



Figure S1. Phylogenetic tree of 16S rRNA sequences of the mycobacterium reference sequences, outgroup strains and the *Mycobacterium* spp. strains from this study.

Table S1. Origin and identification of acid-fast bacteria, identified as *Mycobacteria* with the ZN staining. Shown are the identification with PRA, and identification with BLAST with the sequence of the 16S gene. The strains LTR2603, LTS244, LTO3295, and LTN4726 were identified with the *hsp65* sequence, and the strains LTP1387, LTQ2392, and LTS855 with the *rpoB* sequences. The final column is the species identification based on a phylogenetic analysis.

	Group s	Code ^a	Year ^b	Age	Sex	Sample Type ^c	Pre- identificati on with PRA ^d	Blast Identification: 16S, rpoB and/or Hsp65 Sequence	Species identification by phylogenetic Analysis
COMPLE X 1	G1	LTN1047	2016	39	F	Secretion	<i>M. spp.</i>	<i>M. mucogenicum</i>	<i>M. mucogenicum</i> and <i>phocaicum</i> ^e
	G2	LTQ372	2019	28	F	NA ^f	<i>M. abscessus</i>	<i>M. phocaicum</i>	<i>M. porcinum</i> ^e
		LTM488 4	2015	NA	F	NA	<i>M. spp.</i>	<i>M. porcinum</i> / <i>M. fortuitum</i>	<i>M. porcinum</i>
		LTM501 8	2015	86	M	Sputum	<i>M. spp.</i>	<i>M. phocaicum</i> / <i>M. mucogenicum</i>	<i>M. porcinum</i>
		LTN2236	NA	NA	NA	Tap water medical consultin g room	<i>M. spp.</i>	<i>M. phocaicum</i> / <i>M. mucogenicum</i>	<i>M. porcinum</i>
			LTR2603	2021	74	M	Secretion	<i>M. tuberculosis</i> / <i>M. fortuitum</i>	<i>M. fortuitum</i> / <i>M. spp.</i> / <i>M. gilvum</i>
	G3	LTS244	2018	70	M	Secretion	<i>M. tuberculosis</i> / <i>M. fortuitum</i>	<i>M. fortuitum</i> <i>M. fortuitum</i> / <i>M. spp.</i> / <i>M. gilvum</i>	<i>M. fortuitum</i>
		LTO3295	2017	45	F	Sputum	<i>M. spp.</i>	<i>M. fortuitum</i> <i>M. fortuitum</i> / <i>M. spp.</i> / <i>M. gilvum</i>	<i>M. fortuitum</i>
		LTS1330	2021	63	F	Sputum	NA	<i>M. fortuitum</i> <i>M. fortuitum</i> / <i>M. spp.</i> / <i>M. gilvum</i>	<i>M. fortuitum</i>
	COMPLE X 2	G4	LTQ3133	2019	42	M	Biopsy	<i>M. peregrinum</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M. houstonense</i> / <i>M. fortuitum</i>
LTP1387			2018	62	F	Pleural fluid	<i>M. spp.</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M. houstonense</i> / <i>M. fortuitum</i>	<i>M. senegalense</i> and <i>farcinogenes</i> ^e
LTQ2392			2019	24	F	Secretion	<i>M. peregrinum</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M.</i>	<i>M. conceptionense</i>

COMPLEX 3							<i>houstonense</i> / <i>M. fortuitum</i>	
		LTS855	2021	19	F	Sputum	<i>M. tuberculosis</i> / <i>M. fortuitum</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M. houstonense</i> / <i>M. fortuitum</i> <i>M. conceptionense</i>
		LTP1795	2018	41	F	Secretion	<i>M. spp.</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M. houstonense</i> / <i>M. fortuitum</i> <i>M. conceptionense, senegalense and farcinogenes</i> ^e
	G5	LTS2245	2021	68	M	Sputum	<i>M. spp.</i>	<i>M. senegalense</i> / <i>M. conceptionense</i> / <i>M. farcinogenes</i> / <i>M. houstonense</i> / <i>M. fortuitum</i> <i>M. conceptionense, senegalense and farcinogenes</i> ^e
		LTN3490	2016	60	M	NA	<i>M. spp.</i>	<i>M. immunogenum</i> / <i>M. chelonae</i> / <i>M. spp.</i> <i>M. immunogenum abscessus and chelonae</i> ^e
		LTN4726	2016	17	M	Biopsy	<i>M. spp.</i>	<i>M. chelonae</i> / <i>M. abscessus</i> <i>M. abscessus</i>
	G6	LTP1604	2018	58	M	Pleural fluid	<i>M. spp.</i>	<i>M. thermoresistibile</i> / <i>M. spp.</i> <i>M. thermoresistibile</i>
		LTO2244	2017	45	F	Sputum	<i>M. spp.</i>	<i>N. cyriacigeorgica</i> / <i>M. vanbaalenii</i> Non-identifiable
	G7	LTS2688	2021	8	M	Pleural fluid	NA	<i>M. wolinskyi</i> / <i>M. jacuzzii</i> <i>M. wolinskyi</i>
		LTN3283	2016	-	-	Sauce	<i>M. goodii</i> / <i>M. smegmatis</i>	<i>M. wolinskyi</i> / <i>M. jacuzzii</i> <i>M. wolinskyi</i>
		LTL3271	2014	27	F	Secretion	<i>M. spp.</i>	<i>M. jacuzzii</i> / <i>M. peregrinum</i> / <i>M. spp.</i> <i>M. wolinskyi</i>
	G8	LTN478	2016	43	F	Sputum	<i>M. spp.</i>	<i>M. bacteremicum</i> / <i>M. neoaurum</i> / <i>M. spp.</i> <i>M. neoaurum</i>
		LTM655 7	2015	77	F	Sputum	<i>N. spp.</i> / <i>M. neonarum</i>	<i>M. bacteremicum</i> / <i>M. neonarum</i> <i>M. neoaurum</i>
		LTQ339	2019	19	M	Pleural fluid	<i>M. spp.</i>	<i>M. cosmeticum</i> / <i>M. spp.</i> / <i>M. wolinskyi</i> <i>M. cosmeticum</i>

G9	LTM439 1	2015	40	M	Secretion	<i>M. spp.</i>	<i>M. iranicum</i> / <i>M. spp.</i>	<i>M. iranicum</i>	
	L- carnitine	NA	-	-	Cosmetic product	<i>M. mucogenicu m</i>	<i>M. mucogenicum</i> / <i>M. phocaicum</i> / <i>M. spp.</i>	<i>M. llatzerense</i>	
	LTQ5825	2019	48	F	Secretion	<i>N. spp.</i>	<i>M. mucogenicum</i> / <i>M. phocaicum</i> / <i>M. spp.</i> / <i>M. Llatzerense</i>	<i>M. llatzerense</i> ^e	
	LTQ0649	2019	30	F	Pleural fluid	<i>Actinomiceta e</i>	<i>M. obuense</i> / <i>M. spp.</i> / <i>M. kyogaense</i>	<i>M. obuense</i>	
COMPLE X 4	G10	LTS1567	2021	63	F	Sputum	NA	<i>M. crocinum</i> / <i>M. spp.</i> / <i>M. gilvum</i> / <i>M. chitae</i> / <i>M. aquaticum</i>	Non-identifiable
	G11	LTS1933	2021	63	F	Sputum	NA	<i>M. simiae</i>	<i>M. simiae</i>
		LTP1806	2018	65	F	Pleural fluid	<i>M. spp.</i>	<i>M. simiae</i>	<i>M. simiae</i>
	G12	LTL3678	2014	26	M	Sputum	<i>N. spp.</i>	<i>M. szulgai</i> / <i>M. angelicum</i>	<i>M. szulgai</i>
		LTM489 9	2015	79	M	Pleural fluid	<i>N. spp.</i>	<i>M. szulgai</i> / <i>M. angelicum</i> / <i>M. spp.</i>	<i>M. szulgai</i>
COMPLE X 5	G13	LTQ4602	2019	66	M	Sputum	NA	<i>M. kansasii</i> / <i>M. gastri</i> / <i>M. ostraviense</i>	<i>M. kansasii</i>
	G14	LTL2677	2014	59	F	Sputum	<i>M. spp.</i>	<i>M. marseillense</i> / <i>M. yongonense</i> / <i>M. intracellulare</i>	<i>M. marseillense</i> , <i>intracellulare</i> y <i>avium</i> ^e
		LTP4572	2018	76	M	Sputum	<i>M. chimaera</i>	<i>M. paraintracellulare</i> / <i>M. intracellulare</i> subspp. <i>chimaera</i>	<i>M. marseillense</i> , <i>intracellulare</i> y <i>avium</i> ^e
	G15	LTS1805	2021	58	F	Secretion	NA	<i>M. ulcerans</i> / <i>M. marinum</i>	Non-identifiable
	G16	LTL3092	2014	24	F	Biopsy	<i>N. spp.</i>	<i>C. jeikeium</i> / <i>C. amycolatum</i>	Non-identifiable
		LTM338 1	2015	61	F	Urine	<i>N. spp.</i>	<i>C. jeikeium</i>	Non-identifiable
		LTM621 3	2015	82	F	Sputum	<i>M. chimaera</i> / <i>M. intracellulare</i>	<i>C. jeikeium</i> / <i>C. amycolatum</i>	Non-identifiable
	Outgr oup	LTN436	2016	43	F	Sputum	<i>M. spp.</i>	<i>T. pulmonics</i> / <i>T. conjunctivitis</i>	<i>T. pulmonis</i>

LTP1678	2018	70	F	Sputum	<i>Actinomiceta</i> <i>e</i>	<i>N. puris</i> / <i>N. spp.</i>	<i>N. puris</i>
LTS449	2021	62	F	Secretion	<i>N. spp.</i>	<i>N. niwae</i> / <i>N. beijingensis</i>	<i>N. cyriacigeorgica</i> ^e
LTN2051	2016	-	M	Sputum	<i>M. spp.</i>	<i>N. cyriacigeorgica</i> / <i>N. spp.</i>	<i>N. cyriacigeorgica</i>
LTQ5521	2019	29	M	Pleural fluid	<i>M. abscessus</i>	<i>G. bronchialis</i> / <i>G. spp.</i> / <i>Actinomycetia bacterium</i>	Non-identifiable
LTS2331	2021	30	M	Sputum	<i>N. spp.</i>	<i>G. sputi</i> / <i>G. spp.</i>	<i>G. sputi</i>
LTN381	2016	64	M	Pleural fluid	<i>M. spp.</i>	<i>Clostridium perfringens</i>	Non-identifiable

^a Laboratory code Caracas; ^bYear of isolation; ^cType of clinical sample; ^dPre-identification by PRA;

^eNear (NR);

NA = not available. These are patients' samples but unknown is the type of clinical sample.

Genera: M.: *Mycobacterium*, N*: *Nocardia*, T*: *Tsukamurella*, G*: *Gordonia*; C*: *Corynebacterium*. For strains LTM4391, LTM6213, LTN3490, LTL3092 and LTQ5825, indicated in bold red in the table, no PCR product for the hsp65 PCR was obtained.

Table S2. GenBank accession numbers of *Mycobacterium* species sequences used in the phylogenetic analysis.

<i>Mycobacterium</i> Species	ID Genbank		
	16S	<i>rpoB</i>	<i>hsp65</i>
<i>M. bovis</i>	AF547903	AF057451	AF547813
<i>M. africanum</i>	AF547893	JF923622	AF547803
<i>M. tuberculosis</i>	X52917	L27989	AY299144
<i>M. avium</i> Sub. <i>Avium</i>	AJ536037	-	-
<i>M. abscessus</i>	AY457071	AY262741	AY458075
<i>M. cosmeticum</i>	AY449728	DQ124109	AY449730
<i>M. fortuitum</i>	AY457066	AY147165	AY458072
<i>M. wolinskyi</i>	AY457083	-	-
<i>M. obuense</i>	X55597	AY544951	-
<i>M. arupense</i>	DQ157760	JN571215	EU191917
<i>M. monacense</i>	AF107039	EU191923	JF491320
<i>M. neoaurum</i>	AF480593	JF712875	AF547860
<i>M. gordonae</i>	X52923	AY544919	AF547840
<i>M. mucogenicum</i>	AY457074	AY147171	AY458078
<i>M. szulgai</i>	X52926	AY544966	AF547878
<i>M. phocaicum</i>	AY859682	AY859693	DQ987726
<i>M. kansasii</i>	AJ536035	KY933079	AF547849
<i>M. conceptionense</i>	AY859684	AY859695	AM902957
<i>M. senegalense</i>	AY457081	AY262738	AY458067
<i>M. virginiae</i>	KR025879	KR025893	MK587449
<i>M. kumamotoense</i>	JN571176	JN571253	AB239920
<i>M. thermoresistibile</i>	X55602	AY544968	-
<i>M. marseillense</i>	EU266632	EF584444	EU239788
<i>M. intracellulare</i>	AJ536036	JQ411539	AF547848
<i>M. iranica</i>	HQ009482	-	-
<i>M. farcinogenes</i>	AY457084	AY262742	AF547830
<i>M. porcinum</i>	AF480588	AY544955	AF547867
<i>M. peregrinum</i>	AF547956	AY147166	AY458069

<i>M. chelonae</i>	AF547909	AY147163	JX154110
<i>M. simiae</i>	AF547966	AY544963	AF547875
<i>M. aquaticum</i>	KY392537	-	-
<i>M. llatzerense</i>	AJ746070	-	-
<i>M. immunogenum</i>	HE654001	-	-
<i>Nocardia puris</i>	AJ508748	DQ08514	AY903632
<i>Nocardia cyriacigeorgica</i>	KP137526	DQ085116	AY756522
<i>Tsukamurella pulmonis</i>	X92981	KX932012	-
<i>Tsukamurella tyrosinosolvens</i>	Y12248	KX932015	-
<i>Corynebacterium jeikeium</i>	AB470615	AY492231	-
<i>Gordonia iterans</i>	AB624257	-	-
<i>Gordonia sputi</i>	X81923	-	-

Table S3. DNA Models test, divergence percentages, and number of identifications for each gene.

Gene	Model	Outgroup	Ingroup	% DIVERGENCE		Identifications
				Between	Overall	
16S	T92 ^a +G+I	2.99E+05	1.328	1-9	4.631	22
<i>rpoB</i>	GTR ^b +G+I	-	5.068	5.75-13.61	9.232	2
<i>hsp65</i>	GTR+G+I	-	3.606	2.32-12.48	8.288	5

^a:Tamura 3-parameter is the estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases; ^b: General Time Reversible gives nucleotides consisting of parameters of an equilibrium base frequency vector, where frequency at each base occurred at this site and rate matrix.

Table S4. 16S rRNA sequences of the 48 strains that were identified with ZN staining as belonging to the genus *Mycobacteria*.

#Accession	Sequence ID
ON171319	Mycobacterium1
ON171320	Mycobacterium2
ON171321	Mycobacterium3
ON171322	Nocardia4
ON171323	Corynebacterium5
ON171324	Mycobacterium6
ON171325	Gordonia7
ON171326	Mycobacterium8
ON171327	Mycobacterium9
ON171328	Mycobacterium10
ON171329	Mycobacterium11
ON171330	Tsukamurella12
ON171331	Mycobacterium13
ON171332	Nocardia14
ON171333	Mycobacterium15
ON171334	Mycobacterium16
ON171335	Mycobacterium17
ON171336	Gordonia18
ON171337	Mycobacterium19
ON171338	Mycobacterium20
ON171339	Mycobacterium21
ON171340	Mycobacterium22
ON171341	Mycobacterium23

ON171342	Mycobacterium24
ON171343	Mycobacterium25
ON171344	Corynebacterium26
ON171345	Mycobacterium27
ON171346	Mycobacterium28
ON171347	Clostridium29
ON171348	Mycobacterium30
ON171349	Mycobacterium31
ON171350	Mycobacterium32
ON171351	Mycobacterium33
ON171352	Mycobacterium34
ON171353	Mycobacterium35
ON171354	Mycobacterium36
ON171355	Nocardia37
ON171356	Mycobacterium38
ON171357	Mycobacterium39
ON171358	Corynebacterium40
ON171359	Mycobacterium41
ON171360	Mycobacterium42
ON171361	Mycobacterium43
ON171362	Mycobacterium44
ON171363	Mycobacterium45
ON171364	Mycobacterium46
ON171365	Mycobacterium47
ON171366	Mycobacterium48