Cross-transmission is not the source of new *Mycobacterium abscessus* infections in a multi-centre cohort of cystic fibrosis patients

Authors: Ronan M. Doyle[1,2]*, Marc Rubio[3]*, Garth Dixon[1,2], John Hartley[1,2], Nigel Klein[4,2], Pere Coll[3,5]*, Kathryn A. Harris[1,2]*

1. Department of Microbiology, Virology and Infection Control, Great Ormond Street Hospital NHS Foundation Trust, London, UK.
2. National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and UCL, UK.
3. Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain.
4. UCL Great Ormond Street Institute of Child Health, London, UK.
5. Servei de Microbiologia, Fundació de Gestió de l'Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

* Authors contributed equally

Corresponding author: Kathryn Harris (kathryn.harris@gosh.nhs.uk)

Main Summary:
Whole genome sequencing should replace current molecular typing used routinely in clinical microbiology laboratories. Patient-to-patient spread of *M. abscessus* is not common. Environmental screening may provide a better understanding of acquisition of *M. abscessus* infections.

Abstract

Background:

Mycobacterium abscessus is an extensively drug resistant pathogen that causes pulmonary disease particularly in cystic fibrosis (CF) patients. Identifying direct patient-to-patient transmission of *M. abscessus* is critically important in directing infection control policy for the management of risk in CF patients. A variety of clinical labs have used molecular epidemiology to investigate transmission. However there is still conflicting evidence as to how *M. abscessus* is acquired and whether cross-transmission occurs. Recently labs have applied whole-genome sequencing (WGS) to investigate this further and in this study we investigate whether WGS can reliably identify cross-transmission in *M. abscessus*.

Methods:

We retrospectively sequenced the whole genomes of 145 *M. abscessus* isolates from 62 patients seen at four hospitals in two countries over 16 years.

Results:

We have shown that a comparison of a fixed number of core single nucleotide variants (SNVs) alone cannot be used to infer cross-transmission in *M. abscessus* but does provide enough information to replace multiple existing molecular assays. We detected one episode of possible direct patient-to-patient transmission in a sibling pair. We found that patients acquired unique *M. abscessus* strains even after spending considerable time on the same wards with other *M. abscessus* positive patients.

Conclusions:

This novel analysis has demonstrated that the majority of patients in this study have not acquired *M. abscessus* through direct patient-patient transmission or a common reservoir. Tracking transmission using WGS will only realise its full potential with proper environmental screening as well as patient sampling.

Keywords: Nontuberculous mycobacteria, whole-genome sequencing, transmission, cystic fibrosis, phylogenomics

Background

Mycobacterium abscessus (recently renamed as *Mycobacteroides abscessus*) [1], is a group of three closely related subspecies *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* [1,2]. These rapidly-growing, non-tuberculous mycobacteria cause chronic pulmonary disease, particularly in patients with cystic fibrosis (CF) and other chronic lung diseases. *M. abscessus* is an important pathogen that has emerged in the CF patient population and that has been associated with poor clinical outcomes, especially following lung transplantation [3–5]. This is due, at least in part, to the extensive antibiotic resistance that makes infections with this organism difficult to treat [2,6]. CF patients infected with *M. abscessus* are frequently not listed for transplant, therefore the acquisition of this pathogen is considered to be a serious complication in this group.

The epidemiology of *M. abscessus* strains has been studied using Variable Nucleotide Tandem Repeats (VNTR) and Multi Locus Sequence Typing (MLST) [7]□. The clustering of globally spread sequence types was confirmed with whole genome sequencing (WGS) and has provided greater resolution in how the various lineages are related as well as predicting possible transmission routes [8,9]. A dominant method of transmission of *M. abscessus* remains contested [10,11], with evidence for and against patient-to-patient transmission being the common route [8,12–14]□□. *M. abscessus* is ubiquitous in the environment with its niche hypothesised to be free-living amoeba [15,16], but due to the difficulties in isolating the organism, little has been done to track environment-to-patient acquisition. Confirmation of direct patient-to-patient transmission is important as it influences management of high-risk patients and it could increase the effectiveness of infection control interventions by directing the use of limited resources.

In this retrospective study we assessed utility of using WGS to characterise subspecies, antimicrobial resistance (AMR) profiles and typing of *M. abscessus* isolates. We also wanted to utilise the data to investigate the scale of patient-to-patient transmission and whether identification of single nucleotide variants (SNVs) by WGS can confirm transmission. To do this we have sequenced the genomes of 145 *M. abscessus* clinical isolates from a well characterised cohort of 62 patients from four hospitals in two countries over 16 years.

Methods

Patients and Samples collection

We collected 33 *M. abscessus* isolates from 30 patients at Hospital de la Santa Creu I Sant Pau (bcn_hsp), Hospital Clínic (bcn_hcl) and Hospital Vall d'Hebron (bcn_hvh), Barcelona, Spain and 112 isolates from 32 patients from Great Ormond Street Hospital (GOSH), London, UK (Table 1). At GOSH, CF patients were screened for non-tuberculous mycobacterial (NTM) infection when attending clinics as part of their routine management. In addition to this, CF and other patients at all hospitals included in this study were screened for NTM infection when they presented with suggestive clinical symptoms or exacerbations. Demographic and patient location data were obtained from the patient administration system and microbiological data from the laboratory information management system using Structured Query Language (SQL) and Excel spreadsheets. Additional sources of information included CF and transplant databases. American Thoracic Society consensus guidelines were used to verify evidence of non-tubercuolous mycobacterial infection [17]. All investigations were performed in accordance with the Hospitals Research governance policies and procedures.

DNA extraction, Whole-Genome Sequencing and Multi Locus Sequence Typing (MLST)

Information on DNA extraction, whole-genome sequencing and MLST are included in Supplementary Methods.

Read mapping and variant calling

Sequenced reads for all samples were first mapped to *M. abscessus* subsp. *abscessus* ATCC 19977 using BBMap v37.90 (Joint Genome Institute). Single nucleotide variants (SNVs) were called against the reference genome using freebayes v1.2.0 [19] and variants were filtered to only include those at sites with a mapping quality >30, a base quality >30, at least

five supporting reads, where the variant was present on at least two forward and reverse strand reads and present at the 5' and 3' end of at least two reads.

Phylogenetic analysis

Potential regions of recombination were identified from the consensus genome sequences using Gubbins v2.3.1 [20]. Regions within the genome with low coverage (< 5x) were masked on a per sample basis and regions with low coverage across 75% of samples were masked across the entire dataset. A maximum likelihood tree was inferred from all samples using RAxML v8.2.4 [21] using a GTRCAT model with 99 bootstraps. Sub-species were identified for each sample based on their position upon this tree.

Separate sub-trees were also inferred for *M. abscessus* subsp. *massilense* sequences, as well as for *M. abscessus* subsp. *abscessus* ST-1 and ST-26 sequences. All samples in each sub-tree were mapped against a suitable reference. *M. abscessus* subsp. *massilense* str. GO 06 was used as the reference sequencing for study *massilense* sequences and the *de novo* assembly of the earliest ST-26 study sequence (ldn_gos_2_520) was used as a reference for other ST-26 samples. *M. abscessus* subsp. *abscessus* ATCC 19977 was again used as the reference for ST-1 sequences as it is the same sequence type. All sub-trees were generated using the same method outlined above, apart from ST-26 subtree, which did not use Gubbins but instead variants were filtered if 3 SNVs were found within a 100bp window.

Sequence clusters

Sequence clusters to infer possible transmission were generated using three different methods on each subtree. First we used a SNV threshold that was based on the upper bounds of all within patient diversity applied to complete linkage hierarchical clustering based on pairwise SNV matrix. Secondly we assigned clusters using the R package rPinecone as it incorporates

SNV thresholds and root-to-tip distances and so has been useful when applied to clonal populations [22]. Lastly we also used hierBAPS [23] to assign clusters, however due to the fact that all samples are included in the sequence clusters we found it was not appropriate for this study question. We made the assumption that any strains taken from different patients that were within sequence cluster constituted a possible transmission event.

***De novo* assembly**

All samples underwent *de novo* assembly of bacterial genomes using SPAdes and pilon wrapped in the Unicycler v0.4.4 package [24]. Assembled contigs were annotated using prokka v1.13 [25] and comparison of the accessory genome was generated using roary v3.12.0 [26]. To generate a list of genes that could be used to differentiate isolates we filtered the annotated genes to remove coding sequences (CDS) greater than 8000 bp and less than 250 bp, as well as those only present in a single sample and those present in every sample.

Results

M. abscessus population distribution

We obtained whole genome sequences for 145 *M. abscessus* isolates from 62 patients. Thirty-three *M. abscessus* from Barcelona subdivided into 24 *M. abscessus* subsp. *abscessus*, two *M. abscessus* subsp. *bolletii and* seven *M. abscessus* subsp. *massiliense*. A hundred and twelve *M. abscessus* from UK subdivided into 78 *M. abscessus* subsp. *abscessus,* one *M. abscessus* subsp. *bolletii and* 33 *M. abscessus* subsp. *massiliense*. Sample MLST definitions, VNTR and AMR associated mutations are shown in supplementary table 1.

Possible transmission within *M. abscessus* clusters

To confirm possible transmission between patients we required their isolate genomes to be clustered together by two independent methods and epidemiological evidence that both patients were at the same hospital during the same time period. Using WGS data we inferred a phylogenetic tree from reference genome SNV matrix for all patients (Figure 1). We observed two low variant clusters of isolates that corresponded to ST-1 and ST-26 Pasteur MLST profiles (VNTR II and I respectively), as well as other closely related *M. abscessus* subsp. *massilense* isolates between patients. We used a SNV matrix from mapping against a reference (*M. abscessus* subsp. *abscessus* ATCC19977), as well as hierBAPS and rPinecone to predict sequence clusters. The sequence clusters generated from the single reference SNV matrix provided no further information than the MLST profiles, and in many cases provided spurious findings with large groups of isolates clustered with no epidemiological link (Supplementary Figure 1). This included large sequence clusters relating to a single MLST type which included isolates from different hospitals and countries.

Mapping to a single reference genome led to the inability of a single SNV cut-off, or model, to exclude unrelated isolates from sequence clusters because the number of pairwise SNV distances varied greatly between both subspecies and specific lineages (Figure 2). For

example, the pairwise median (interquartile range) SNV distance between just ST-1 isolates was 73 (62 – 81) compared to 29589 (27701 – 63703) for all *M. abscessus* subsp. *abscessus* isolates. The same differences were seen in *M. abscessus* subsp. *massilense* as well with a pairwise median (IQR) SNV distance between ST-23 and ST-48 isolates of 2084 (960 – 7274) compared to 70545 (59947 – 71891) across all isolates from the subspecies.

Sub-tree sequence clusters

The variation in the scale of diversity within subspecies and sequence type hampered efforts to capture possible transmission events. In order to improve accuracy of sequence clustering, multiple sub-trees were made for closely related isolates using a more suitable reference sequence. We separated *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massilense* isolates, as well as further sub-trees for ST-1 (VNTR II), ST-26 (VNTR I) and ST-23/ST-48 (VNTR III) isolates. We also integrated the presence of accessory genes when interrogating possible sequence clusters for transmission (Figures 3, 4 & 5). Sequence clusters were assigned for each sub-tree using both a single SNV threshold (Supplementary Figure 2) and rPinecone. Overall we found that predicting transmission from the sub-trees reduced the number of different patients clustered together from 46 to 19 and the number of possible sequence clusters suggesting patient-to-patient transmission from 11 to seven.

A total of 18 sequence clusters (I – XVIII) were identified (listed in supplementary table 1), 15 of these were within the sub-trees (I – XV), and seven clusters contained samples from more than one patient (IV, V, VI, VIII, XIV, XVI & XVII). We found no sequence clusters that contained samples from both the UK and Spain. We found no evidence of transmission between patients within ST-26. (Figure 3). Within ST-1, four clusters (IV, V, VI and VIII) containing samples from more than one patient were found. Three of these clusters (IV, V and VI) contained isolates from nine patients from multiple hospitals within Barcelona. Only two of these patients were in hospital during the same time period (cluster VI: bcn_hcl_009 and

bcn_hvh_30), but both were treated in different hospitals. Cluster VIII suggested transmission between two patients (ldn_gos_18 and ldn_gos_19) who were siblings with previously assumed either direct transmission or common reservoir (Figure 4) [13]. A single cluster (XIV) containing samples from two patients (ldn_gos_46 and ldn_gos_7) was found among ST-23 isolates. However, the two strains were isolated from samples taken nine years apart (Figure 5). Patient ldn_gos_7 was already positive for *M. abscessus* on first admission to Great Ormond Street Hospital (GOSH), and the two patients were present at the lung function lab within a month of each other on two occasions, but never in the same location at the same day, and never admitted to the same ward.

All samples found within their respective clusters also contained similar accessory gene profiles with the median (IQR) shared percentage of accessory genes within a sequence cluster being 89% (79% – 94%) compared to 18% (12% - 37%) for isolates not in the same sequence cluster.

For the 32 GOSH CF patients included in the study, 16 became infected with *M. abscessus* after their first visit to clinic (Table 1), however transmission confirmed by both WGS and epidemiological data could only be identified in one case (gos_19) thus suggesting a different route of acquisition for the rest of these patients.

Discussion

This study has shown that whole genome sequencing of *M. abscessus* isolates can determine sub-species, identify previously reported AMR associated mutations and provide common typing definitions in a single workflow. This single method can replace the multiple existing molecular assays used in clinical microbiology laboratories to provide the same information and could be used to predict novel resistance variants [27]. We used the WGS data to investigate the likelihood of cross-transmission and found 43 (69%) patients had unique

isolates that did not cluster with other patients. We identified seven sequence clusters from the remaining 19 patients but only one pair of patients (ldn_gos_18 and ldn_gos_19) had a plausible epidemiological link to support possible patient-to-patient transmission, as they were siblings. All other patients with genetically similar strains were either isolated in different countries, different hospitals or isolated from samples that were taken years apart, making direct transmission of these strains extremely unlikely.

Every *M. abscessus* isolated from a GOSH patient was sequenced and so the dataset generated represents a complete picture of *M. abscessus* infection in this hospital, which is vital for inferring transmission. Most of these patients were only attending clinics at GOSH, therefore this study has captured all of their *M. abscessus* isolates and they are unlikely to have been in contact with *M. abscessus* positive patients at other hospitals (Table 1). Therefore, if direct patient-patient transmission was occurring frequently we would expect to see evidence of it here. In contrast to this we found that the majority of patients in this study had unique strains and the majority of sequence clusters were multiple isolates from the same patients. This study confirms previous findings that despite many *M. abscessus* negative patients spending considerable time on the same wards as patients with ongoing *M. abscessus* infections they did not subsequently acquire genetically similar isolates [13,14,28].

We have therefore found that a fixed number of SNVs cannot be reliably used to infer cross-transmission across all *M. abscessus* isolates as there seems to be irreconcilable differences in the substitution rate between both sub-species and dominant clones. These difficulties are similar to those seen in *Legionella pneumophila* outbreaks where the majority of cases can belong to only a few sequence types [26]. *L. pneumophila* can also display different scales of genetic diversity within different sequence or genotypes and so it is also recognised that a single SNV threshold cut-off will not provide sufficient discriminatory power [27]. When using WGS to infer relatedness in *M. abscessus* there has previously been an attempt to find an absolute threshold which can rule in or rule out strains into a transmission event. This has

previously been placed as below 25-30 SNVs [8,14,29,30]. From our findings we would advocate using a suitable genetically similar reference sequence when carrying out core genome SNV calling, especially for the dominant clones such as ST-1 and ST-26. There is a large amount of variation within the genomes of *M. abscessus* [31] and so the use of a single reference such as *M. abscessus* subsp. *abscessus* ATCC 19977 will mask many differences between strains and generate spurious clusters of genetically similar sequences. Where a suitable reference is not available we recommend using a high quality draft de-novo assembly of the first isolated sample to compare other isolates against as in the example of the ST-26 samples in this study (Figure 3).

In addition to core genome SNV analysis we have also found the integration of accessory genome information is a useful indicator of relatedness within *M. abscessus* isolates that can be used to further interrogate assigned sequence clusters. Generally there was good concordance between the proportion of putative genes shared and the SNV distance between two samples. This is helped by using a closely related reference sequences to map sequence reads against. We have seen in this study, and previously [32], diversity in the accessory genome profiles as well as in the number of SNPs and AMR associated mutations taken from multiple samples from the same patient on the same day. However we have always found inter-patient diversity to be greater than that seen within the same patient. This would suggest that any direct transmission between patients of even minority populations would still be identified by WGS and, taken together, the data suggests that person-to-person transmission of *M. abscessus* in paediatric patients in our institution is very uncommon. In this study we have an example of two patients with transmission predicted by genomic epidemiology (ldn_gos_7 and ldn_gos_46) that had attended a lung function laboratory on three occasions within a month of each other. In this case, the only way transmission could have occurred is if ldn_gos_7 who was already infected contaminated the environment and this then transmitted to ldn_gos_46. The predominant view [8] that human-to-human transmission occurs via

contamination of fomites by respiratory secretions could explain this, although no other instances of this appeared to have occurred, despite numerous other CF patients attending the unit over many years. What is harder to explain is that for this to be the case, the interval between exposure and culture positivity was nine years. It could be that *M. abscessus* remains present but undetectable by conventional methods for this time period, or intriguingly could cause latent infection, like what occurs with *Mycobacterium tuberculosis*. To the best of our knowledge, this has never been a demonstrated part of the pathogenesis of *M. abscessus* infection, and maybe worthy of further investigation.

In agreement with previous studies we have found an international distribution of *M. abscessus* dominant clones [8]. We have found WGS to be useful to confirm whether different patient's strains are unrelated, even within the dominant clones, but it has been far more difficult to reach definite conclusions about cross-transmission. Without environmental samples we cannot rule out the possibility of intermediate sources of infection and so WGS as a tool for tracking cross-transmission in *M. abscessus* will only realise its full potential with proper screening of environmental sources alongside longitudinal patient sampling.

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Conflict of Interest

All authors have no potential conflicts of interest to disclose.

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Table 1: Study patient information.

Patient	Hospital	Subspecies	Sex	Underlying condition	Source of isolate	Infection status at first contact	Date of first isolate	Date of first contact	Date of first ward admission
bcn_hcl_002	HCL	*abscessus*	M	Cystic Fibrosis	Lung	Already Infected	30/12/13	30/12/13	30/12/13
bcn_hcl_004	HCL	*abscessus*	F	Bronchiectasis	Lung	Not infected	09/05/13	01/01/12	01/01/12
bcn_hcl_005	HCL	*abscessus*	F	None	Lung	Already Infected	01/08/14	01/08/14	01/08/14
bcn_hcl_007	HCL	*abscessus*	M	Liver Neoplasi	Blood	Not infected	10/09/14	2013	2013
bcn_hcl_008	HCL	*massilense*	M	None	Lung	Already Infected	30/06/14	30/06/14	30/06/14
bcn_hcl_009	HCL	*abscessus*	F	Bronchiectasis	Lung	Not infected	08/05/13	2008	2008
bcn_hsp_011	HSP	*bolletii*	M	None	Lung	Already Infected	01/12/00		
bcn_hsp_012	HSP	*abscessus*	F	None	Lung	Already Infected	31/10/08	31/10/08	31/10/08
bcn_hsp_014	HSP	*abscessus*	F	None	Lung	Already Infected	30/01/01	30/01/01	30/01/01
bcn_hsp_019	HSP	*abscessus*	M	None	Lung	Already Infected	23/01/14	03/12/04	23/01/14
bcn_hsp_021	HSP	*abscessus*	F	Chronic bronchial infection	Lung	Not infected	24/04/15	02/02/14	02/02/14
bcn_hsp_1	HSP	*abscessus*	F	None	Lung	Already Infected	17/09/09	17/09/09	17/09/09
bcn_hsp_2	HSP	*abscessus*	F	None	Lung	Already Infected	24/03/09	24/03/09	24/03/09
bcn_hsp_3	HSP	*abscessus*	F	None	Lung	Already Infected	05/06/07	05/06/07	05/06/07
bcn_hvh_030	HVH	*abscessus*	F	Cystic Fibrosis	Lung		09/01/12		
bcn_hvh_031	HVH	*abscessus*	F	Cystic Fibrosis	Lung		06/05/09		
bcn_hvh_033	HVH	*abscessus*	M	Cystic Fibrosis	Lung		15/07/13		
bcn_hvh_034	HVH	*massilense*	F	Cystic Fibrosis	Lung		23/01/09		
bcn_hvh_035	HVH	*abscessus*	F	Cystic Fibrosis	Lung		14/02/14		
bcn_hvh_036	HVH	*massilense*	M	Cystic Fibrosis	Lung		29/03/11		
bcn_hvh_037	HVH	*abscessus*	F	Cystic Fibrosis	Lung		30/01/13		
bcn_hvh_038	HVH	*abscessus*	F	Cystic Fibrosis	Lung		25/07/14		
bcn_hvh_039	HVH	*abscessus*	M	Cystic Fibrosis	Lung		22/01/07		
bcn_hvh_040	HVH	*massilense*	M	Cystic Fibrosis	Lung		04/09/09		
bcn_hvh_041	HVH	*bolletii*	F	Lung transplant	Lung		29/08/12		

bcn_hvh_042	HVH	*massilense*	F	Lung transplant	Lung		18/04/12		
bcn_hvh_043	HVH	*abscessus*	M	Lung transplant	Lung		23/02/13		
bcn_hvh_045	HVH	*massilense*	M	Lung transplant	Lung		10/04/13		
bcn_hvh_046	HVH	*massilense*	M	Lung transplant	Lung		08/11/13		
bcn_hvh_047	HVH	*abscessus*	F	Lung transplant	Lung		28/03/13		
ldn_gos_1	GOSH	*bolletii*	F	Cystic Fibrosis	Lung	Already Infected	29/06/2004	27/06/2004	27/06/2004
ldn_gos_11	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Not infected	07/10/2008	09/06/1997	15/09/1998
ldn_gos_14	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Already Infected	25/04/2005	24/04/2005	24/04/2005
ldn_gos_15	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	26/06/2006	29/08/1995	26/05/2003
ldn_gos_17	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	21/04/2009	04/07/1995	01/01/1997
ldn_gos_18	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	13/12/2004	27/02/2001	03/07/2002
ldn_gos_19	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	18/06/2007	16/04/1991	27/07/1994
ldn_gos_2	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Already Infected	18/08/2005	15/08/2005	15/08/2005
ldn_gos_21	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	28/10/2008	11/11/1996	20/10/1997
ldn_gos_22	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	08/05/2008	15/02/1994	05/01/1998
ldn_gos_23	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Not infected	03/02/2009	17/06/2003	10/11/2006
ldn_gos_24	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Already Infected	30/03/2009	30/03/2009	30/03/2009
ldn_gos_27	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	16/10/2010	07/05/2003	07/05/2003
ldn_gos_28	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Already Infected	06/06/2011	06/06/2011	06/06/2011
ldn_gos_3	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Already Infected	05/09/2005	04/09/2005	04/09/2005
ldn_gos_30	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	28/06/2012	11/11/1997	13/01/1999
ldn_gos_32	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Already Infected	08/11/2010	08/11/2010	08/11/2010
ldn_gos_35	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	21/10/2013	05/08/2000	05/08/2000
ldn_gos_36	GOSH	*abscessus*	F	CNS Tumour	Faeces	Not infected	11/01/2014	10/10/2013	N/A
ldn_gos_37	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	20/02/2014	01/10/2013	28/10/2013
ldn_gos_38	GOSH	*massilense*	M	Cystic Fibrosis	Lung	Already Infected	31/07/2014	28/04/2014	28/04/2014
ldn_gos_39	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Already Infected	29/09/2014	29/09/2014	29/09/2014
ldn_gos_40	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Already Infected	29/03/2015	16/02/2015	16/02/2015
ldn_gos_41	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Already Infected	02/06/2015	01/06/2015	01/06/2015
ldn_gos_42	GOSH	*abscessus*	M	SCID	Lung	Not infected	30/07/2015	27/09/2002	

ldn_gos_43	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	03/08/2015	02/11/1999	25/02/2001
ldn_gos_44	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Already Infected	27/10/2015	26/10/2015	26/10/2015
ldn_gos_45	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Already Infected	26/02/2016	05/01/2015	05/01/2015
ldn_gos_46	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Not infected	09/01/2017	10/11/2004	10/11/2004
ldn_gos_7	GOSH	*massilense*	F	Cystic Fibrosis	Lung	Already Infected	17/12/2007	17/12/2007	17/12/2007
ldn_gos_8	GOSH	*abscessus*	M	Cystic Fibrosis	Lung	Not infected	29/06/2007	17/06/1997	17/06/1998
ldn_gos_9	GOSH	*abscessus*	F	Cystic Fibrosis	Lung	Not infected	04/12/2007	20/08/2007	10/06/2008

Abbreviations: HCL, Hospital Clínic; HSP, Hospital de la Santa Creu I Sant Pau; HVH, Hospital Vall d'Hebron; GOSH, Great Ormond Street Hospital

Figure legends

Figure 1. Maximum likelihood single nucleotide variant (SNV) tree using only the earliest isolated sample from all 62 patients. SNVs were identified from mapping reads to ATCC19977 *M. abscessus* subsp. *abscessus* reference genome. Sample names are highlighted in colour based on what hospital they were isolated from: Great Ormond Street Hospital, London, UK, Hospital Clínic, Barcelona, Spain, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, and Hospital Vall d'Hebron, Barcelona, Spain. The scale bar represents the number of single nucleotide variants and node bootstrap scores below are shown if below 75.

Figure 2. Frequency of pairwise single nucleotide variant (SNV) distances between all isolates. SNVs were identified from mapping sequence reads to *M. abscessus* subsp. *abscessus* ATCC19977. The full plot includes all samples while the bottom subsidiary plot only includes isolates that have a pairwise difference between zero and 1000 SNVs.

Figure 3. Maximum likelihood single nucleotide variant (SNV) tree for all ST-26 isolates. SNVs were identified from mapping reads to a de-novo assembled study isolate genome (ldn_gos_2_520). Samples are highlighted based on inclusion in sequence clusters. The tree is annotated with the presence (black) and absence (white) of accessory genes as well as the presence of AMR associated genes and mutations. This included presence of a functional *erm(41)* gene conferring inducible resistance to macrolides, presence of two *rrl* mutations conferring high level macrolide resistance and the presence of mutation in *rrs* conferring high level amikacin resistance. The scale bar represents the number of single nucleotide variants and node bootstrap scores below are shown if below 75.

Figure 4. Maximum likelihood single nucleotide variant (SNV) tree for all ST-1 isolates. SNVs were identified from mapping reads to *M. abscessus* subsp. *abscessus* ATCC19977. Samples are highlighted based on inclusion in sequence clusters. The tree is annotated with the presence (black) and absence (white) of accessory genes as well as the presence of AMR associated genes and mutations. This included presence of a functional *erm(41)* gene conferring inducible resistance to macrolides, presence of two *rrl* mutations conferring high level macrolide resistance and the presence of mutation in *rrs* conferring high level amikacin resistance. The scale bar represents the number of single nucleotide variants and node bootstrap scores below are shown if below 75.

Figure 5. Maximum likelihood single nucleotide variant (SNV) tree for all ST-23 and ST-48 isolates. SNVs were identified from mapping reads to *M. abscessus* subsp. *massilense* GO 06. Samples are highlighted based on inclusion in sequence clusters. The tree is annotated with the presence (black) and absence (white) of accessory genes as well as the presence of AMR associated genes and mutations. This included presence of a functional *erm(41)* gene conferring inducible resistance to macrolides, presence of two *rrl* mutations conferring high level macrolide resistance and the presence of mutation in *rrs* conferring high level amikacin resistance. The scale bar represents the number of single nucleotide variants and node bootstrap scores below are shown if below 75.

Figure 1

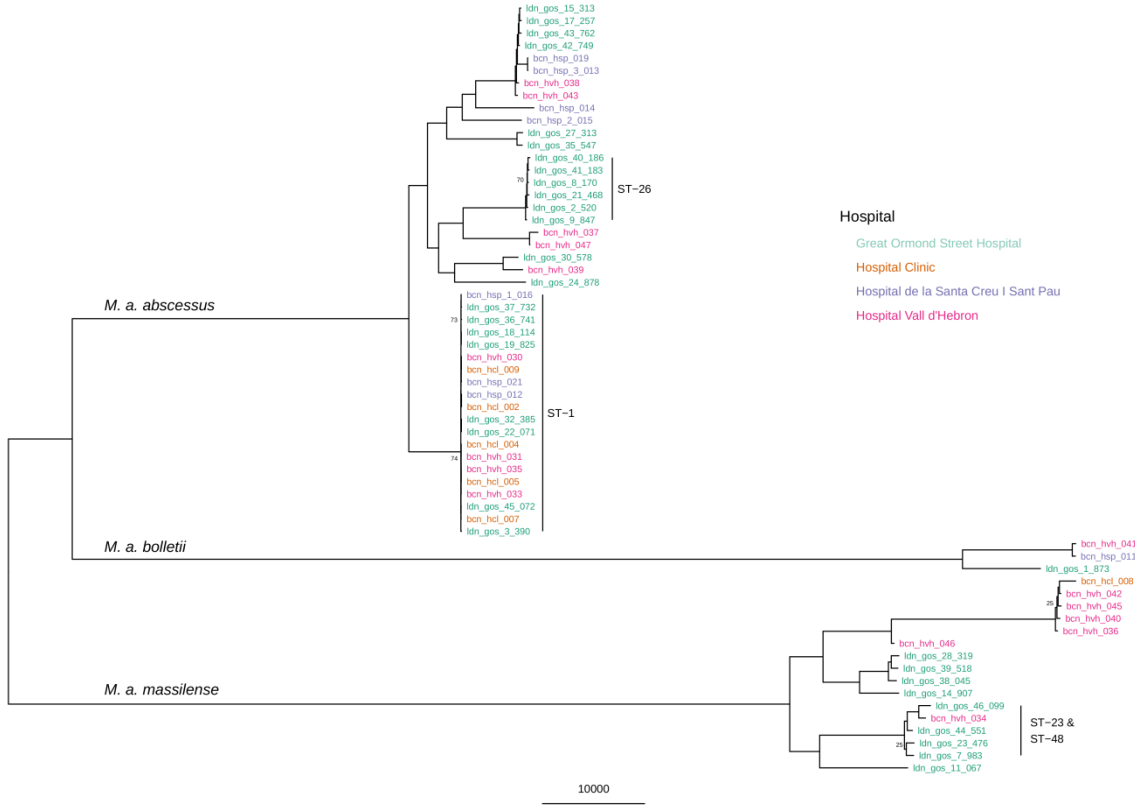

Figure 2

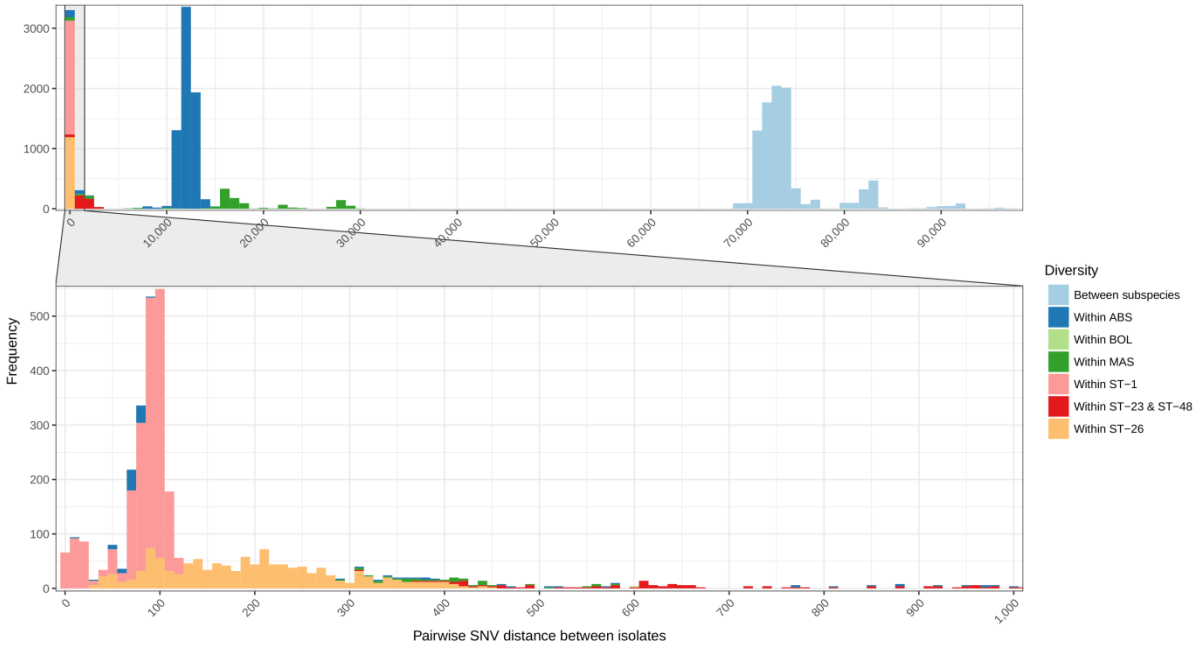

Figure 3

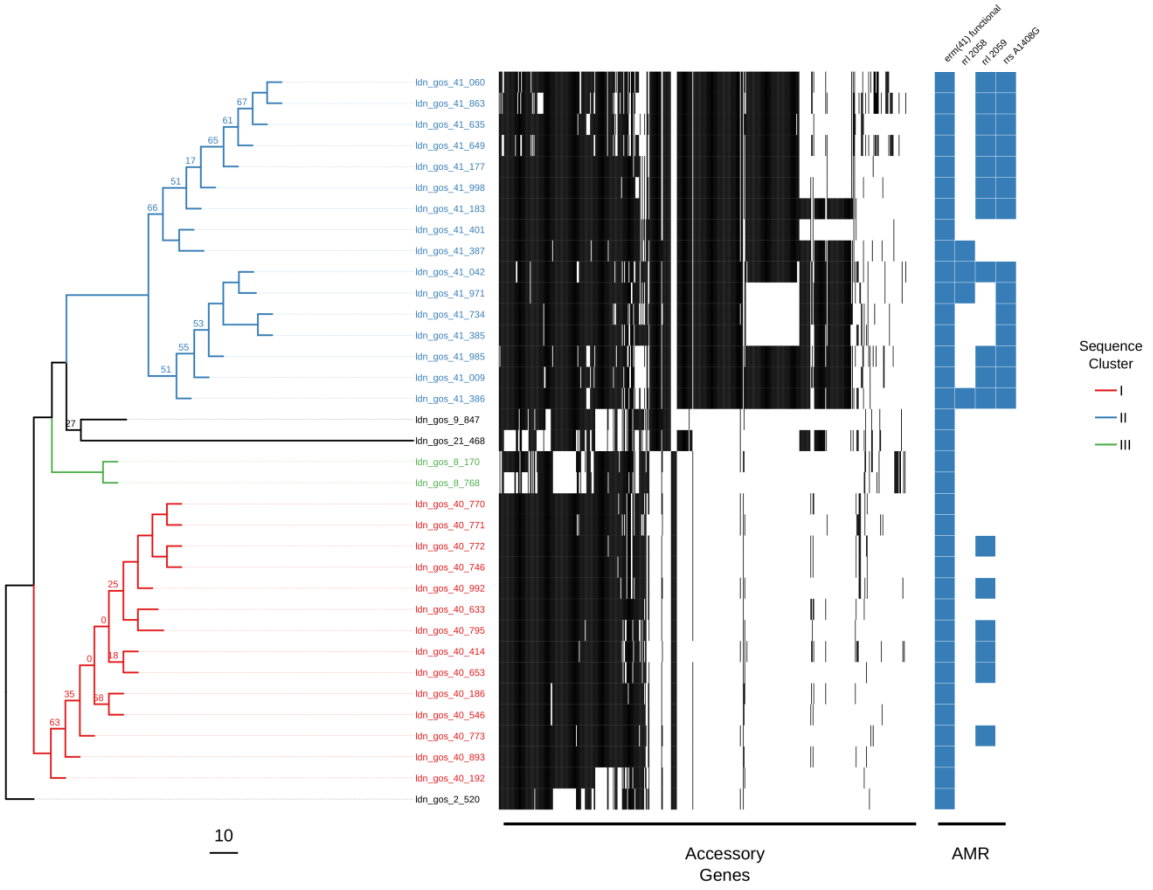

Figure 4

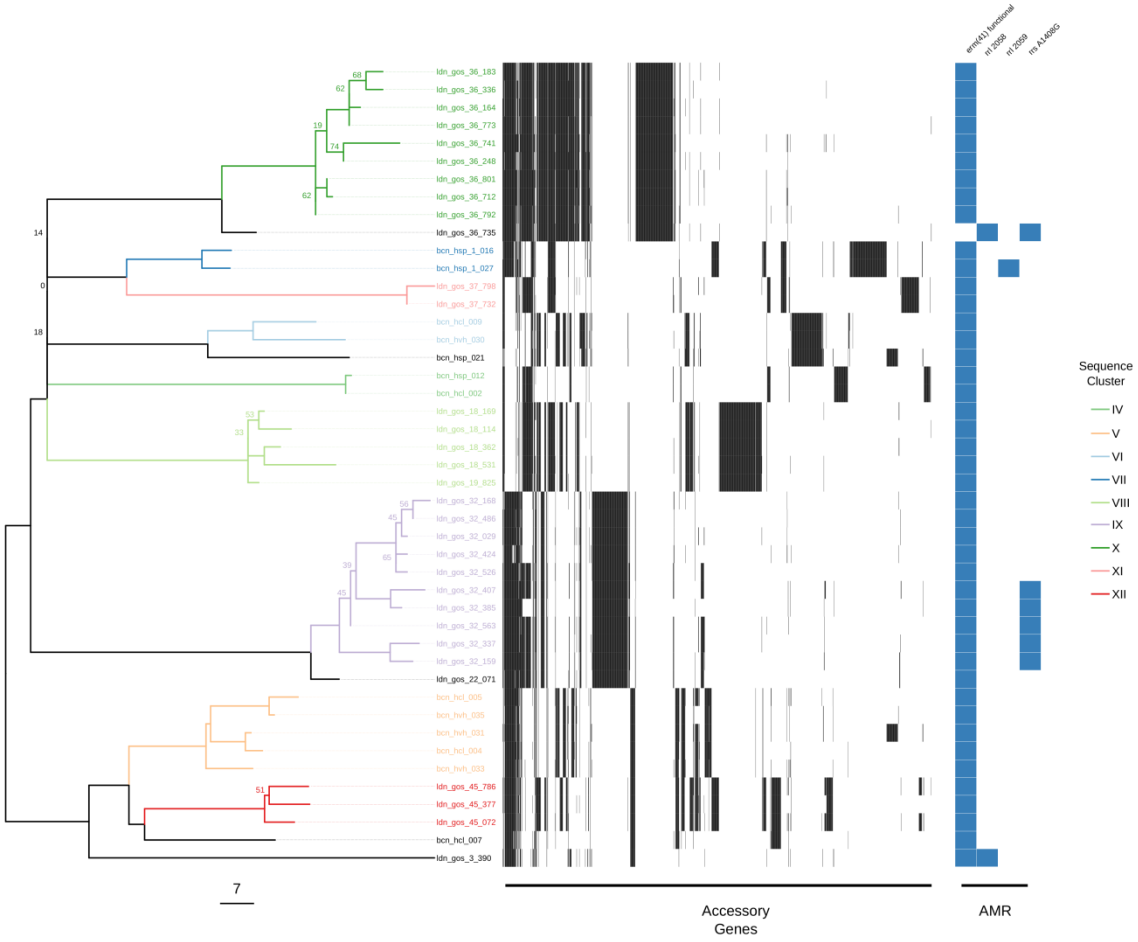

Figure 5