus*	95.7	98.2	99.6	96.6	**100**	98.0	95.9	**99.4**	?
				M. massiliense	98.3	99.1	**100**	97.2	98.0	**99.6**	97.5	98.6	
				M. bolletii	**99.8**	**100**	98.2	**100**	98.6	97.0	**98.6**	97.9	
38 (La Réunion, 2005)	S	>32	>32	*M. abscessus*	95.7	98.2	97.7	96.6	**99.1**	95.8	95.7	98.1	*M. bolletii*
				M. massiliense	98.5	**99.7**	98.2	97.2	98.2	97.0	96.9	97.3	
				M. bolletii	**100**	99.4	**100**	**100**	98.8	**100**	**100**	**100**	

[a] Isolate no. (city in France and year of collection).
[b] CLA, clarithromycin; MNO, minocycline.
[c] Reference strains used for comparison were *M. abscessus* CIP 104536^T (same as ATCC 19977^T), *M. massiliense* CIP 108297^T, and *M. bolletii* CIP 108541^T.
[d] Values shown in boldface type indicate the highest percentage of identity.
[e] ?, unknown.

studied all the isolates from our collection, which were obtained by different laboratories at different geographical locations and on different dates. The only potential bias could arise from the fact that all our isolates were from CF patients. However, this is very unlikely because *M. abscessus* sensu lato isolates found in CF patients show extensive genetic diversity and are probably acquired in the community, from various reservoirs, rather than acquired in hospitals or by patient-to-patient transmission (6, 29).

Several recent studies have reported similar findings. Viana-Niero et al. reported two *M. bolletii* isolates with a *sodA* sequence identical to that in *M. massiliense* (35). Kim et al. reported two isolates from South Korea which were identified as *M. massiliense* with *rpoB* and *sodA* and as *M. abscessus* with *hsp65* (18). Very recently, Zelazny et al. reported 7 out of 42 clinical isolates showing ambiguous identification by partial sequencing of *rpoB*, *hsp65*, and *secA* (36). These findings, similar to our own observations, are unlikely to be related to mutations (the presence of specific genetic signatures). Rather, they suggest genetic exchange among members of the *M. abscessus* group, most probably leading to legitimate recombination events between homologous genes. As shown for the first time here, these events also involve housekeeping genes, such as *cya* and *glpK*, and are undoubtedly relatively frequent in this group of bacteria. Certain *M. abscessus* isolates thus seem to have a composite genetic structure, resulting from genetic exchange between the members of this group.

The practical implication of these results is that an *M. abscessus* sensu lato isolate cannot be reliably identified to the species level by sequencing a single genetic locus, whatever it is. As reported in the study by Devulder et al. (13), *sodA* was the most variable gene in our study; in 10 composite strains with concordant *rpoB* and *hsp65* sequences, the *sodA* sequence diverged, and in five of these strains, *sodA* was the only one that diverged. However, *rpoB* and *hsp65*, the genes most frequently used for RGM identification (11–13, 18, 35), also gave diverging data for a number of strains. For two of our isolates (isolates 19 and 31), the sequence data for the eight genes studied, comprising ~4,330 bp, did not allow us to distinguish between *M. abscessus*, *M. massiliense*, and *M. bolletii*.

Furthermore, our findings, together with those of Viana-Niero et al. (35), Kim et al. (18) and Zelazny et al. (36), question the distinction between *M. abscessus*, *M. massiliense*, and *M. bolletii* within the *M. abscessus* group. *M. massiliense* and *M. bolletii* were identified as novel species on the basis of >3% divergence of their *rpoB* gene sequence from the sequences of the *M. abscessus* type strain (1, 5, 7, 36). The difference between *M. abscessus* and *M. massiliense* or *M. bolletii rpoB* nucleotide sequences is indeed about 3%, but there is only a 1.5% difference between those of *M. massiliense* and *M. bolletii*. Our results do not call into question the existence of these three entities within the *M. abscessus* group; indeed, it is clear that composite strains arose from exchanges between these three entities. But the definition of species based on just one or two gene sequences should be questioned (17). Further studies are needed to determine whether these three bacterial entities should be considered truly distinct species within the *M. abscessus* group.

Nucleotide sequence accession numbers. The sequences of the following genes were submitted to GenBank: *argH*, *glpK*, *murC*, *gnd*, and *cya* of *M. bolletii* CIP 108541^T under accession numbers FJ609780, FJ609781, FJ609782, FJ609783, and FJ609784, respectively, and *argH*, *glpK*, *murC*, *gnd*, and *cya* of *M. massiliense* CIP 108297^T under accession numbers FJ609785, FJ609786, FJ609787, FJ609788, and FJ609789, respectively.

REFERENCES

1. **Adekambi, T., P. Berger, D. Raoult, and M. Drancourt.** 2006. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. Int. J. Syst. Evol. Microbiol. **56:**133–143.
2. **Adékambi, T., P. Colson, and M. Drancourt.** 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41:**5699–5708.
3. **Adékambi, T., and M. Drancourt.** 2004. Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, *hsp65*, *sodA*, *recA* and *rpoB* gene sequencing. Int. J. Syst. Evol. Microbiol. **54:**2095–2105.
4. **Adékambi, T., and M. Drancourt.** 2009. Mycobacterium bolletii respiratory infections. Emerg. Infect. Dis. **15:**302–305.
5. **Adekambi, T., M. Reynaud-Gaubert, G. Greub, M. J. Gevaudan, B. La Scola, D. Raoult, and M. Drancourt.** 2004. Amoebal coculture of "*Mycobacterium massiliense*" sp. nov. from the sputum of a patient with hemoptoic pneumonia. J. Clin. Microbiol. **42:**5493–5501.
6. **Bange, F. C., B. A. Brown, C. Smaczny, R. J. Wallace, Jr., and E. C. Bottger.** 2001. Lack of transmission of *Mycobacterium abscessus* among patients with cystic fibrosis attending a single clinic. Clin. Infect. Dis. **32:**1648–1650.
7. **Blackwood, K. S., C. He, J. Gunton, C. Y. Turenne, J. Wolfe, and A. M. Kabani.** 2000. Evaluation of *recA* sequences for identification of *Mycobacterium* species. J. Clin. Microbiol. **38:**2846–2852.
8. **Brown-Elliott, B. A., and R. J. Wallace, Jr.** 2002. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. Clin. Microbiol. Rev. **15:**716–746.
9. **Catherinot, E., J. Clarissou, G. Etienne, F. Ripoll, J. F. Emile, M. Daffe, C. Perronne, C. Soudais, J. L. Gaillard, and M. Rottman.** 2007. Hypervirulence of a rough variant of the *Mycobacterium abscessus* type strain. Infect. Immun. **75:**1055–1058.
10. **Chernenko, S. M., A. Humar, M. Hutcheon, C. W. Chow, C. Chaparro, S. Keshavjee, and L. G. Singer.** 2006. *Mycobacterium abscessus* infections in lung transplant recipients: the international experience. J. Heart Lung Transplant. **25:**1447–1455.
11. **Cheunoy, W., T. Prammananan, A. Chaiprasert, and S. Foongladda.** 2005. Comparative evaluation of polymerase chain reaction and restriction enzyme analysis: two amplified targets, *hsp65* and *rpoB*, for identification of cultured mycobacteria. Diagn. Microbiol. Infect. Dis. **51:**165–171.
12. **Cooksey, R. C., M. A. Jhung, M. A. Yakrus, W. R. Butler, T. Adekambi, G. P. Morlock, M. Williams, A. M. Shams, B. J. Jensen, R. E. Morey, N. Charles, S. R. Toney, K. C. Jost, Jr., D. F. Dunbar, V. Bennett, M. Kuan, and A. Srinivasan.** 2008. Multiphasic approach reveals genetic diversity of environmental and patient isolates of *Mycobacterium mucogenicum* and *Mycobacterium phocaicum* associated with an outbreak of bacteremias at a Texas hospital. Appl. Environ. Microbiol. **74:**2480–2487.
13. **Devulder, G., M. Perouse de Montclos, and J. P. Flandrois.** 2005. A multigene approach to phylogenetic analysis using the genus *Mycobacterium* as a model. Int. J. Syst. Evol. Microbiol. **55:**293–302.
14. **Feizabadi, M. M., I. D. Robertson, D. V. Cousins, D. J. Dawson, and D. J. Hampson.** 1997. Use of multilocus enzyme electrophoresis to examine genetic relationships amongst isolates of *Mycobacterium intracellulare* and related species. Microbiology **143:**1461–1469.
15. **Griffith, D. E.** 2003. Emergence of nontuberculous mycobacteria as pathogens in cystic fibrosis. Am. J. Respir. Crit. Care Med. **167:**810–812.
16. **Hill, L. R., V. B. D. Skerman, and P. H. A. Sneath.** 1984. Corrigenda to the approved lists of bacterial names edited for the international committee on systematic bacteriology. Int. J. Syst. Bacteriol. **34:**508–511.
17. **Janda, J. M., and S. L. Abbott.** 2002. Bacterial identification for publication: when is enough enough? J. Clin. Microbiol. **40:**1887–1891.
18. **Kim, H. Y., Y. Kook, Y. J. Yun, C. G. Park, N. Y. Lee, T. S. Shim, B. J. Kim, and Y. H. Kook.** 2008. Proportion of *Mycobacterium massiliense* and *Mycobacterium bolletii* in Korean *Mycobacterium chelonae-Mycobacterium abscessus* group isolates. J. Clin. Microbiol. **46:**3384–3390.
19. **Kubica, G. P., I. Baess, R. E. Gordon, P. A. Jenkins, J. B. G. Kwapinski, C. McDurmont, S. R. Pattyn, H. Saito, V. Silcox, J. L. Stanford, K. Takeya, and M. Tsukamura.** 1972. A co-operative numerical analysis of rapidly growing mycobacteria. J. Gen. Microbiol. **73:**55–70.
20. **Kusunoki, S., and T. Ezaki.** 1992. Proposal of *Mycobacterium peregrinum* sp. nov., nom. rev., and elevation of *Mycobacterium chelonae* subsp. *abscessus* (Kubica et al.) to species status: *Mycobacterium abscessus* comb. nov. Int. J. Syst. Bacteriol. **42:**240–245.
21. **Mahapatra, S., D. C. Crick, and P. J. Brennan.** 2000. Comparison of the UDP-*N*-acetylmuramate:L-alanine ligase enzymes from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. J. Bacteriol. **182:**6827–6830.
22. **Moore, M., and J. B. Frerichs.** 1953. An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region; report of a case with a study of the organism, Mycobacterium abscessus, n. sp. J. Investig. Dermatol. **20:**133–169.
23. **Mussaffi, H., J. Rivlin, I. Shalit, M. Ephros, and H. Blau.** 2005. Nontuberculous mycobacteria in cystic fibrosis associated with allergic bronchopulmonary aspergillosis and steroid therapy. Eur. Respir. J. **25:**324–328.
24. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing. Document no. M100-S12. NCCLS, Wayne, PA.
25. **Olivier, K. N., D. J. Weber, R. J. Wallace, Jr., A. R. Faiz, J. H. Lee, Y. Zhang, B. A. Brown-Elliot, A. Handler, R. W. Wilson, M. S. Schechter, L. J. Edwards, S. Chakraborti, and M. R. Knowles.** 2003. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am. J. Respir. Crit. Care Med. **167:**828–834.
26. **Ringuet, H., C. Akoua-Koffi, S. Honore, A. Varnerot, V. Vincent, P. Berche, J. L. Gaillard, and C. Pierre-Audigier.** 1999. *hsp65* sequencing for identification of rapidly growing mycobacteria. J. Clin. Microbiol. **37:**852–857.
27. **Ripoll, F., S. Pasek, C. Schenowitz, C. Dossat, V. Barbe, M. Rottman, E. Macheras, B. Heym, J. L. Herrmann, M. Daffe, R. Brosch, J. L. Risler, and J. L. Gaillard.** 2009. Nonmycobacterial virulence genes in the genome of the emerging pathogen *Mycobacterium abscessus*. PLoS ONE **4:**e5660.
28. **Sassetti, C. M., D. H. Boyd, and E. J. Rubin.** 2003. Genes required for mycobacterial growth defined by high density mutagenesis. Mol. Microbiol. **48:**77–84.
29. **Sermet-Gaudelus, I., M. Le Bourgeois, C. Pierre-Audigier, C. Offredo, D. Guillemot, S. Halley, C. Akoua-Koffi, V. Vincent, V. Sivadon-Tardy, A. Ferroni, P. Berche, P. Scheinmann, G. Lenoir, and J. L. Gaillard.** 2003. *Mycobacterium abscessus* and children with cystic fibrosis. Emerg. Infect. Dis. **9:**1587–1591.
30. **Shenoy, A. R., N. Srinivasan, and S. S. Visweswariah.** 2002. The ascent of

nucleotide cyclases: conservation and evolution of a theme. J. Biosci. **27:**85–91.
31. **Springer, B., E. C. Bottger, P. Kirschner, and R. J. Wallace, Jr.** 1995. Phylogeny of the *Mycobacterium chelonae*-like organism based on partial sequencing of the 16S rRNA gene and proposal of *Mycobacterium mucogenicum* sp. nov. Int. J. Syst. Bacteriol. **45:**262–267.
32. **Stanford, J. L., S. R. Pattyn, F. Portaels, and W. J. Gunthorpe.** 1972. Studies of *Mycobacterium chelonei*. J. Med. Microbiol. **5:**177–182.
33. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
34. **Tortoli, E.** 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin. Microbiol. Rev. **16:**319–354.
35. **Viana-Niero, C., K. V. Lima, M. L. Lopes, M. C. Rabello, L. R. Marsola, V. C. Brilhante, A. M. Durham, and S. C. Leao.** 2008. Molecular characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in isolates collected from outbreaks of infections after laparoscopic surgeries and cosmetic procedures. J. Clin. Microbiol. **46:**850–855.
36. **Zelazny, A. M., J. M. Root, Y. R. Shea, R. E. Colombo, I. C. Shamputa, F. Stock, S. Conlan, S. M. McNulty, B. A. Brown-Elliott, R. J. Wallace, Jr., K. N. Olivier, S. M. Holland, and E. P. Sampaio.** 2009. Cohort study of molecular identification and typing of *Mycobacterium abscessus*, *Mycobacterium massiliense* and *Mycobacterium bolletii*. J. Clin. Microbiol. **47:**1985–1995.
37. **Zhang, Y., M. A. Yakrus, E. A. Graviss, N. Williams-Bouyer, C. Turenne, A. Kabani, and R. J. Wallace, Jr.** 2004. Pulsed-field gel electrophoresis study of *Mycobacterium abscessus* isolates previously affected by DNA degradation. J. Clin. Microbiol. **42:**5582–5587.