Molecular Epidemiology of *Mycobacterium abscessus* Isolates Recovered from German Cystic Fibrosis Patients

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ABSTRACT Infections due to *Mycobacterium abscessus* are a major cause of mortality and morbidity in cystic fibrosis (CF) patients. Furthermore, *M. abscessus* has been suspected to be involved in person-to-person transmissions. In 2016, dominant global clonal complexes (DCCs) that occur worldwide among CF patients have been described. To elucidate the epidemiological situation of *M. abscessus* among CF patients in Germany and to put these data into a global context, we performed whole-genome sequencing of a set of 154 isolates from 123 German patients treated in 14 CF centers. We used MTBseq pipeline to identify clusters of closely related isolates and correlate those with global DCCs (Absc 1, Absc 2, and Mass 1) in our cohort. Intrapersonal isolates showed higher genetic relatedness than interpersonal isolates (median 3 SNPs versus 16 SNPs; *P* < 0.001). We further identified four clusters with German patients from same centers clustering with less than 25 SNPs distance (range 3 to 18 SNPs) but did not find representatives of all major DCCs (Absc 1, Absc 2, and Mass 1) in our cohort. Intrapersonal isolates showed higher genetic relatedness than interpersonal isolates (median 3 SNPs versus 16 SNPs; *P* < 0.001). We further identified four clusters with German patients from same centers clustering with less than 25 SNPs distance (range 3 to 18 SNPs) but did not find....

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any hint for in-hospital person-to-person transmission. This is the largest study investigating phylogenetic relations of M. abscessus isolates in Germany. We identified representatives of all reported DCCs but evidence for nosocomial transmission remained inconclusive. Thus, the occurrence of genetically closely related isolates of M. abscessus has to be interpreted with care, as a direct interhuman transmission cannot be directly deduced.

**IMPORTANCE** Mycobacterium abscessus is a major respiratory pathogen in cystic fibrosis (CF) patients. Recently it has been shown that dominant global clonal complexes (DCCs) have spread worldwide among CF patients. This study investigated the epidemiological situation of M. abscessus among CF patients in Germany by performing whole-genome sequencing (WGS) of a set of 154 M. abscessus from 123 German patients treated in 14 CF centers. This is the largest study investigating the phylogenetic relationship of M. abscessus CF isolates in Germany.

**KEYWORDS** Mycobacterium abscessus, cystic fibrosis, whole-genome sequencing, dominant circulating clones, hospital transmission, German CF registry, nontuberculous mycobacteria

*M. abscessus* is a multidrug-resistant rapid growing nontuberculous mycobacterium (NTM) causing lung infections in predisposed individuals or soft tissue infections after surgical procedures (1, 2). It is a major cause of morbidity and mortality in patients with predisposing lung diseases, such as cystic fibrosis (CF) or non-CF-bronchiectasis (3). In CF patients, infection with *M. abscessus* leads to an accelerated decline in lung function (4, 5) and is considered a contraindication for lung transplantation in most CF-centers (6–8). Over the last 2 decades, increasing prevalence rates for NTM lung infection in CF patients have been reported. However, they vary widely among countries and between different centers, currently ranging from 0% to 7.2% in European countries (in adults from 0% to 11.1%) (9). *M. abscessus* is the predominant species in the European CF population (9). In contrast, the majority of pulmonary NTM infections in people with CF in the US are caused by *M. avium* complex (MAQ) (10).

As *M. abscessus* is inherently drug resistant to most anti-infective agents, infections are extremely hard to treat and eradication remains often unsuccessful. Treatment success rates for *M. abscessus* pulmonary disease have been shown to be only 25% to 58% (5, 11). Current treatment guidelines recommend a prolonged and intense combination therapy consisting of several antibiotic agents with significant adverse effects (12, 13), with macrolides and aminoglycosides (intravenously or inhalative) being the backbones of the therapy (12–14).

*M. abscessus* comprises three subspecies, *M. abscessus* subsp. *abscessus* (*mabs*), *M. abscessus* subsp. *massiliense* (*mmnas*), and *M. abscessus* subsp. *bolletii* (*mbol*) (15). This taxonomic distinction is clinically relevant as most *mabs* and *mbol* isolates show inducible resistance to macrolides due to a functional *erm*(41) gene that encodes an erythromycin ribosome methyltransferase. In *mmnas*, this gene is truncated, rendering this subspecies generally susceptible to macrolides, which is associated with better treatment response and clinical outcome (16). Constitutive macrolide resistance in all three subspecies can be conferred via mutations in the *rrl* gene (A2058C, A2058G, A2058T, A2059C, A2059G, and A2059T *Escherichia coli* numbering) encoding 23S rRNA (17), whereas mutations in the *rrs* gene (A1408G, T1406A, and C1409T) encoding 16S rRNA can lead to aminoglycoside resistance (18).

In recent years, so-called dominant circulating clones (DCCs) have been described among CF patients suggesting a global spread and the possibility of human-to-human transmission (direct and indirect) (19, 20). Moreover, representatives of the DCCs have been shown to be more virulent *in vivo* and *in vitro* (20, 21). Recently, it has been hypothesized that niche adaptation of *M. abscessus* in smokers preceded its spread in the CF community in the 1960s (22). Until now, DCCs have been described in CF-patients from different countries (23, 24), and interestingly in non-CF-patients, as well (25). In a prior single center analysis, we demonstrated that they are also present in Germany, but comprehensive data from CF patients in Germany is missing so far (26).

The aim of this work was to evaluate the prevalence and species distribution of NTM among CF patients in Germany, as well as testing frequency, using data from the German
TABLE 1 Summarized NTM data from the German CF registry 2015–2020

| Patient characteristics   | 2015   | 2016   | 2017   | 2018   | 2019   | 2020   |
|---------------------------|--------|--------|--------|--------|--------|--------|
| Patients in registrya     | 5,462  | 5,512  | 5,869  | 6,031  | 6,108  | 6,295  |
| Patients tested for NTM   | 1,989  | 1,775  | 1,775  | 2,026  | 2,178  | 2,316  |
| Patients with positive cultureb | 106(1.9%;5.3%) | 133(2.4%;7.5%) | 177(3.0%;8.7%) | 176(2.9%;8.1%) | 174(2.9%;7.5%) | 179(2.8%;8.5%) |

M. abscessus

Mabs 66(62.3%) 79(59.4%) 91(51.4%) 82(46.6%) 84(47.5%) 94(52.5%)

M. milletiae

Mmmb 2 6 19 17 24 26

M. massiliense

Mmbol 3 3 7 3 3 1

Subspecies not known

61 67 53 54 51 57

MAC 16(15.1%) 38(28.6%) 48(27.1%) 55(31.3%) 51(28.8%) 65(36.3%)

Others

22(20.8%) 14(10.5%) 37(20.9%) 39(22.2%) 42(23.7%) 35(19.6%)

M. kansasii

2 1 2 4 2 0

M. fortuitum

2 0 3 0 6 3

M. gordoniae

1 4 15 15 17 13

M. chelonae

2 3 4 4 7 9

Miscellaneous

15 6 13 16 10 10

Unknown

2(1.9%) 4(3.0%) 4(2.3%) 5(2.8%) 1(0.6%) 1(0.6%)

aIn each year, patients who had a prior transplant were excluded.

bPercentage of all patients in registry.

Percentage of tested patients.

Percentage of patients with positive NTM culture. NTM, nontuberculous mycobacteria; mabs, M. abscessus subsp. abscessus; mmas, M. abscessus subsp. massiliense; mbol, M. abscessus subsp. bolletii; MAC, Mycobacterium avium complex.

Negative patients included those who do not produce sputum or were not investigated for NTM.

CF patient registry (27). In addition, we aimed to investigate whether the DCCs are present in a comprehensive set of M. abscessus isolates from German CF patients and to identify possible transmission clusters using a whole-genome sequencing approach.

RESULTS

NTM epidemiology in German CF patients. From 2015 to 2020, the number of patients documented in the CF registry increased from 5,462 patients to 6,295 patients (Table 1). Of those, between 32.2% and 37.9% were tested for NTM and 1.9% to 3.0% had positive test results (Table 1, Fig. 1A). The most frequently isolated mycobacterial species was M. abscessus accounting for 46.6% to 62.3% of cultured isolates per year, followed by MAC (15.1% to 36.3%) and others (19.6% to 23.7%, including M. kansasii, M. fortuitum, M. gordoniae and M. chelonae). Among children, M. abscessus is the leading NTM in Germany (45.9% to 78.1%), whereas MAC was cultured in 9.4% to 36.1% (Fig. 1B). In adults, other NTM such as MAC become more important (17.6% to 36.4%), but M. abscessus remains predominant (40.3% to 56.1%). For the majority of M. abscessus isolates, the subspecies was not reported to the registry (69.2%). Of those specified (n = 153), mabs was the most frequent one with 61.4% during the 6-year period (Table 1, Fig. 1C).

Included isolates and general characteristics. In total, whole-genome data were obtained for 154 M. abscessus isolates from 123 German CF patients in 14 centers (Table 2, Fig. 2). Mean age at first positive culture was 22 years (range 5 to 75 years). Bacterial isolates were initially cultured from sputum (n = 104, 84.6%), bronchoalveolar lavage (n = 5, 4.1%), bronchial secretion (n = 3, 2.4%) and oropharyngeal swabs (n = 11, 8.9%). Of the isolates included, 123 were primary isolates and 31 sequential isolates. In primary isolates, subspecies identification by whole-genome sequencing resulted in 83 isolates of subspecies mabs (67.5%), 35 isolates of mmas (28.5%), and 5 isolates of mbol (4.1%) (Table 2, Fig. 3).

The duration of culture positivity ranged between 0 and 14 years. It was longer in mabs (mean 2.68 years, range 0–12 years) and mmas (mean 2.93 years, range 0 to 14 years) than in mbol (mean 0.8 years, range 0–3 years).

In primary isolates, we could detect 15 isolates (12.2%; 5 mabs, 2 mbol, 8 mmas) that were predicted to be constitutively resistant to macrolides due to mutations in the rrl gene, 78 isolates (63.4%, 71 mabs, 5 mbol, 2 mmas) with inducible resistance (functional erm (41) gene) and 6 isolates that carried both mechanisms of resistance (4.9%, 4 mabs and 2
Therefore, 87/123 isolates were genotypically resistant to macrolides (70.7%). Interestingly, 2 \textit{mmb} isolates showed a fully functional \textit{erm}(41) gene. A predicted aminoglycoside resistance mediated by mutations in the \textit{rrs} gene could be detected in 5 isolates (4.1%), resulting in 118 genotypically susceptible isolates in our patient cohort (95.9%).

**Phylogenetic relations.** SNP distribution for closely related isolates (SNP-distance below 125) showed two distinct peaks: one below 25 SNPs and another below 125 SNPs (Fig. S1), confirming our previously determined thresholds of 25 SNPs (d25) for highly related isolates, and 125 SNPs for closely related isolates (d125) (26). Therefore, we identified 25 d125 clusters with 170 isolates (72.0% of all isolates) and 27 d25 clusters with 129 isolates (54.6% of all isolates) (Fig. 4). Based on a d125 threshold, 37.5% of all German isolates clustered with the DCCs Absc 1, Absc 2 and Mass 1 (n = 63, including 3 non-CF isolates). Isolates from CF-patients did not cluster significantly more often with the DCCs than those of non-CF controls (45/123 patients versus 4/14 patients, \(P = 0.378\)) (Table 2). Isolates from all centers except one (13/14; 92.9%) grouped with the DCCs with a maximum of 125 SNPs distance. However, German isolates did not group with international isolates other than the DCCs with less than 25 SNPs distance.

**Transmission analysis.** Out of the 27 d25 clusters, we could identify 4 clusters that consisted only of patients that have been treated at the same German center (Clusters 1 to 4, Fig. 4C). In center 1, one cluster consisted of two isolates from two twin siblings (\textit{mmb}), suggesting a possible human-to-human transmission or a shared environmental source (Cluster 1). Another cluster at center 1 comprised two isolates from two individuals that were treated at different hospitals and lived in different cities (Cluster 2, \textit{mmb}). Both isolates derived from the same microbiological laboratory that provided diagnostics for the two but distant hospitals (thus originally counted as one center). In addition, these two isolates clustered with international isolates with less than 125 SNPs. In center 2, we identified a \textit{mmb}-cluster comprised of three patients that did not group with international isolates and shared a specific mutation in the \textit{rrs} gene (Cluster 3). However, precise analysis of hospital stays and visits of these three patients could not identify simultaneous hospital visits. In addition, two patients were regularly segregated due to different CF-pathogen status (one colonized with \textit{Achromobacter xylosoxidans}/one free of \textit{Pseudomonas aeruginosa}) and the third was a non-CF-patient. Finally, we identified another cluster from a single German center which did not
group within the DCCs but with other international isolates (Cluster 4). Unfortunately, more detailed data about these two patients were not available.

**DISCUSSION**

This study is the first to provide comprehensive data on colonization and infection with nontuberculous mycobacteria, especially *M. abscessus*, in CF patients across Germany. We demonstrate that *M. abscessus* is the most relevant NTM in German CF patients. This is in line with other reports from Europe, whereas in North America MAC is the NTM most frequently isolated among CF patients (10). According to registry data, prevalence of *M. abscessus* has remained relatively constant between 2015 and 2020 which is in contrast to several previous reports where an increase in prevalence was observed (28, 29). However, in Germany, segregation of NTM-positive CF patients was recommended since 2014 (30). *M. abscessus* dominates in younger CF patients (including children), whereas the proportion of MAC increases with age. The overall prevalence of NTM of 1.9% to 3.0% in Germany is similar to that reported from other European countries (9). Finally, in more than 50% of all patients NTM culture was not available. Thus, the frequency of *M. abscessus* may be underestimated. Many patients, particularly younger children, are unable to expectorate and mycobacterial culture might not have been possible (13). Nevertheless, these data clearly indicate that in Germany, the adherence to the CF Foundation/European Cystic Fibrosis Guideline Committee recommendation to annually screen for NTM infection in expectorating patients needs to be improved.

Whole genome sequencing of 154 *M. abscessus* isolates from 123 CF patients showed that *mabs* is the predominant subspecies in German CF patients (67.5% of all *M. abscessus* isolates). The dominant circulating clones (Absc 1, Absc 2, and Mass 1) are present in a majority of German CF centers. In total, 37.5% of German isolates grouped within the three dominant DCCs with less than 125 SNPs, but evidence for direct human-to-human transmission is still

**TABLE 2** Metadata, subspecies, genotypic resistance, and clustering with DCCs of included *M. abscessus* isolates from German sites

| Isolate characteristics | CF Patients | CF Isolates | Non-CF Patients | Non-CF Isolates | P value* |
|-------------------------|-------------|-------------|-----------------|----------------|---------|
| Total                   | 123         | 154         | 14              | 14             |         |
| Material                |             |             |                 |                |         |
| Sputum                  | 104         | 129         | 5               | 5              | <0.001  |
| Oropharyngeal swab      | 11          | 13          | 0               | 0              | 0.60    |
| Bronchoalveolar lavage  | 5           | 9           | 4               | 4              | <0.01   |
| Bronchial secretion     | 3           | 3           | 2               | 2              | 0.081   |
| Soft tissue             | 0           | 0           | 3               | 3              | <0.001  |
| Isolate type            |             |             |                 |                |         |
| Primary isolates        | 123         | 123         | 14              | 14             |         |
| Sequential isolates     | 17          | 31          | 0               | 0              |         |
| Subspecies              |             |             |                 |                |         |
| mabs                    | 83          | 107         | 6               | 6              | 0.081   |
| mmas                    | 35          | 42          | 5               | 5              | 0.55    |
| mbol                    | 5           | 5           | 3               | 3              | <0.05   |
| Genotypic antibiotic resistance |     |             |                 |                |         |
| Functional *erm*(41)   | 78          | 102         | 7               | 7              | 0.39    |
| rrl mutation            | 15          | 17          | 1               | 1              | 1       |
| rrs mutation            | 5           | 5           | 0               | 0              | 1       |
| Clusters                |             |             |                 |                |         |
| Absc 1                  | 23          | 29          | 3               | 3              | 0.73    |
| Absc 2                  | 15          | 21          | 0               | 0              | 0.36    |
| Mass 1                  | 7           | 10          | 0               | 0              | 1       |
| Boll 1                  | 0           | 0           | 1               | 1              | 0.10    |
| Unclustered             | 30          | 30          | 6               | 6              | 0.20    |

*Bold values denote statistical significance with a P value < 0.05.*
elusive. On the other hand, we could identify four clusters of highly related isolates that contained only patients that were treated at the same centers in Germany suggesting the possibility of indirect or direct person-to-person transmission. One cluster consisted of two siblings, the second of two patients living in different cities, treated at different hospitals indicating that in these cases in-hospital transmission is not very likely. Two further clusters comprised three and two patients. Although epidemiological analyses did not identify opportunities for direct transmission between the patients in cluster 3, the genetic relatedness and the specific rs mutation suggest that an indirect transmission via an environmental hospital source is possible.

Prior studies from Germany covering only few patients did not find evidence for cross transmission so far (26, 31). However, in CF, the carriage of identical clones, e.g., for *P. aeruginosa* and *B. cepacia*, among siblings is common, suggesting that there might be a household transmission risk for *M. abscessus* as well. Recent studies have found evidence for cross-infection by *M. abscessus* (19, 20). Even patients across different centers or even continents may share very closely related isolates (32). Thus, the detection of genotypically related *M. abscessus* strains during transmission analysis should be interpreted carefully and always with respect to the global molecular epidemiology. It has been hypothesized, that current analytic techniques omitting accessory genes and plasmids might play a role in the seemingly relatedness of some isolates (33). This might be an alternative explanation for the fact that in our study 37.5% of included German isolates cluster with the dominant circulating clones with less than 125 SNPs distance. In addition, d25 clusters that consisted only of ‘one German’ center isolates did not have significant higher SNP distances than those that

FIG 2 (A) Geographic provenance and subspecies distribution of sequenced CF patient isolates included in this study. (B) Collection date and subspecies distribution of included *M. abscessus* isolates (*n* = 154).
contained ‘different German center’ isolates. Nevertheless, SNP distances were significantly lower in (sequential) isolates that were only from one patient. On the other hand, our results may also be interpreted with respect to a recent evolutionary model proposed by Bryant et al.: (i) in-host evolution is constrained fitting our observation of highly related intrapersonal isolates with low SNP distances between single isolates, (ii) saltatory evolutionary events are supposed to take place in the environment explaining higher SNP distances in interpersonal isolate-to-isolate comparisons within the clusters (21).

Much more isolates were predicted to be resistant to macrolides than to aminoglycosides (70.7% versus 4.1%). Macrolide resistance was mainly mediated by functional \textit{erm}(41) genes in \textit{mabs} and \textit{mbol}. These results are in line with those from global studies, where aminoglycoside resistance remains a rare event (34).

This study has several limitations. First, we could not obtain clinical meta-data for all included patients, as well as information on whether the patients met outside the hospital (for example during private meeting, at patient gatherings etc.). Second, we did not investigate environmental specimens from hospitals to verify if isolates from closely related clusters are present. However, in sporadic and epidemic \textit{M. abscessus} infections, the pathogen is almost never isolated from the closest environment (35). Third, the control group of non-CF isolates was quite small. However, to the best of our knowledge, this is the largest phylogenomic study on \textit{M. abscessus} in CF patients in Germany so far.

To conclude, whether the presence of closely related isolates among CF patients has to be regarded as indicator for inter-human transmission remains debatable, especially in the light
of current genomic analytic methods. Long read techniques might increase typing resolution in the future by generating closed genomes and by adding accessory genes to improve the discriminatory power of comparative genetic analysis. Nevertheless, we could identify clusters with patients that are infected or colonized with highly related isolates with less than 25 SNPs distance. As health care-associated transmission events cannot be ruled out, hospital infection control practices should be maintained such as segregation of NTM-positive CF patients in order to prevent possible transmission via fomites.

**Conclusion.** The impact of *M. abscessus* transmission among CF patients varies greatly between different epidemiological studies. In this work, we found no evidence for nosocomial patient-to-patient transmissions, although patients with closely related isolates and treated at the same center were identified. As nosocomial transmission could not be completely ruled out, hospital infection control practices for NTM as part of best practice guidelines for the management of CF patients should be maintained. Improved NTM surveillance combined with continuous and systematic epidemiologic investigation of potential transmissions and high-resolution comparative genome analysis techniques will help to guide future evidence-based infection control measures for patients with CF.

**MATERIALS AND METHODS**

**Ethical approval.** Ethical consent for this study was given by the ethics committee at University Hospital Frankfurt under file number 20–791. No experiments on animals were involved. As this is a retrospective study, no patient consent was needed.

**German CF registry data.** To assess the overall epidemiological situation in Germany, a database query from the German CF registry was conducted (27, 36). This registry currently includes annual data.
from over 6,000 CF patients from specialized CF sites, representing approximately 80% of all CF patients in Germany. The database collects demographic, clinical and microbiological data from consenting people with CF since 1995, whereas detailed data for NTM was available since 2015. Thus, for patients with no prior transplant, test results for NTM for the time span of 2015 to 2020 were recorded, as well as testing frequency. NTM-species were divided into M. abscessus, M. avium complex (MAC), others (including M. kansasi, M. fortuitum, M. gordonae, and M. chelonae) and unknown. In addition, patient age at the time of cultivation was recorded. Children were defined as patients with an age <18 years.

Included isolates. A representative set of M. abscessus isolates has been collected from 14 German CF centers (located in the cities of Essen, Oldenburg, Cologne, Munich, Berlin, Münster, Dresden, Erlangen, Gießen, Tübingen, Würzburg, Hamburg, Heidelberg, and Frankfurt) for the time period of 2004 to 2020. We recorded the date of cultivation, patient age, type of specimen (sputum, bronchoalveolar lavage, endotracheal swabs) and if isolates were primary or sequential isolates. In addition, the duration between the first and last positive culture was recorded. From Frankfurt University Hospital sequential isolates were included, if available. Bacterial culture was performed on Middlebrook 7H10 agar with oleic albumin dextrose catalase (OADC, Becton, Dickinson, Heidelberg, Germany) at 37°C until visible growth could be detected. M. abscessus species identification was verified by Matrix-assisted-laser desorption ionization-time of flight analysis (MALDI-TOF; Vitek MS; bioMérieux, Nürtingen, Germany) and in case of no identification with the GenoType NTM-DR VER 1.0 (Hain Lifescience, Nehren, Germany). Bacterial cultures were then transferred to the German Reference Center for Mycobacteria at Research Center Borstel for DNA extraction and whole-genome sequencing. Furthermore, 14 M. abscessus isolates from non-CF patients were included as controls.

Data availability. All sequence data used in this study was deposited in the European Nucleotide Archive (ENA) under bioproject number PRJEB44160 and can also be accessed via https://ntmscope.github.io/moma_cf_microreact.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

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