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Early View

Original article

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Longitudinal non-cystic fibrosis trends of pulmonary *Mycobacterium abscessus* disease from 2010-2017 - spread of the "globally successful clone" in Asia

Short Title: Molecular epidemiology of ST23 of *Mycobacterium abscessus* in Taiwan.

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ABSTRACT

Background

Mycobacterium abscessus (MAB) has emerged as the predominant pulmonary non-tuberculous mycobacterial pathogen in parts of Asia, including Taiwan. The reasons for the significant increase in MAB infections in the non-cystic fibrosis (CF) populations are poorly understood. The study aimed to elucidate whether this increase is related to the spread of the globally successful clone of MAB.

Methods

We performed multi-locus sequence typing (MLST) of 371 non-duplicated MAB pulmonary isolates from 371 patients sampled between 2010-2017 at 7 hospitals across Taiwan.

Results

In total, 183 (49.3%) isolates were *M. abscessus* subsp. *abscessus* (MAB-a), 187 (50.4%) were *M. abscessus* subsp. *massiliense* (MAB-m), and 1 (0.3%) was *M. abscessus* subsp. *bolletii* (MAB-b). MAB-a sequence type 1 (ST1) [23.7%] and ST127 [3.8%], followed by MAB-m ST48 [16.2%], ST117 [15.1%], ST23 [8.6%] were commonest overall. Of MAB-a strains, 50 (27.3%) belonged to novel STs and 38 (10.2%) were singleton strains, while of MAB-m strains, only 10 (5.3%) were novel and 8 (2.2%) were singletons. From 2010 to 2017, the frequency of the historically dominant ST1 declined from 28.6% to 22.5%, whereas the recently emerged globally successful clonal cluster 3, ST23 and ST48, increased from 14.3% to 40.0%.

Conclusions

The dominance of ST1 particularly in the last 2 years of this study appears to be declining whilst ST23, reported in outbreaks among CF and post-surgical cohorts across the Americas and Europe, alongside the closely related ST48, is present among non-CF populations in

Taiwan. These trends need to be confirmed with further ongoing studies to track the molecular epidemiology of clinical MAB isolates worldwide.

INTRODUCTION

Mycobacterium abscessus (MAB) are the commonest rapidly growing non-tuberculous mycobacteria (NTM) causing pulmonary infections worldwide [1-3]. In recent years, MAB have undergone multiple taxonomic revisions [4-8]. However, three closely related entities are recognised: *M. abscessus* subsp. *abscessus* (MAB-a), *M. abscessus* subsp. *bolletii* (MAB-b) and *M. abscessus* subsp. *massiliense* (MAB-m) [6, 7]. They differ in terms of drug susceptibility, and may have differences related to transmissibility [9-11]. Pulmonary infection is the most typical clinical presentation, but extrapulmonary infection either due to direct inoculation into the skin or due to disseminated disease, often in association with neutralising anti-interferon gamma (IFN-γ) autoantibodies is recognised with increased frequency [12-14].

Previous studies have indicated great diversity within MAB among cystic fibrosis (CF) patients, suggesting independent acquisitions from the environment [15, 16]. However, suspicion of patient-to-patient transmission arose after two reports of respiratory outbreaks with MAB-m at different CF centers across the Atlantic [10, 17, 18]. One outbreak occurred in Seattle, USA, wherein the index case-patient and 4 additional patients were infected with near identical MAB-m isolates with resistance to amikacin and clarithromycin [17]. The second outbreak occurred in UK, involving 11 patients who all had MAB-m infections sharing the same constitutive resistance to clarithromycin and amikacin, despite some individuals being naive to long-term macrolide or aminoglycoside therapy [10].

By whole-genome sequencing (WGS), isolates from these two CF centers, were subsequently found to be highly related, belonging to sequence type 23 (ST23) and clonal

cluster 3 (CC3) [18, 19]. Meanwhile, an epidemic of at least 2032 post-surgical infections between 2004-2011 across Brazil was also due to ST23 (CC3), and an outbreak of post-procedural infections between 2010-2012 in Taiwan was due to ST48, differing from ST23 only at the *murC* locus (also within CC3), thereafter referred to as the "globally successful clone" [20-22].

In parts of Asia including Singapore, Okinawa and southern Taiwan, MAB has overtaken the *Mycobacterium avium* complex as the commonest NTM causing lung disease [23-25]. CF is extremely rare in such populations with an estimated incidence of 3.12 per million live births in the Japanese population and less than one in 90 000 live births among Orientals [26]. One hypothesis for the rising dominance of MAB is that evolutionary changes affecting environmental adaptation, transmissibility, and virulence to humans may have enhanced the spread of the globally successful clone (CC3). This clone may not follow traditional patterns of MAB infection and may affect the general population without pre-requisite for abnormal lungs or airways. However, comparable molecular epidemiology studies are lacking in Asia and non-CF populations. Longitudinal population studies are unreported worldwide. Thus, the aim of this study is to investigate the molecular epidemiology of MAB in Taiwan which has not been reported before.

METHODS

Hospital Sites

Seven hospitals participated in this nationwide study (Figure 1). The institutional ethic committees of the participating sites approved the study (NTUH REC 201605114RIND, KMUHIRB-E(I)-20180008, TSGH REC 2-108-05-113) or waived the need for formal review under the auspices of infection control.

Mycobacterial isolates

Pulmonary MAB isolates were identified using the hospital laboratory database. Random sampling was performed by date criteria (samples submitted every 1st and 15th of each month starting from January 2010 -ending in December 2017) were collected from each hospital site and submitted to the National Taiwan University Hospital, Taipei (site A) for genotypic verification and typing. Only the first MAB isolate for each patient was included (duplicates were excluded). The maximum number of MAB isolates submitted for genotypic identification by multilocus sequence analysis (MLSA) per year per site was 35. Excessive isolates per site were randomly excluded by the following method: the first two of every triplet was included (every 3rd isolate was excluded). The maximum number of genotypically confirmed MAB isolates submitted for multilocus sequence typing (MLST) per year per site was 24. All mycobacterial isolates were stored at -80 degrees Celsius in GermBank [Creative Media Products (Wugu Shiang, Taipei County, Taiwan)]. Immediately prior to use, the strains were subcultured onto sheep blood agar at 35° Celsius as described previously [27].

Multilocus sequence analysis (MLSA)

Molecular confirmation of MAB isolates was done by concatenating the partial sequences of 3-genes (*hsp65, rpoB, secA1*) according to Zelazny *et al.* [28]. Only isolates molecularly identified as MAB were included in this study for MLST.

Multilocus sequence typing (MLST)

MLST was performed using 7 housekeeping genes (*argH, cya, glpK, gnd, murC, pta, and purH*) according to http://bigsdb.pasteur.fr/mycoabscessus/mycoabscessus.html [21]. Phylogenetic trees were constructed using the BioNumerics software (Version 6.6; Applied Maths, BioMèrieux, Belgium).

Retrospective Chart Review

The clinical relevance of each MAB isolate was determined according to the 2007 ATS/IDSA guidelines [29]. Patient demographics and underlying comorbidities were retrieved. The presence of concomitant respiratory tract pathogens were recorded. All-cause mortality and date of last visit or death were recorded.

RESULTS

Six-hundred and forty-nine unique *M. abscessus* (MAB) pulmonary isolates were retrieved by study criteria from the microbiology laboratory databases of seven hospitals across Taiwan (Figures 1 and 2). After excluding isolates in excess of the maximum number of isolates allowed per site by random deletion to avoid over-representation of NTUH-Taipei, 468 routine microbiological laboratory identified MAB isolates underwent MLSA. Subsequently, 24 were excluded because they were molecularly identified as species other than MAB and another 73 isolates randomly excluded to avoid over-representation of KMUH. Thus, a total of 371 genotypically-confirmed MAB isolates were included in this MLST study (Figure 2). These strains were isolated from the expectorated sputum (n = 343), endotracheal aspirates (n = 10), bronchial washings (n = 10), broncho-alveolar lavage specimens (n = 2), and biopsied lung tissue (n = 5) of 371 patients. Approximately half were men (n = 189, 50.9%) with a median age (interquartile range, IQR) of 67 (55 - 77) years

(Table 1). Patients in this cohort were followed for an average duration of 2.8 years after their cultures yielded *M. abscessus* during which 30.5% (n = 113) died. The majority of deaths within hospital were due to severe sepsis, mostly secondary to pneumonia (n = 30, 45.5%), and only five were attributed directly to progressive non-tuberculous mycobacterial pulmonary disease (n = 5, 7.6%). Overall, two-thirds of the patients in this cohort (n = 260, 70.1%) were classified as having NTM-pulmonary disease (NTM-PD) by the consensus definitions published in 2007 by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA), of those, 137 (52.7%) had nodular-bronchiectatic pattern on computed tomography, 14 (5.4%) had fibro-cavitary lung disease, and 109 (42.9%) had a mixed or non-classifiable pattern [29].

The majority of patients had one or more co-morbid conditions (Table 1). Underlying lung disease was the most common comorbidity (n = 183, 49.3%), although of note, there were no patients with known CF. The second most common underlying disease was malignancy (n = 89, 24.0%), notably of the head and neck (n = 22, 5.9%), the upper gastrointestinal tract (n = 16, 4.4%) and the lung (n = 13, 3.5%). However immunocompromising conditions in the context of susceptibility to mycobacterial infections as listed in Table 1, were not overtly common in this cohort of mainly pulmonary and not disseminated disease. Interestingly, a fifth of this elderly population had neurological conditions (n = 76, 20.5%) that often resulted in tracheotomy to avert aspiration, similar to the risks posed by head and neck cancers. The spectrum of neurological disease included ischemic stroke (n = 28, 7.5%), movement disorders such as amyotrophic lateral sclerosis and Parkinsonism (n = 19, 5.1%), intracranial hemorrhages (n = 10, 2.7%), senile dementia (n = 9, 2.4%), brain tumors (n = 5, 1.3%), metabolic or hypoxic encephalopathy (n = 2, 0.5%) and other cognitive disorders (n = 2, 0.5%).

Of the 371 MAB isolates, 183 (49.3%) belonged to the subspecies *abscessus* (MAB-a) and 187 (50.4%) belonged to the subspecies *massiliense* (MAB-m) (Table 2). Only 1 isolate (0.3%) was identified as the subspecies *bolletii* (MAB-b). For MAB-a, 55 sequence types (STs) were identified in total, including 48 novel sequence types (combinations of alleles not matching any of the current 270 STs in the international database) (Table 2 & Figure 3). The most common MAB-a sequence types was ST1 (comprising 23.7% of all strains and 48.1% of all subspecies abscessus strains included in this study), followed by ST127 (3.8% of all strains and 7.7% of all MAB-a). There were five isolates each of ST22 (a common ST in Ireland) and ST49 [30].

The 187 strains of MAB-m were assigned to 19 sequence types, including 7 novel sequence types. The commonest MAB-m sequence types was ST48 (16.2% of all strains and 32.1% of all subspecies *massiliense*), followed by ST117 (15.1% overall and 29.9% of MAB-m), and ST23 (8.6% overall and 17.1% of MAB-m). Only 2 MAB-m isolates belonged to ST2, previously reported as the predominant ST in non-outbreak settings among CF patients [21]. A smaller proportion of MAB-m isolates compared to MAB-a isolates (2.2% vs.10.2%) were represented in single STs.

The only isolate of MAB-b in this study had a novel allele at the *gnd* locus and thus, represented a novel singleton ST.

Geographic distribution of M. abscessus subspecies and sequence types

MAB-a compared to MAB-m was more common in the north (60.4% vs. 39.6%), present in equal proportions (50% vs. 50%) in central Taiwan, and less common in the south (41.8% vs. 57.5%). For MAB-a, ST1 represented 27.6%, 27.8% and 20.4% of all strains from northern, central and southern Taiwan, respectively, whereas ST127 represented 2.2%,

2.8% strains and 5.0% strains from northern, central, and southern Taiwan. For MAB-m, the prevalence of ST48 versus ST23 in northern, central and southern Taiwan were 11.2%, 8.3%, and 20.9%, versus 4.5%, 11.1%, and 10.9%, respectively, while the prevalence of ST117 gradually increased from north southwards from 10.4%, to 16.7% and 17.9%.

Changing trends of M. abscessus subspecies and sequence types over time

The frequencies of the common sequence types for each consecutive year from 2010 to 2017 are shown in Table 3 and Figure 4. A possible declining trend of ST1 (MAB-a) and ST117 (MAB-m) over the study period ($p < 0.01$) in contrast to a possible increase in the globally successful clone (ST48 and ST23 combined p = 0.02) awaits confirmation by further study. ST23 was not detected among northern Taiwan isolates until 2014, whereas ST23 had been present among southern Taiwan isolates since 2010. ST23 was detected in all regions of Taiwan by 2017.

Clinical descriptions of M. abscessus sequence types

Patients with ST23 tended to be younger than patients with ST1, ST48, ST117, or this cohort overall (median (IQR) age for ST23 was 62 (50-73) years versus 65 (55-79) years for ST1, versus 70 (57-77) years for ST48, versus 66 (53-76) for ST 117 and versus 67 (55-77) years for entire cohort [p = 0.29, 0.08, 0.21, 0.15, respectively]) . Deaths during follow-up were not significantly higher among patients with ST23 (n = 14/32, 43.2%) than for ST1 (n = 26/88, 29.5%), for ST48 (n = 18/60, 30.0%), for ST117 (n = 16/56, 28.6%) or for the cohort overall (n = 113/371, 30.5%) [p = 0.19, 0.25, 0.18, 0.16, respectively].

DISCUSSION

Over recent years, there has been a large increase in the number of cases of *M. abscessus* (MAB) infections worldwide, and the reasons for this are poorly understood [1-3, 31, 32]. MLST typing enables a better understanding of the epidemiology of MAB and helps to establish whether there are major changes in the population structure of clinically relevant MAB. Despite the frequency of reports of MAB colonizing and infecting lungs in the largely non-CF populations in Asia, molecular epidemiology studies are scarce in this region [11, 23-25].

The preliminary findings of this study are twofold: first, ST23, and the genetically-related, ST48 of clonal cluster 3 (CC3) are clinically nascent in non-CF patients in Taiwan, second and more disconcertingly, is that this globally successful CC3, might be increasing, in contrast to the declining dominance of more genetically diverse ST1 and ST117. The observation that CC3 clones appear to spread among clinical environments without a specific common underlying host immune-defect such as CF or cytokine blockade by autoantibody or therapy, may contribute to the increasing incidence of human infections. Our study also highlight that current recommendations for infection control policies to be implemented to minimize risks of person-to-person transmission of MAB in CF clinics may be misdirected for two reasons: one, for limiting these policies to CF populations, two, for blanket treatment of all MAB, when the threat may lie specifically with CC3 [33].

Our cohort featured a predominance of patients with head and neck squamous cell carcinoma, lung and esophageal cancer patients and those requiring tracheotomy for functional disorders such as advanced Parkinson's disease, amyotrophic lateral sclerosis, post-stroke vocal cord or bulbar palsies and dysphagia that accompanies neurodegenerative diseases such as senile dementia. Anatomical or functional abnormalities of the upper

aerodigestive tract might be an underappreciated risk factor for MAB pulmonary disease. MAB has been demonstrated in 74% of patients with gastric symptoms in their biopsied gastric epithelium [34]. More studies are warranted to quantify the risks of such upper aerodigestive tract abnormalities.

Although microdroplet nuclei aerosolised after an individual coughs, with subsequent inhalation by an uninfected host, is not an inferred mode of spread of MAB, this possibility has not been excluded, specifically for CC3 [19]. ST23 is the only MAB so far reported to be unusually conserved in widespread outbreaks without a specific or linked environmental source [18, 19]. Previous studies using pathogenomic analyses suggests MAB share features such as cording, a virulence associated phenomenon, with *M. tuberculosis* [35]. However, the ST of the MAB strains in these biological studies and in other clinical or environmental surveillance reports are not known [36, 37].

To our knowledge, this is the largest longitudinal molecular epidemiological study conducted of MAB pulmonary isolates. The previous largest collection of MAB reported in a cross-sectional manner was used to establish the international MLST database, and comprised 227 isolates including both pulmonary and soft tissue isolates, a large proportion of which were obtained from French and CF patients, from which 100 STs and 11 clonal clusters or complexes were identified [21]. Subsequent studies employing this MLST scheme are summarized in Table 4, including one small study conducted in Ireland of 36 isolates obtained between 2006-2012 from 36 patients, 18 of whom had CF [30] and the other in Scotland of 178 strains sequentially isolated from 12 patients, 10 of whom had CF [38]. The only small single-center study typing 55 isolates from 55 patients with pulmonary MAB in a non-CF cohort was conducted in Shanghai [39]. In all the above studies, ST23 was detected more than once, but ST48 was found only once in the Shanghai study and not at all in the

European studies [30, 38, 39]. Although ST23 (5 of 26 isolates) and not ST48 (1 of 26) was the most prevalent MAB-m in the Shanghai study, the second most common MAB-m was also ST117 (2 of 26 isolates). At the time of publication, 7 of 36 (19%) and 32 of 55 (58%) strains in the Irish and mainland Chinese study were novel STs. On an individual level, ST23 has been shown to replace an MAB-a strain (ST122) in 1 of 12 patients studied longitudinally, whilst the remaining 11 were persistently colonized with the same ST over years [38].

The major limitation of this study was the lack of WGS and access to the previously reported Seattle/Papworth outbreak strains, to confirm the discriminatory power of MLST. Nevertheless, MLST as previously used by Tettelin *et al.* to cluster MAB-m outbreak strains, has been validated by WGS [18, 19]. In addition, MLST as used by Tettelin *et al.* has been shown to be in high agreement with the MLST scheme used here [40]. Since MLST is more accessible than WGS to the developing world, and the MLST scheme used here is publicly available on the Institut Pasteur's database unlike Tettelin's scheme, most studies conducted from 2014 onwards have used this scheme (Table 4) [18, 40].

Given that Taiwan is an island and that our population has one of the lowest incidence of CF worldwide, it is not surprising that a fair number of our isolates were novel STs. By the same token, ST48 was uniquely more common than ST23 in Taiwan. This finding challenges the assumption that ST23 is the common ancestor of CC3, but due to the predominance of ST23 over ST48 in mainland China and in Europe, ST23 may have recently been imported into southern Taiwan, where the largest trade harbor, the Port of Kaohsiung accounting for an annual volume of more than 10 million TEU, is located.

One might also expect individual MAB-a strain prevalence in Taiwan to differ from other countries, given its isolation as an island. However, like previously published studies conducted in Europe, ST1 remains the most common isolate overall in our study and the

diversity of MAB-a isolates exceeded that of MAB-m [21, 30]. While it has been suggested that Asians possess an undefined genetic susceptibility to MAB infection, and our previous outbreak of extrapulmonary post-procedural infections was also due to ST48, whether ST48 is indeed more common or specific among Asians compared to Caucasians or Hispanics, remains to be determined. More studies from other continents such as Australia and Africa using this MLST scheme rather than WGS, which may be too cost-prohibitive, are necessary to clarify whether only ST23 (or all members of CC3) should be highlighted for relative evolutionary deviations from traditional MAB and preventative infection control measures.

In conclusion, this population-level, longitudinal molecular epidemiology study documents a possible recent decline in the traditionally dominant and diverse ST1 of MAB-a and increasing recognition of genetically-related, MAB-m clones. As reported in outbreaks and non-outbreak studies among cystic fibrosis in the US, UK, France, Ireland, Germany, Switzerland, and Brazil, CC3 appears to be increasingly clinically prevalent among respiratory isolates of non-cystic fibrosis patients in Taiwan. These findings are likely to be explained by the environmental prevalence of CC3 that are more likely to infect humans, perhaps associated with their exceptional resistance to disinfectants such as alcoholic chlorhexidine and povidone-iodine[41]. Novel features from this study include the prominence of ST48, which is closely related to ST23 and assigned to the same clonal complex 3, and the lower percentage of singleton isolates with new MLST types compared to the earlier MLST studies. Further global epidemiology using a shared common molecular language are needed to understand the evolution and dissemination of potentially "fitter" clones of MAB.

• **Conflict of interest:** There are no conflicts of interests to declare for the authors.

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Table 1. Clinical characteristics of the patients with *Mycobacterium abscessus* respiratory isolates

	All patients n = 371
Age, median (IQR[a]), years	67 (55-77)
Men (%)	189 (50.9)
NTM-pulmonary disease (%)	260 (70.1)
Nodular-bronchiectatic type	137 (36.9)
Fibro-cavitary type	14 (3.8)
Mixed or other type	109 (29.3)
Underlying lung diseases (%)	183 (49.3)
Bronchiectasis	131 (35.3)
Chronic obstructive pulmonary disease	57 (15.4)
Remote history of pulmonary tuberculosis	58 (15.6)
Interstitial lung disease	3 (0.8)
Pneumoconioses	1 (0.3)
Diabetes mellitus (%)	72 (19.4)
Congestive heart failure (%)	39 (10.5)
Chronic kidney disease stage IV-V (%)	26 (7.0)
Chronic hepatitis B (%)	28 (7.5)
Chronic hepatitis C (%)	11 (3.0)
Autoimmune disease (%)	18 (4.9)
Anti-interferon-gamma autoantibodies (%)	9 (2.4)
Anti-GM-CSF[b] autoantibodies (%)	1 (0.3)
Gastroesophageal reflux disease (%)	43 (11.6)
Human immunodeficiency virus infection (%)	1 (0.3)
Hematological/bone marrow transplantation (%)	5 (1.3)
Solid organ transplantation (%)	3 (0.8)
Thyroid disease (%)	22 (5.9)
Neurological disease (%)	76 (20.5)
Malignancy (%)	89 (24.0)
Head and neck cancer (%)	22 (5.9)
Upper gastrointestinal cancer (%)	16 (4.4)
Lung cancer (%)	13 (3.5)
Renal, bladder and prostate cancer (%)	11 (3.0)
Colorectal cancer (%)	9 (2.4)
Breast cancer (%)	9 (2.4)
Hematological (%)	5 (1.3)
Neuroendocrine (%)	3 (0.8)
Other (%)	4 (1.1)
Deaths during follow-up (%)	113 (30.5)
In-hospital deaths (%)	66 (17.8)
Cause of death: sepsis (including pneumonia)	34 (9.2)
Cause of death: pneumonia	30 (8.1)
Cause of death: NTM-PD	5 (1.3)
Cause of death: cancer	6 (1.6)
Cause of death: hemorrhage	4 (1.1)
Mean duration of follow-up (SD[c]), years	2.8 (8.7)

[a] IQR = interquartile range, [b]GM-CSF = granulocyte-macrophage colony stimulating factor, [c] SD = standard deviation

Table 2. ***Mycobacterium abscessus*** **complex isolates from Taiwan (371 isolates from 371 patients).**

Subspecies	Sequence Type (ST)	No. of strains of each ST n = 371 (% of total)	No. of strains in North Taiwan n = 134 (% of northern strains)	No. of strains in Central Taiwan n = 36 (% of central strains)	No. of strains in South Taiwan n = 201 (% of southern strains)
M. abscessus **subsp.** ***abscessus***		183 (49.3)	81 (60.4)	18 (50.0)	84 (41.8)
	ST 1	88 (23.7)	37 (27.6)	10 (27.8)	41 (20.4)
	ST 127	14 (3.8)	3 (2.2)	1 (2.8)	10 (5.0)
	ST 22	5 (1.3)	3 (2.2)	0 (0.0)	2 (1.0)
	ST 49	5 (1.3)	2 (1.5)	0 (0.0)	3 (1.5)
	ST 40	3 (0.8)	2 (1.5)	0 (0.0)	1 (0.5)
	ST 63	3 (0.8)	1 (0.7)	1 (2.8)	1 (0.5)
	ST 173	3 (0.8)	1 (0.7)	0 (0.0)	2 (1.0)
	ST 33	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	ST 59	2 (0.5)	0 (0.0)	1 (2.8)	1 (0.5)
	ST 142	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	ST 315	4 (1.1)	3 (2.2)	0 (0.0)	1 (0.5)
	ST 299	3 (0.8)	0 (0.0)	0 (0.0)	3 (1.5)
	ST 324	3 (0.8)	1 (0.7)	1 (2.8)	1 (0.5)
	ST 274	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	ST 322	2 (0.5)	1 (0.7)	0 (0.0)	1 (0.5)
	ST 323	2 (0.5)	1 (0.7)	0 (0.0)	1 (0.5)
	ST 318	2 (0.5)	1 (0.7)	1 (2.8)	0 (0.0)
Total singleton strains 38 (10.2%)	ST 34, 58, 64, 96, 128, 134, 135, 172	8 (2.2)	4 (3.0)	0 (0.0)	4 (2.0)
	NewSTs singletons	30 (8.0)	15 (11.2)	3 (8.3)	12 (5.5)
M. abscessus **subsp.** ***massiliense***		187 (50.4)	53 (39.6)	18 (50.0)	116 (57.7)
	ST 48	60 (16.2)	15 (11.2)	3 (8.3)	42 (20.9)
	ST 117	56 (15.1)	14 (10.4)	6 (16.7)	36 (17.9)
	ST 23	32 (8.6)	6 (4.5)	4 (11.1)	22 (10.9)
	ST 115	9 (2.4)	2 (1.5)	2 (5.6)	5 (2.5)
	ST 34	7 (1.9)	4 (3.0)	1 (2.8)	2 (1.0)
	ST 69	3 (0.8)	1 (0.7)	0 (0.0)	2 (1.0)
	ST 2	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	ST 110	2 (0.5)	1 (0.7)	0 (0.0)	1 (0.0)
	ST 129	2 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)
	ST 176	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	ST 279	4 (1.1)	4 (3.0)	0 (0.0)	0 (0.0)
Total singleton strains 8 (2.2%)	ST 88, 151	2 (0.5)	2 (1.5)	0 (0.0)	0 (0.0)
	New STs singletons	6 (1.6)	0 (0.0)	1 (2.8)	5 (2.5)
M. abscessus **subsp.** ***bolletii***	ST 311	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.5)

Table 3. ***Mycobacterium abscessus*** **sequence types over consecutive years from 2010 to 2017 in Taiwan. Sites TSGH, TVGH, NTUH-HC could not be included in the longitudinal analysis since they did not submit isolates preceding 2016.**

	2010	2011	2012	2013	2014	2015	2016	2017
Total no. of MAB isolates	35	34	38	35	34	42	42	40
ST1	28.6 (10)	26.5 (9)	23.7 (9)	22.9 (8)	23.5 (8)	23.8 (10)	11.9 (5)	22.5 (9)
ST23	5.7 (2)	5.9 (2)	2.6 (1)	11.4 (4)	2.9 (1)	11.9 (5)	7.1 (3)	22.5 (9)
ST48	8.6 (3)	17.6 (6)	26.3 (10)	11.4 (4)	23.5 (8)	16.7 (7)	19.0 (8)	17.5 (7)
ST117	22.9 (8)	14.7 (5)	15.8 (6)	14.3 (5)	11.8 (4)	16.7 (7)	16.7 (7)	5.0 (2)
Other STs	34.3 (12)	35.3 (12)	31.6 (12)	40.0 (14)	38.2 (13)	42.8 (18)	45.2 (19)	32.5 (13)

Table 4. Published molecular epidemiological studies using the Pasteur Institut's International MLST database of *M. abscessus* pulmonary isolates from different countries.

Year, Country, [Reference]	No. of isolates (patients)	Cystic fibrosis (%)	MAB-a (%)	MAB-b (%)	MAB-m (%)	Dominant STs (%)	Novel STs (%)	Singleton STs (%)
1998-2010, Scotland [35]	178 (12)	83	38	16	46	ST23 (42)	50	67
2004-2012, Ireland [30]	36 (36)	50	78	0	22	ST1 (19) ST26 (14) ST126 (8) ST22 (6) ST23 (6)	19	47
2013-2014, China [39]	55 (55)	0	76	4	20	ST1 (9) ST23 (9) ST117 (4) ST48. (2)	58	NA
2010-2017 Taiwan [this study]	371 (371)	0	49	1	50	ST1 (24) ST48 (16) ST117 (15) ST23 (9)	16	13

Figure 1. Map showing the location of participating hospitals and the included patient numbers at each site (modified from the Map Taiwan with Counties - Multicolor by FreeVectorMaps.com)

Figure 2. Flow diagram of the study.

Figure 3. Minimum spanning phylogenetic trees derived from multilocus sequence typing using the integrated concatenated sequences of 7 housekeeping genes: *argH, cya, glpK, gnd, murC, pta, and purH* (BioNumerics V.6.6, Applied Maths). Strains clustered together by the 7-gene MLST scheme was depicted as circles, strains from different hospitals were depicted by different colors with shades of green representing southern Taiwan, shades of yellow representing central Taiwan, and shades of red representing northern Taiwan.

Figure 4. Trends in relative frequencies of the four predominant *M. abscessus* sequence types (ST) between 2010-2017 across Taiwan. Sites TSGH, TVGH, NTUH-HC could not be included in the longitudinal analysis since they did not submit isolates preceding 2016.

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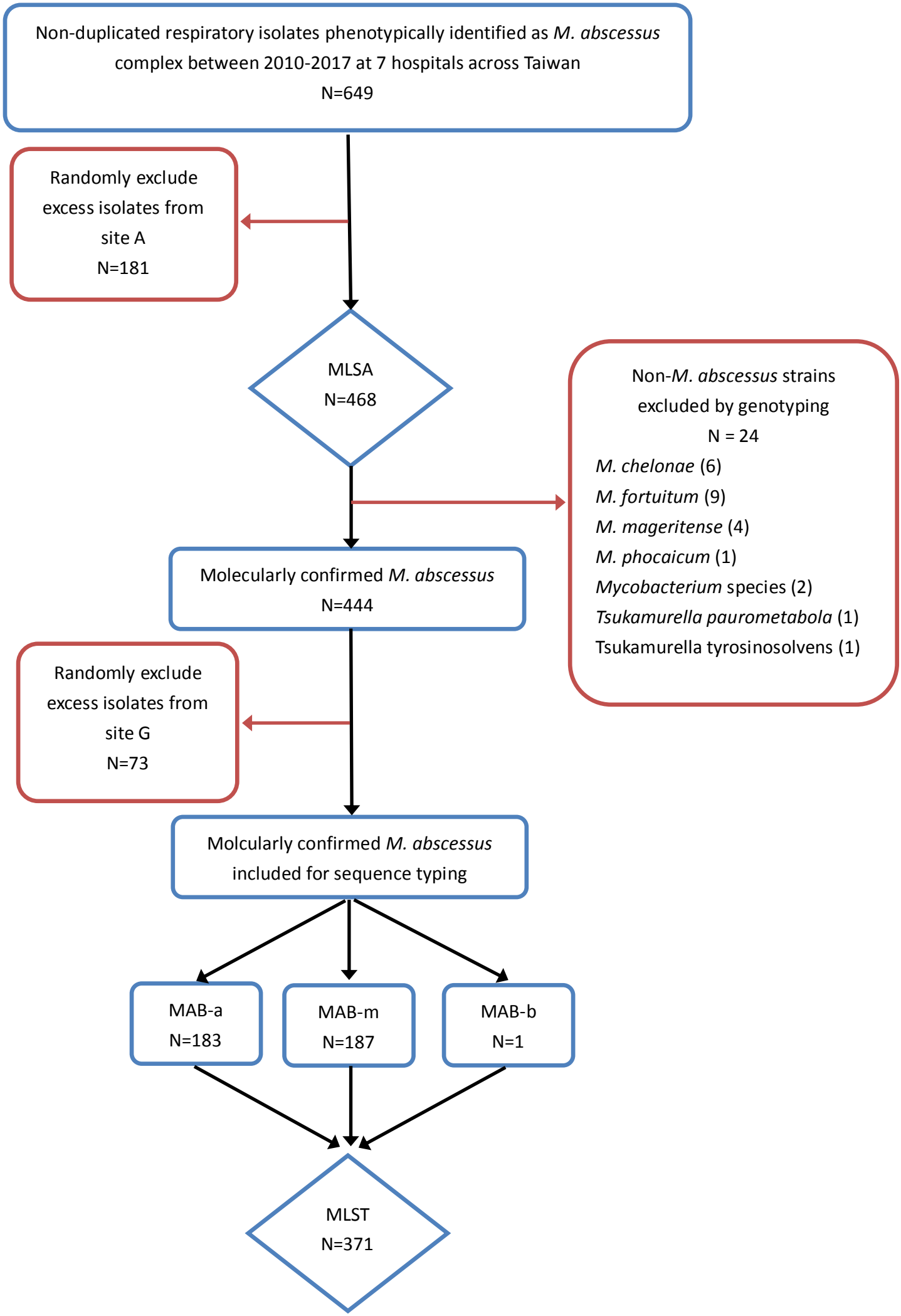
Non-duplicated respiratory isolates phenotypically identified as *M. abscessus* complex between 2010-2017 at 7 hospitals across Taiwan
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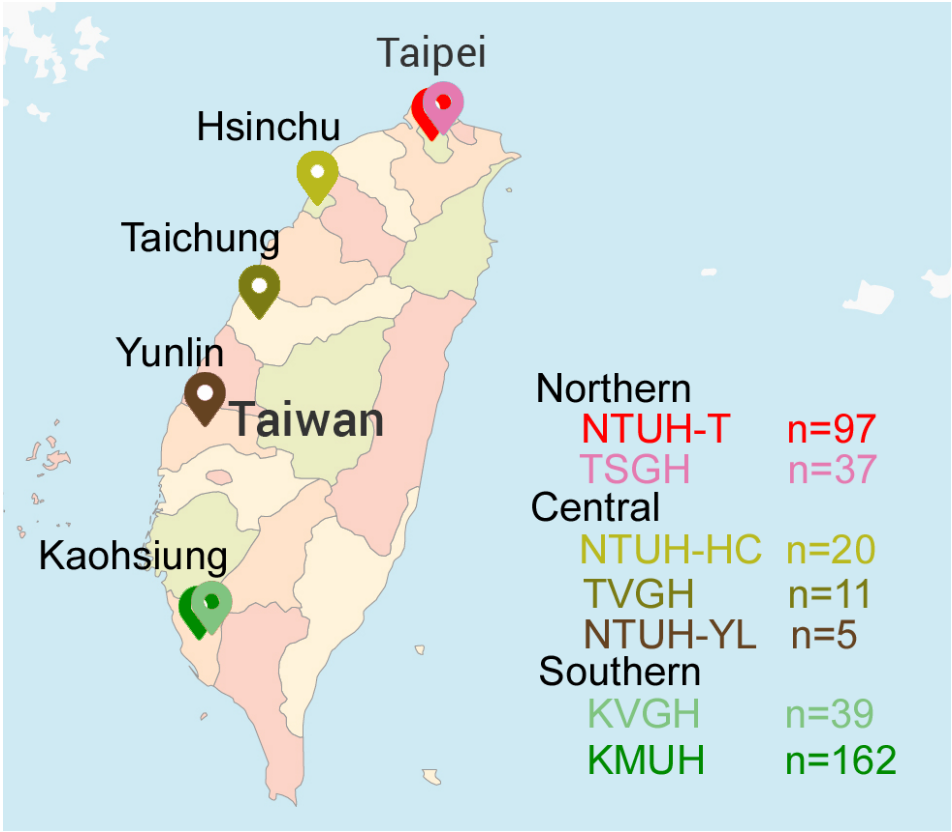
Randomly exclude excess isolates from site A
N=181
MLSA
N=468
Non-*M. abscessus* strains excluded by genotyping
N = 24
M. chelonae (6)
M. fortuitum (9)
M. mageritense (4)
M. phocaicum (1)
Mycobacterium species (2)
Tsukamurella paurometabola (1)
Tsukamurella tyrosinosolvens (1)
Molecularly confirmed *M. abscessus*
N=444
Randomly exclude excess isolates from site G
N=73
Molcularly confirmed *M. abscessus* included for sequence typing
MAB-a
N=183
MAB-m
N=187
MAB-b
N=1
MLST
N=371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Hsinchu
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Kaohsiung
Northern
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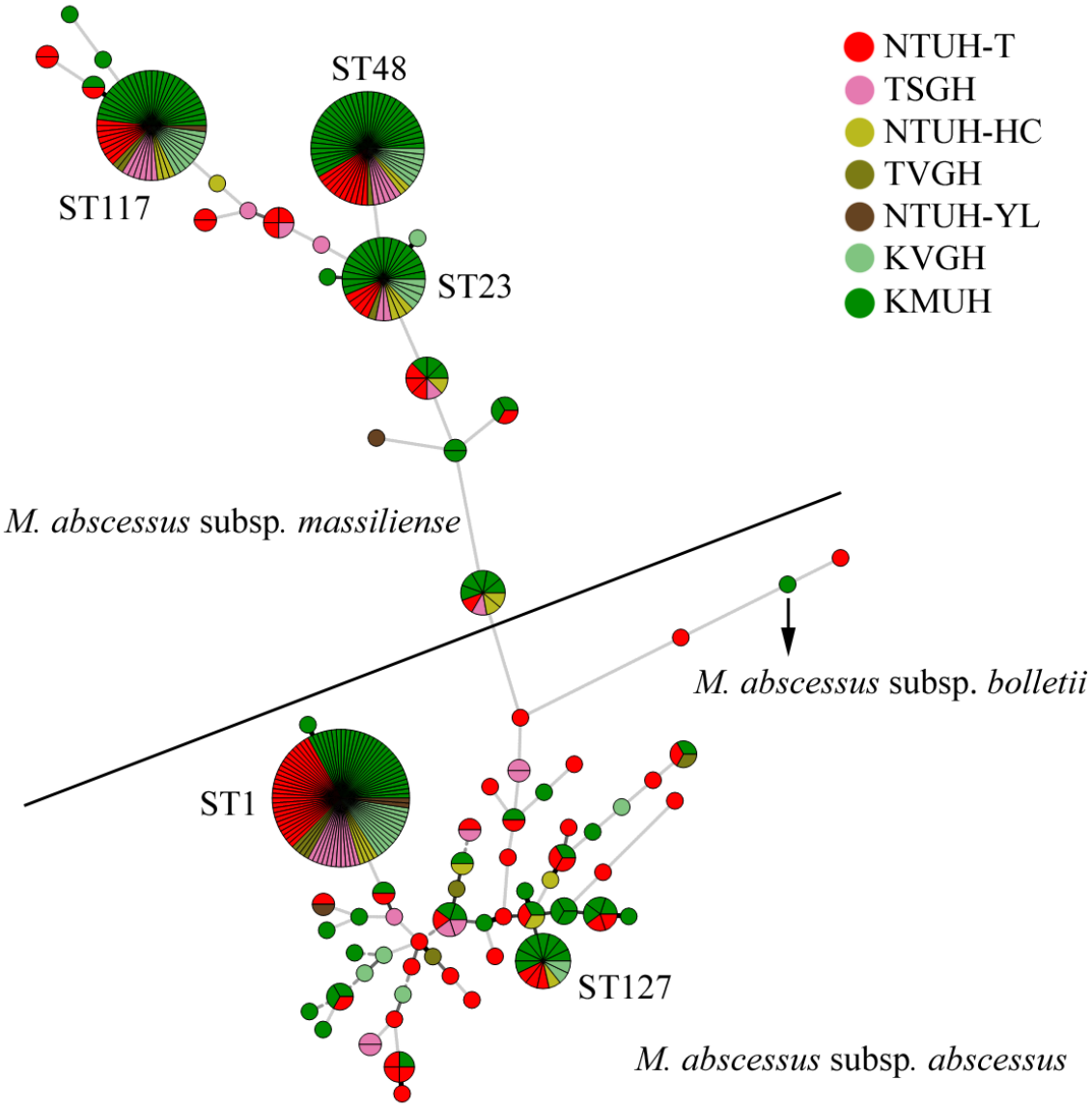
NTUH-T
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KVGH
KMUH
ST48
ST117
ST23
M. abscessus subsp. massiliense
M. abscessus subsp. bolletii
ST1
ST127
M. abscessus subsp. abscessus

Linear Regression Analysis of Trends in the Relative Frequency of Predominant *M. abscessus* Sequence Types in Taiwan over Time