

University of Dundee

## Immunological corollary of the pulmonary mycobiome in bronchiectasis

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1           **IMMUNOLOGICAL COROLLARY OF THE PULMONARY MYCOBIOME IN**  
2                           **BRONCHIECTASIS: THE CAMEB STUDY**

3  
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43 **ABSTRACT**

44 **Introduction:** Understanding the composition and clinical importance of the fungal  
45 mycobiome was recently identified as a key topic in a “research priorities” consensus  
46 statement for bronchiectasis **Methods:** Patients were recruited as part of the CAMEB study:  
47 an international multicentre cross-sectional Cohort of Asian and Matched European  
48 Bronchiectasis patients. The mycobiome was determined in 238 patients by targeted  
49 amplicon shotgun sequencing of the 18S-28S rRNA internally transcribed spacer regions  
50 ITS1 and ITS2. Specific qPCR for detection of and conidial quantification for a range of  
51 airway *Aspergillus* species was performed. Sputum galactomannan, *Aspergillus*-specific IgE,  
52 IgG and Thymus and Activation Regulated Chemokine levels were measured systemically  
53 and associated to clinical outcomes. **Results:** The bronchiectasis mycobiome is distinct, and  
54 characterised by specific fungal genera including *Aspergillus*, *Cryptococcus*, and *Clavispora*.  
55 *A. fumigatus* (in Singapore/Kuala Lumpur) and *A. terreus* (in Dundee) dominated profiles,  
56 the latter associating with exacerbations. High frequencies of *Aspergillus*-associated disease  
57 including sensitization and allergic bronchopulmonary aspergillosis were detected. Each  
58 revealed distinct mycobiome profiles and associated with more severe disease, poorer  
59 pulmonary function and increased exacerbations. **Conclusion:** The pulmonary mycobiome is  
60 of clinical relevance in bronchiectasis. Screening for *Aspergillus*-associated disease should be  
61 considered even in apparently stable patients.

62

63

64 **Key words:** Fungi, *Aspergillus*, microbiome, mycobiome, bronchiectasis

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67

68 **Abbreviations:**

69 AC: Aspergillus colonized

70 AS: Aspergillus sensitized

71 BMI: Body mass index

72 BSI: Bronchiectasis severity index

73 CAMEB: Cohort of Asian and Matched European Bronchiectasis

74 COPD: Chronic obstructive pulmonary disease

75 DD: Dundee

76 GM: Galactomannan

77 HRCT: High resolution computed tomography

78 ND: Non-diseased

79 OTU: Operational taxonomic unit

80 qPCR: Quantitative polymerase chain reaction

81 sABPA: Serological allergic bronchopulmonary aspergillosis

82 SAFS: Severe asthma with fungal sensitization

83 sCPA: Suspected chronic pulmonary aspergillosis

84 SG-KL: Singapore-Kuala Lumpur

85 sIgE: Specific immunoglobulin E

86 TARC: Thymus and activation regulated chemokine

87

88 **INTRODUCTION**

89

90 Bronchiectasis is a chronic respiratory disease characterised by progressive bronchial  
91 dilatation. To date, no therapy has been licensed for its treatment. Geographic regions  
92 illustrate preponderance for particular aetiologies, for instance post-tuberculous disease in  
93 countries of endemic infection [1]. Incipient infection in bronchiectasis incites deleterious  
94 host inflammatory responses and disease progression. A ‘vicious cycle’ of impaired  
95 mucociliary clearance, recurrent infection and chronic inflammation, with established links to  
96 bacteria, lead to progressive disease however, the role of fungi is poorly understood.

97

98 The pulmonary microbiome and its association with chronic respiratory disease is an  
99 emerging area of research. Culture-independent airway sequencing has revealed novel  
100 associations between the airway microbiome and lung disease [2]. To date, most airway  
101 microbiome studies have a bacterial focus; an approach that provides disease insight into  
102 pathogenesis and prognosis[3, 4]. In contrast however, sequencing of the fungal microbiome  
103 (the mycobiome) has lagged behind and is applied in relatively few studies and, to our  
104 knowledge none in bronchiectasis [5].

105

106 Fungal spores are environmentally ubiquitous hence their inhalation is an inevitable  
107 consequence of breathing [6]. While prompt mucociliary and phagocytic clearance occurs in  
108 the healthy lung, anatomically abnormal and immunocompromised airways such as in  
109 bronchiectasis are at higher risk of fungal acquisition, colonization and potential disease [6].  
110 Our group and others have performed extensive work characterising fungi in cystic fibrosis  
111 (CF) related bronchiectasis where they colonize and act as pathogens associated with poorer  
112 clinical outcome [7-10].

113

114 Fungi, particularly *Aspergillus spp.* cause a range of pulmonary consequences including  
115 allergic, chronic and/or invasive disease [11, 12]. An overzealous host response to  
116 *Aspergillus* for instance is clinically important in severe asthma with fungal sensitization  
117 (SAFS), chronic obstructive pulmonary disease (COPD) and CF [13-15]. The role of fungi in  
118 bronchiectasis not due to CF is, however, less well defined. *Aspergillus* sensitization and/or  
119 allergic bronchopulmonary aspergillosis (ABPA) is a cause (~10%) and importantly a  
120 consequence of bronchiectasis [6, 10, 16]. The role of the mycobiome in bronchiectasis is yet  
121 to be comprehensively investigated and its importance and relevance is clearly highlighted as  
122 a priority research area in this field [17].

123

124 We characterise for the first time, the mycobiome in bronchiectasis assessing its clinical  
125 relevance in two geographically distinct cohorts from the CAMEB study, encompassing four  
126 Asian sites across Singapore and Kuala Lumpur and a single European site in Dundee.

127

128 **METHODS**

129 **Study Population:** Patients with stable bronchiectasis were recruited across three countries  
130 as part of the CAMEB study; a cross-sectional Cohort of Asian and Matched European  
131 Bronchiectasis. Recruitment included three sites in Singapore (Singapore General Hospital,  
132 Changi General Hospital and Tan Tock Seng Hospital; n=124), one Malaysian site (UKM  
133 Medical Centre, Kuala Lumpur; n=14) and an age-, sex- and disease-severity matched group  
134 from a single European site (Ninewells Hospital, Dundee, UK; n=100) and was conducted  
135 between March 2016 and July 2017. Full details on patient inclusion and exclusion criteria,  
136 non-diseased controls and the CAMEB population are provided in the supplementary  
137 methods. Clinical characteristics, bronchiectasis aetiology and patient demographics are  
138 shown in Table 1. The study was approved by the institutional review boards of all  
139 participating institutes and all patients gave written informed consent to participate. Further  
140 details are in the supplementary methods.

141

142 Full details on clinical data and specimen collection, statistical analysis, molecular methods  
143 including mycobiome analyses, sputum qPCR detection of fungi and immunological  
144 bioassays are provided in the supplementary methods.

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153 **RESULTS**

154 **The pulmonary mycobiome in bronchiectasis is distinct:** Culture-independent analysis of  
155 the pulmonary mycobiome in bronchiectasis reveals that Ascomycota dominate the airway at  
156 the phylum level ( $p < 0.01$ , Mann-Whitney U-test) (Figure 1A). Fungal diversity was  
157 significantly lower in bronchiectasis, as measured by the number of identified taxa ( $p < 0.01$ )  
158 and the Shannon Diversity Index; SDI ( $p < 0.05$ ) (Mann-Whitney U-test) (Figure 1A).  
159 Limitations of fungal ITS databases may account for the lower classification percentages  
160 observed (95.3%) compared to bacteria where genus-level OTU classification of ~99% can  
161 be achieved [18]. Despite Ascomycota dominating overall mycobiome profiles, a significant  
162 number of bronchiectasis patients (49%,  $n=116$ ) harboured some degree of airway  
163 Basidiomycota. Ten percent ( $n=23$ ) had Basidiomycota making up at least one quarter of  
164 their overall profile while 4% ( $n=9$ ) exhibited Basidiomycota-dominant profiles (Figure 1B).  
165 *Candida*, *Saccharomyces* and *Penicillium* were the most frequently detected genera in both  
166 groups while differentially abundant, bronchiectasis-associated genera included *Aspergillus*,  
167 *Cryptococcus*, *Clavispora*, *Botrytis* and *Alternaria* (Figures 1C and 2A).

168

169 In the design of the CAMEB study,  $n=100$  Asian patients (Singapore / Kuala Lumpur)  
170 were matched individually to patients from a European cohort (Dundee) by age; gender and  
171 total bronchiectasis severity index (BSI) score (Table 1). This allowed assessment of findings  
172 in two geographically distinct cohorts as well as providing insight into potential regional  
173 differences in the bronchiectasis mycobiome. Geographic variation in mycobiome signature  
174 was detected (Figure 2A). Patients from Singapore and Kuala Lumpur exhibited significant  
175 and higher average relative abundance of *Simplicillium*, *Trichosporon* and *Aspergillus* while  
176 patients from Dundee were distinguished by higher abundances of *Wickerhamomyces*,  
177 *Clavispora* and *Cryptococcus* ( $p < 0.05$ ) following assessment of group differences using

178 metastats statistical analysis[19]. *Candida*, by far the most frequently observed fungal genera,  
179 was observed across both cohorts at equal frequency while patients from Dundee exhibited  
180 higher *Saccharomyces*, *Penicillium*, *Cryptococcus*, *Clavispora*, and *Botrytis* in particular.  
181 The only fungal genus in the top ten with higher prevalence in Singapore and Kuala Lumpur  
182 was *Aspergillus*, although this was detected at all the sampled sites (Figure 2B). Given our  
183 identification of *Aspergillus* as an exclusive bronchiectasis-associated fungal genus with high  
184 airway abundance; and its established pathogenic role in other chronic respiratory diseases  
185 [10, 12, 13, 15], we pursued it for further investigation.

186

187 ***A. fumigatus* and *A. terreus* are identified in bronchiectasis and associate with**  
188 **exacerbations:** We next further characterised the presence of specific *Aspergillus* species in  
189 the airway by qPCR using established protocols published by our group and others [7, 20].  
190 We assessed four major *Aspergillus* species: *fumigatus*, *terreus*, *flavus* and *niger* and found  
191 that non-diseased individuals had no detectable airway *Aspergillus*, in agreement with our  
192 mycobiome analysis (Figure 3A). In contrast, high proportions of bronchiectasis patients  
193 (from all sites) had detectable *A. fumigatus* and/or *A. terreus* with no patient demonstrating *A.*  
194 *flavus* or *A. niger* (Figure 3A). Interestingly, similar proportions of those recruited from an  
195 Asian site had either *A. fumigatus* and/or *A. terreus* whilst patients from Dundee  
196 demonstrated higher *A. terreus* (Figure 3A) which associates with increased exacerbations  
197 (Figures 3B). Importantly, significant numbers of patients (40%, n=96) harboured both  
198 species concurrently. Of these, an equal distribution between groups recruited from  
199 Singapore/Kuala Lumpur and Dundee respectively was observed (44% vs 52%,  $p = 0.22$ )  
200 (Figure 3C). Patients from Singapore and Kuala Lumpur were equally likely to have none,  
201 either or both species in their airway in contrast to patients from Dundee who exhibit a higher  
202 likelihood for *A. terreus* (Figure 3C). Greater exacerbations are seen in patients with *A.*

203 *terreus* alone or where both species were present compared to those without any fungi or *A.*  
204 *fumigatus* alone (Figure 3D).

205

206 **Quantification of *A. fumigatus* to *A. terreus* conidial burden ratio and associated**  
207 **exacerbations:** We next quantified *A. fumigatus* and *A. terreus* conidial burden using  
208 established protocols published by our group [7]. We then classified the detected loads as low  
209 (<500), intermediate (500-2000 or high (>2000) based on the number of conidia per gram of  
210 sputum. Measured conidial burden varied among *Aspergillus*-positive patients and was  
211 comparable between patients recruited from Singapore/Kuala Lumpur and Dundee  
212 respectively (Figure 4A). Interestingly, where patients harboured both species concurrently,  
213 their proportionality varied: patients from sites in Singapore and Kuala Lumpur exhibited  
214 higher proportions of *A. fumigatus* (median = 69% of conidial load) whilst the Dundee cohort  
215 had a higher *A. terreus* burden (median = 89% of total conidial load) (Figure 4B). This agrees  
216 with our earlier observation of regional variation; where cohorts in Singapore/Kuala Lumpur  
217 tended toward more *A. fumigatus* and the Dundee cohort greater *A. terreus* either when the  
218 fungi exist alone or together. When patients were grouped by detectable *Aspergillus* species  
219 and accompanying conidial burden (using high load as cut-off); a significant association  
220 existed between high concurrent conidial burdens of both *Aspergillus* species and greater  
221 exacerbations (Figure 4C). Specifically, however, when a high conidial burden of *A. terreus*  
222 was identified, either alone or in combination with *A. fumigatus*, significantly more  
223 exacerbations also occurred (Figure 4C).

224

225 **A high frequency of clinically relevant *Aspergillus*-associated disease occurs in**  
226 **bronchiectasis:** Prior work from our group and others highlights the association of  
227 *Aspergillus*-associated disease (Aspergillosis) with adverse clinical outcomes across a range

228 of chronic respiratory diseases [7, 13, 21]. Given the high frequencies of *Aspergillus*  
229 detected, we next evaluated the occurrence of Aspergillosis and its clinical relevance in  
230 bronchiectasis. To do this, we used a modified immunological classification system  
231 developed for CF (Table E4) [14]. In addition to the criteria described by Baxter *et al.*, our  
232 modified version includes incorporation of potential *A. terreus*-associated disease (by  
233 inclusion of *A. terreus* sIgE and qPCR) along with other minor modifications detailed in  
234 Table E4. Of note; total IgE although measured, was not used in our classification of patients  
235 because it is described that ABPA may occur at IgE concentrations below the described cut-  
236 offs in the established criteria [22]. Patients were grouped into the following five categories:  
237 non-diseased (ND); *Aspergillus*-colonized (AC); *Aspergillus*-sensitized (AS); serological  
238 allergic bronchopulmonary aspergillosis (sABPA) and suspected chronic pulmonary  
239 aspergillosis (sCPA). Significant numbers in the CAMEB cohort met criteria for an  
240 *Aspergillus*-associated disease state (ND; 1.7%, AC; 3.4%, AS; 76.5%, sABPA; 18.1%,  
241 sCPA; 0.3%) (Figure 5). Measured total serum IgE, *Aspergillus*-specific IgG and sputum  
242 galactomannan varied between patient groups (Figure E1). Specific-IgE against crude  
243 antigens of *A. fumigatus* and *A. terreus* respectively were highest in AS (0.77 and 0.57 kU/L)  
244 and sABPA (0.92 and 0.93 kU/L) ( $p < 0.001$ ) (Figures 5A-B). Thymus and Activation  
245 Regulated Chemokine (TARC); previously identified as a marker of CF-ABPA was also  
246 assessed [23]. We found TARC to be a poor indicator of AS and/or sABPA in bronchiectasis  
247 with high false negative rates in both groups (Figure 5C). Additionally, false positives were  
248 also observed in a small number of ND patients (Figure 5C). Patients with sABPA exhibited  
249 significantly more exacerbations, poorer pulmonary function and the severest disease  
250 compared to other groups (Figure 5D-F). AS was associated with greater exacerbations while  
251 AC portended toward more exacerbations with preserved pulmonary function and less severe  
252 disease (Figure 5D-F). AC, AS and sABPA therefore appear to exist along an increasing

253 continuum of disease severity. Only a single patient met criteria for sCPA precluding further  
254 analysis of this group. *Aspergillus*-associated disease in bronchiectasis is therefore frequent,  
255 variable and clinically relevant including in patients appearing clinically stable; a  
256 characteristic inclusion criteria for our study.

257

258 ***Aspergillus*-associated disease in bronchiectasis has distinct mycobiome profiles:** As high  
259 frequencies of *Aspergillus*-associated disease were observed in bronchiectasis based on our  
260 modified classification system, we next assessed for the presence of distinct taxonomic  
261 profiles that may associate with four of the five clinical states: ND, AC, AS and sABPA,  
262 excluding sCPA because this group contained only a single patient. Although unsupervised  
263 beta-diversity analysis revealed three distinct mycobiome groups (Figure E3) their  
264 association with clinical phenotype was not evident. We therefore adopted a supervised  
265 strategy comparing mycobiome profiles between our identified immunological classes. Each  
266 clinical state revealed mycobiome profiles characterised by a combination of varying fungal  
267 genera (Figure 6). The ND group exhibited apparent high abundance of *Phellinus*,  
268 *Magnusiomyces* and *Phlebia* with lower relative abundance of *Saccharomyces*, *Aspergillus*  
269 and *Penicillium* (Figure 6A). These latter three genera however increased in their relative  
270 abundance across a spectrum from ND to AC, AS and sABPA (Figure 6, Table E5). The  
271 genera *Cryptococcus* and *Clavispora* followed similar and comparable spectra with  
272 increasing relative abundance from AC, AS to sABPA. *Mycosphaerella*, and *Botrytis* were  
273 present only in diseased states without specific pattern (Figure 6B-D). Other characteristic  
274 fungal genera included *Trichosporon* and *Cladosporium* in the AC state and;  
275 *Wickerhamomyces* and *Alternaria* in AS and sABPA (Figure 6B). Importantly, the relative  
276 abundance of *Aspergillus* detected from mycobiome profiles were in agreement with our  
277 qPCR-based quantification of conidial burden for *A. fumigatus* and *A. terreus* across all

278 disease categories with greater exacerbations noted in AS and sABPA (Figure 6).  
279 Mycobiome profiles unique to each *Aspergillus*-associated disease state are therefore  
280 potentially useful for future bronchiectasis studies that focus on the diagnosis and endo-  
281 phenotyping of fungal disease.

282

## 283 **DISCUSSION**

284 We describe the first, culture-independent analysis of the pulmonary mycobiome performed  
285 to date in bronchiectasis and, to our knowledge, the first such respiratory study to  
286 meticulously match geographically distinct patient cohorts allowing us to assess potential  
287 regional variation in the mycobiome. By applying high-throughput 18S-28S ITS sequencing  
288 and other molecular techniques, we delineated the mycobiome constituents and their  
289 associated clinically relevant states. Our analysis identified *Aspergillus* as a major fungal  
290 genus in bronchiectasis, similar to that described in other chronic respiratory diseases [6, 12].  
291 An interesting observation was the prevalence of specific *Aspergillus* species characterising  
292 our Singapore/Kuala Lumpur and Dundee cohorts with *A. fumigatus* and *A. terreus* most  
293 frequently detected within each respective group, the latter associated to exacerbations.  
294 Further patient stratification into groups including AC, AS and sABPA revealed high  
295 occurrences of clinically significant disease, even in ‘stable’ patients and, the existence of  
296 distinct mycobiome profiles of discriminant taxa for each group.

297         Changes to regional lung growth conditions creates a conducive environment for  
298 colonisation and infection by pathogenic microbes [2]. Given the gross anatomical distortion  
299 characteristic of bronchiectasis, it is therefore unsurprising that microbial differences are  
300 observed when compared to non-diseased states. The chronic bronchial insult coupled to  
301 anatomic distortion, compromised mucociliary clearance, sustained neutrophilic response and

302 recurrent infection all favour the growth of pathogenic fungi and their ensuing clinical  
303 consequences in bronchiectasis [6, 24].

304 As observed in this and other studies, the airway mycobiome is dominated by  
305 Ascomycota [25]. Ascomycota-dominant profiles are characteristic of bronchiectasis  
306 although an important minority have high airway Basidiomycota. Basidiospores are  
307 environmentally more abundant and smaller than ascospores, explaining their airway  
308 presence [26]. Compared to Ascomycota, which includes the established respiratory  
309 pathogens *Aspergillus* and *Candida*, the specific role of Basidiomycota in bronchiectasis is  
310 unclear. Notably pathogenic Basidiomycota including *Cryptococcus*, *Schizophyllum*,  
311 *Phellinus*, *Ceriporia* and *Trichosporon* were all detected in our work [27]. *Cryptococcus* is  
312 significantly elevated in bronchiectasis; an observation previously documented, which now  
313 warrants further investigation [28]. The existence of pulmonary Basidiomycota might reflect  
314 dynamic trafficking of ‘rare biosphere’ fungi (‘immigration and elimination’), suggestive of a  
315 healthier respiratory state rather than the sustained fungal outgrowth and unfavourable  
316 environment associated with Ascomycota [2, 5]. The cross-sectional design of our study  
317 however precluded longitudinal assessment of the stability and transiency of the mycobiome  
318 over time, a key consideration for future work.

319 Bronchiectasis-associated fungal genera include *Aspergillus*, *Issatchenkia*,  
320 *Wickerhamomyces* and *Simplicillium*. Where dedicated species-specific qPCR for *Aspergillus*  
321 was performed, only *A. fumigatus* and *A. terreus* were identifiable in bronchiectasis.  
322 Interestingly, *A. fumigatus* had preponderance within the Asian cohorts from Singapore and  
323 Kuala Lumpur whilst *A. terreus* exhibited higher prevalence and burden in patients from  
324 Dundee. Even in patients with both species, geographic origin dictated which proportionally  
325 dominated. Our patients were stringently matched for age, sex and disease severity to

326 confront the heterogeneity that plagues bronchiectasis research and allowed us to better  
327 assess potential geographic variation. Studies of additional cohorts from Europe and Asia will  
328 be required to determine if these are truly geographical differences or reflect differences in  
329 patient selection or referral patterns at the participating sites. These geographic phenotypes  
330 were accompanied by differences in lung mycobiome profiles: *Penicillium*, *Clavispora* and  
331 *Cryptococcus* had markedly higher frequency in patients from Dundee while Basidiomycota  
332 of the genera *Phlebia*, *Trichosporon*, *Ceriporia*, *Phellinus*, *Schizophyllum*, *Psathyrella* and  
333 *Peniophora* were exclusive to patients from Singapore/Kuala Lumpur who exhibit higher  
334 overall Basidiomycota. Our identified differences may reflect a myriad of regionally variable  
335 factors such as contrasting atmospheric conditions between temperate and tropical climates  
336 including temperature and humidity, each documented to affect fungi [29, 30]. Interestingly,  
337 our identified geographic variation of *A. terreus* predominance in Dundee occurred despite  
338 low reported UK prevalence rates of this fungus suggestive of enrichment in the  
339 bronchiectasis population [31]. CF-related bronchiectasis also associates with *A. terreus* and  
340 an unidentified ‘environmental exposure’ of likely relevance in our Dundee cohort is ascribed  
341 to this species [32, 33]. Other key geographic factors to consider include dietary preferences,  
342 genetics, air quality and/or lifestyle, all influences on acquisition and persistence of microbes  
343 including fungi [34]. Interestingly, the marked BMI difference between our ‘matched’  
344 cohorts raises questions about potential differences in gut microbiomes that in turn may  
345 associate with the airway mycobiome given the emerging lung-gut axis relationship; an area  
346 of increasing relevance to respiratory health [35]. Other confounders to consider include  
347 regional differences in pharmacological prescribing and their consequent implications for the  
348 mycobiome; important considerations given differences between our Dundee and  
349 Singapore/Kuala Lumpur cohorts in terms of inhaled corticosteroids, long-term prophylactic  
350 antibiotics (greater in Dundee) and mucolytic use (greater in Singapore/Kuala Lumpur)



351 (Table 1) – all features of potential influence on mycobiome composition which warrant  
352 further study. While our findings could be peculiar to Dundee, Singapore and Malaysia;  
353 generalizability is supported by data showing that Dundee patients are similar and data  
354 generated in Scotland has been generalizable across more than 10 European cohorts in recent  
355 analyses [36, 37].

356

357 Our group and others have previously investigated the role of *Aspergillus*-associated  
358 disease in CF-related bronchiectasis; however focused work in non-CF bronchiectasis is  
359 lacking [7, 34, 38-40]. Importantly however, Everaerts *et al* assessed occurrence of *A.*  
360 *fumigatus* sensitisation in COPD and, when detectable, associated it with a high risk for  
361 bronchiectasis [21]. These data support the role for *Aspergillus* as a bronchiectasis pathogen  
362 either as a cause or consequence. Our data further corroborates such associations with high  
363 observable sIgE levels to *A. fumigatus* and *A. terreus*. Using a modified immunologic  
364 classification, adapted from CF, we describe high rates of AS and sABPA in the CAMEB  
365 cohort outlining its clinical relevance even in patients appearing clinically stable [14]. TARC,  
366 a proposed marker of CF-ABPA was found to have poor sensitivity and specificity when  
367 applied to the CAMEB cohort illustrating difficulties in translating findings from CF to non-  
368 CF bronchiectasis [23]. Each of our classified clinical states is accompanied by a distinct  
369 mycobiome pattern suggestive of mechanistic links necessitating future studies.

370

371 A potential role for *A. terreus* in bronchiectasis is a novel finding of this work. When  
372 compared to *A. fumigatus*, *A. terreus* is lesser-studied. It is however noted for colonising the  
373 CF lung, is capable of causing ABPA, and associates with opportunistic infections[41-43]. Its  
374 resistance to polyene antifungals confers survival advantages in the host compared to *A.*  
375 *fumigatus* [44-45]. For instance, *A. terreus* conidia are rapidly phagocytosed through dectin-1

376 and mannose receptors, however, exhibit an increased macrophage survival compared to *A.*  
377 *fumigatus* [45]. Rapid germination of *A. fumigatus* within the macrophage comes at the cost  
378 of its increased vulnerability to host immunity. In contrast, *A. terreus* germinates at lower  
379 rates favouring its persistence. Our work highlights high proportions of bronchiectasis  
380 patients with both *A. fumigatus* and *A. terreus*. Moreover, such patients demonstrate greater  
381 exacerbations, suggestive that, in combination these fungi have important clinical effects on  
382 distinct host pathways that worsen disease. As a consequence, assessment of fungal airway  
383 conidial burden is important in bronchiectasis.

384

385         While our work is novel, it does have limitations including its cross sectional design.  
386 This is a consequence of our attempt to robustly match Asian and European cohorts within  
387 the study to address disease heterogeneity, a key issue in bronchiectasis research. In addition,  
388 we must consider the ‘generalizability’ of our three participating countries in comparison to  
389 the wider Asian and European sub-continent including the potential variation in aetiology  
390 for exacerbations, their management including bronchiectasis treatments and geographic  
391 differences in climate, temperature and air quality. Epidemiologic fungal studies have in fact  
392 illustrated species differences between countries even within the same sub-continent [31].  
393 Furthermore, we included only a small group of non-diseased (healthy) individuals (all from  
394 Singapore) which precluded us from defining a ‘healthy’ mycobiome clearly which was not  
395 the primary aim of this work. Future studies however should focus on assessing the non-  
396 diseased lung mycobiome and include individuals from more than a single region to assess  
397 potential geographic variation. In addition, targeted amplicon sequencing – even through our  
398 parallel shotgun sequencing of ITS1 and ITS2 - has lower resolution compared to the more  
399 costly and analytically challenging whole genome shotgun metagenomics that provides  
400 superior speciation and functional annotation. We focused on *Aspergillus* as the predominant

401 bronchiectasis-associated fungal pathogen however our mycobiome analysis suggests that  
402 other fungal genera may also have roles. Finally, when the CAMEB cohort was conceived, a  
403 decision was made to match across Singapore/Kuala Lumpur and Dundee based on age, sex  
404 and disease severity. This matching on total BSI rather than its specific components including  
405 exacerbations, radiology or microbiology limits our ability to perform more specific analyses.  
406 Our cohort enrolled predominantly severe patients with bronchiectasis with a relatively low  
407 number of patients with mild disease (BSI 0-4).

408

409         We have performed the first fungal profiling study in bronchiectasis to date using two  
410 ‘matched’ cohorts across three countries. We illustrate high levels of airway fungi and key  
411 differences between cohorts. These data suggest that the role for fungi and specifically  
412 *Aspergillus* in bronchiectasis is significant and routine screening for *Aspergillus*-associated  
413 diseases should be considered for inclusion in future bronchiectasis guidelines. Screening  
414 may include skin testing, *Aspergillus*-associated sIgE and *Aspergillus*-specific IgG with  
415 potential follow up immunological monitoring for those clinically affected. Our identified  
416 mycobiome profiles that relate to clinical disease should also be explicated in future work.

417

418 **REFERENCES**

- 419 1. Polverino, E., P.C. Goeminne, M.J. McDonnell, S. Aliberti, S.E. Marshall, M.R.  
420 Loebinger, M. Murriss, R. Canton, A. Torres, K. Dimakou, A. De Soyza, A.T. Hill,  
421 C.S. Haworth, M. Vendrell, F.C. Ringshausen, D. Subotic, R. Wilson, J. Vilaro, B.  
422 Stallberg, T. Welte, G. Rohde, F. Blasi, S. Elborn, M. Almagro, A. Timothy, T.  
423 Ruddy, T. Tonia, D. Rigau, and J.D. Chalmers, *European Respiratory Society*  
424 *guidelines for the management of adult bronchiectasis*. Eur Respir J, 2017. **50**(3).
- 425 2. Dickson, R.P., J.R. Erb-Downward, F.J. Martinez, and G.B. Huffnagle, *The*  
426 *Microbiome and the Respiratory Tract*. Annu Rev Physiol, 2016. **78**: p. 481-504.
- 427 3. Rogers, G.B., N.M. Zain, K.D. Bruce, L.D. Burr, A.C. Chen, D.W. Rivett, M.A.  
428 McGuckin, and D.J. Serisier, *A novel microbiota stratification system predicts future*  
429 *exacerbations in bronchiectasis*. Ann Am Thorac Soc, 2014. **11**(4): p. 496-503.
- 430 4. Cox, M.J., E.M. Turek, C. Hennessy, G.K. Mirza, P.L. James, M. Coleman, A. Jones,  
431 R. Wilson, D. Bilton, W.O. Cookson, M.F. Moffatt, and M.R. Loebinger,  
432 *Longitudinal assessment of sputum microbiome by sequencing of the 16S rRNA gene*  
433 *in non-cystic fibrosis bronchiectasis patients*. PLoS One, 2017. **12**(2): p. e0170622.
- 434 5. Huffnagle, G.B. and M.C. Noverr, *The emerging world of the fungal microbiome*.  
435 Trends Microbiol, 2013. **21**(7): p. 334-41.
- 436 6. Chotirmall, S.H. and M.T. Martin-Gomez, *Aspergillus Species in Bronchiectasis:*  
437 *Challenges in the Cystic Fibrosis and Non-cystic Fibrosis Airways*. Mycopathologia,  
438 2017.
- 439 7. Coughlan, C.A., S.H. Chotirmall, J. Renwick, T. Hassan, T.B. Low, G. Bergsson, A.  
440 Eshwika, K. Bennett, K. Dunne, C.M. Greene, C. Gunaratnam, K. Kavanagh, P.M.  
441 Logan, P. Murphy, E.P. Reeves, and N.G. McElvaney, *The effect of Aspergillus*

- 442 *fumigatus* infection on vitamin D receptor expression in cystic fibrosis. Am J Respir  
443 Crit Care Med, 2012. **186**(10): p. 999-1007.
- 444 8. Chotirmall, S.H., E. O'Donoghue, K. Bennett, C. Gunaratnam, S.J. O'Neill, and N.G.  
445 McElvaney, *Sputum Candida albicans* presages FEV(1) decline and hospital-treated  
446 exacerbations in cystic fibrosis. Chest, 2010. **138**(5): p. 1186-95.
- 447 9. McMahan, M.A., S.H. Chotirmall, B. McCullagh, P. Branagan, N.G. McElvaney, and  
448 P.M. Logan, *Radiological abnormalities associated with Aspergillus* colonization in a  
449 *cystic fibrosis* population. Eur J Radiol, 2012. **81**(3): p. e197-202.
- 450 10. Chotirmall, S.H. and N.G. McElvaney, *Fungi in the cystic fibrosis lung: bystanders*  
451 *or pathogens?* Int J Biochem Cell Biol, 2014. **52**: p. 161-73.
- 452 11. Chotirmall, S.H., M. Al-Alawi, B. Mirkovic, G. Lavelle, P.M. Logan, C.M. Greene,  
453 and N.G. McElvaney, *Aspergillus-associated airway disease, inflammation, and the*  
454 *innate immune response*. Biomed Res Int, 2013. **2013**: p. 723129.
- 455 12. Yii, A.C., M.S. Koh, T.S. Lapperre, G.L. Tan, and S.H. Chotirmall, *The emergence of*  
456 *Aspergillus species in chronic respiratory disease*. Front Biosci (Schol Ed), 2017. **9**:  
457 p. 127-138.
- 458 13. Goh, K.J., A.C.A. Yii, T.S. Lapperre, A.K. Chan, F.T. Chew, S.H. Chotirmall, and  
459 M.S. Koh, *Sensitization to Aspergillus species is associated with frequent*  
460 *exacerbations in severe asthma*. J Asthma Allergy, 2017. **10**: p. 131-140.
- 461 14. Baxter, C.G., G. Dunn, A.M. Jones, K. Webb, R. Gore, M.D. Richardson, and D.W.  
462 Denning, *Novel immunologic classification of aspergillosis in adult cystic fibrosis*. J  
463 Allergy Clin Immunol, 2013. **132**(3): p. 560-566.e10.
- 464 15. Leung, J.M., P.Y. Tiew, M. Mac Aogain, K.F. Budden, V.F. Yong, S.S. Thomas, K.  
465 Pethe, P.M. Hansbro, and S.H. Chotirmall, *The role of acute and chronic respiratory*

- 466            *colonization and infections in the pathogenesis of COPD*. *Respirology*, 2017. **22**(4):  
467            p. 634-650.
- 468    16.    Lonni, S., J.D. Chalmers, P.C. Goeminne, M.J. McDonnell, K. Dimakou, A. De  
469            Soyza, E. Polverino, C. Van de Kerkhove, R. Rutherford, J. Davison, E. Rosales, A.  
470            Pesci, M.I. Restrepo, A. Torres, and S. Aliberti, *Etiology of Non-Cystic Fibrosis*  
471            *Bronchiectasis in Adults and Its Correlation to Disease Severity*. *Ann Am Thorac*  
472            *Soc*, 2015. **12**(12): p. 1764-70.
- 473    17.    Aliberti, S., S. Masefield, E. Polverino, A. De Soyza, M.R. Loebinger, R. Menendez,  
474            F.C. Ringshausen, M. Vendrell, P. Powell, J.D. Chalmers, and E.S. Group, *Research*  
475            *priorities in bronchiectasis: a consensus statement from the EMBARC Clinical*  
476            *Research Collaboration*. *Eur Respir J*, 2016. **48**(3): p. 632-47.
- 477    18.    Ong, S.H., V.U. Kukkillaya, A. Wilm, C. Lay, E.X. Ho, L. Low, M.L. Hibberd, and  
478            N. Nagarajan, *Species identification and profiling of complex microbial communities*  
479            *using shotgun Illumina sequencing of 16S rRNA amplicon sequences*. *PLoS One*,  
480            2013. **8**(4): p. e60811.
- 481    19.    White, J.R., N. Nagarajan, and M. Pop, *Statistical methods for detecting differentially*  
482            *abundant features in clinical metagenomic samples*. *PLoS Comput Biol*, 2009. **5**(4):  
483            p. e1000352.
- 484    20.    Walsh, T.J., M.C. Wissel, K.J. Grantham, R. Petraitiene, V. Petraitis, M. Kasai, A.  
485            Francesconi, M.P. Cotton, J.E. Hughes, L. Greene, J.D. Bacher, P. Manna, M.  
486            Salomoni, S.B. Kleiboeker, and S.K. Reddy, *Molecular detection and species-specific*  
487            *identification of medically important Aspergillus species by real-time PCR in*  
488            *experimental invasive pulmonary aspergillosis*. *J Clin Microbiol*, 2011. **49**(12): p.  
489            4150-7.

- 490 21. Everaerts, S., K. Lagrou, A. Dubbeldam, N. Lorent, K. Vermeersch, E. Van  
491 Hoeyveld, X. Bossuyt, L.J. Dupont, B.M. Vanaudenaerde, and W. Janssens,  
492 *Sensitization to Aspergillus fumigatus as a risk factor for bronchiectasis in COPD*. Int  
493 J Chron Obstruct Pulmon Dis, 2017. **12**: p. 2629-2638.
- 494 22. Agarwal, R., A. Chakrabarti, A. Shah, D. Gupta, J.F. Meis, R. Guleria, R. Moss, D.W.  
495 Denning, and A.c.a.I.w. group, *Allergic bronchopulmonary aspergillosis: review of*  
496 *literature and proposal of new diagnostic and classification criteria*. Clin Exp  
497 Allergy, 2013. **43**(8): p. 850-73.
- 498 23. Hartl, D., P. Latzin, G. Zissel, M. Krane, S. Krauss-Etschmann, and M. Griese,  
499 *Chemokines indicate allergic bronchopulmonary aspergillosis in patients with cystic*  
500 *fibrosis*. Am J Respir Crit Care Med, 2006. **173**(12): p. 1370-6.
- 501 24. Chalmers, J.D. and A.T. Hill, *Mechanisms of immune dysfunction and bacterial*  
502 *persistence in non-cystic fibrosis bronchiectasis*. Mol Immunol, 2013. **55**(1): p. 27-34.
- 503 25. Ghannoum, M.A., R.J. Jurevic, P.K. Mukherjee, F. Cui, M. Sikaroodi, A. Naqvi, and  
504 P.M. Gillevet, *Characterization of the oral fungal microbiome (mycobiome) in*  
505 *healthy individuals*. PLoS Pathog, 2010. **6**(1): p. e1000713.
- 506 26. Yamamoto, N., K. Bibby, J. Qian, D. Hospodsky, H. Rismani-Yazdi, W.W. Nazaroff,  
507 and J. Peccia, *Particle-size distributions and seasonal diversity of allergenic and*  
508 *pathogenic fungi in outdoor air*. ISME J, 2012. **6**(10): p. 1801-11.
- 509 27. Chowdhary, A., S. Kathuria, K. Agarwal, and J.F. Meis, *Recognizing filamentous*  
510 *basidiomycetes as agents of human disease: A review*. Med Mycol, 2014. **52**(8): p.  
511 782-97.
- 512 28. Li, S.S. and C.H. Mody, *Cryptococcus*. Proc Am Thorac Soc, 2010. **7**(3): p. 186-96.
- 513 29. Lim, S.H., F.T. Chew, S.D. Binti Mohd Dali, H.T. Wah Tan, B.W. Lee, and T.K. Tan,  
514 *Outdoor airborne fungal spores in Singapore*. Grana, 1998. **37**(4): p. 246-252.

- 515 30. O’Gorman, C.M. and H.T. Fuller, *Prevalence of culturable airborne spores of*  
516 *selected allergenic and pathogenic fungi in outdoor air*. Atmospheric Environment,  
517 2008. **42**(18): p. 4355-4368.
- 518 31. Risslegger, B., T. Zoran, M. Lackner, M. Aigner, F. Sanchez-Reus, A. Rezusta, A.  
519 Chowdhary, S.J. Taj-Aldeen, M.C. Arendrup, S. Oliveri, D.P. Kontoyiannis, A.  
520 Alastruey-Izquierdo, K. Lagrou, G. Lo Cascio, J.F. Meis, W. Buzina, C. Farina, M.  
521 Drogari-Apiranthitou, A. Grancini, A.M. Tortorano, B. Willinger, A. Hamprecht, E.  
522 Johnson, L. Klingspor, V. Arsic-Arsenijevic, O.A. Cornely, J. Meletiadis, W.  
523 Prammer, V. Tullio, J.J. Vehreschild, L. Trovato, R.E. Lewis, E. Segal, P.M. Rath, P.  
524 Hamal, M. Rodriguez-Iglesias, E. Roilides, S. Arikani-Akdagli, A. Chakrabarti, A.L.  
525 Colombo, M.S. Fernandez, M.T. Martin-Gomez, H. Badali, G. Petrikos, N. Klimko,  
526 S.M. Heimann, J. Houbraken, O. Uzun, M. Edlinger, S. Fuente, and C. Lass-Flörl, *A*  
527 *prospective international Aspergillus terreus survey: an EFISG, ISHAM and ECMM*  
528 *joint study*. Clin Microbiol Infect, 2017. **23**(10): p. 776 e1-776 e5.
- 529 32. Lackner, M., S. Coassin, M. Haun, U. Binder, F. Kronenberg, H. Haas, M. Jank, E.  
530 Maurer, J.F. Meis, F. Hagen, and C. Lass-Flörl, *Geographically predominant*  
531 *genotypes of Aspergillus terreus species complex in Austria: a microsatellite typing*  
532 *study*. Clin Microbiol Infect, 2016. **22**(3): p. 270-6.
- 533 33. Rougeron, A., S. Giraud, B. Razafimandimby, J.F. Meis, J.P. Bouchara, and C.H.  
534 Klaassen, *Different colonization patterns of Aspergillus terreus in patients with cystic*  
535 *fibrosis*. Clin Microbiol Infect, 2014. **20**(4): p. 327-33.
- 536 34. Chotirmall, S.H., S.L. Gellatly, K.F. Budden, M. Mac Aogain, S.D. Shukla, D.L.  
537 Wood, P. Hugenholtz, K. Pethe, and P.M. Hansbro, *Microbiomes in respiratory*  
538 *health and disease: An Asia-Pacific perspective*. Respiriology, 2017. **22**(2): p. 240-  
539 250.



- 540 35. Budden, K.F., S.L. Gellatly, D.L. Wood, M.A. Cooper, M. Morrison, P. Hugenholtz,  
541 and P.M. Hansbro, *Emerging pathogenic links between microbiota and the gut-lung*  
542 *axis*. Nat Rev Microbiol, 2017. **15**(1): p. 55-63.
- 543 36. McDonnell, M.J., S. Aliberti, P.C. Goeminne, K. Dimakou, S.C. Zucchetti, J.  
544 Davidson, C. Ward, J.G. Laffey, S. Finch, A. Pesci, L.J. Dupont, T.C. Fardon, D.  
545 Skrbic, D. Obradovic, S. Cowman, M.R. Loebinger, R.M. Rutherford, A. De Soyza,  
546 and J.D. Chalmers, *Multidimensional severity assessment in bronchiectasis: an*  
547 *analysis of seven European cohorts*. Thorax, 2016. **71**(12): p. 1110-1118.
- 548 37. Chalmers, J.D., S. Aliberti, A. Filonenko, M. Shteinberg, P.C. Goeminne, A.T. Hill,  
549 T.C. Fardon, D. Obradovic, C. Gerlinger, G. Sotgiu, E. Operschall, R.M. Rutherford,  
550 K. Dimakou, E. Polverino, A. De Soyza, and M.J. McDonnell, *Characterisation of*  
551 *the "Frequent Exacerbator Phenotype" in Bronchiectasis*. Am J Respir Crit Care  
552 Med, 2018.
- 553 38. Hector, A., S.H. Chotirmall, G.M. Lavelle, B. Mirkovic, D. Horan, L. Eichler, M.  
554 Mezger, A. Singh, A. Ralhan, S. Berenbrinker, I. Mack, R. Ensenaer, J. Riethmuller,  
555 U. Graepler-Mainka, M.A. Murray, M. Griese, N.G. McElvaney, and D. Hartl,  
556 *Chitinase activation in patients with fungus-associated cystic fibrosis lung disease*. J  
557 Allergy Clin Immunol, 2016. **138**(4): p. 1183-1189 e4.
- 558 39. Mirkovic, B., G.M. Lavelle, A.A. Azim, K. Helma, F.S. Gargoum, K. Molloy, Y.  
559 Gernez, K. Dunne, J. Renwick, P. Murphy, R.B. Moss, C.M. Greene, C. Gunaratnam,  
560 S.H. Chotirmall, and N.G. McElvaney, *The basophil surface marker CD203c*  
561 *identifies Aspergillus species sensitization in patients with cystic fibrosis*. J Allergy  
562 Clin Immunol, 2016. **137**(2): p. 436-443 e9.
- 563 40. Gernez, Y., J. Walters, B. Mirkovic, G.M. Lavelle, D.E. Colleen, Z.A. Davies, C.  
564 Everson, R. Tirouvanziam, E. Silver, S. Wallenstein, S.H. Chotirmall, N.G.

- 565 McElvaney, L.A. Herzenberg, and R.B. Moss, *Blood basophil activation is a reliable*  
566 *biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis*. Eur Respir J,  
567 2016. **47**(1): p. 177-85.
- 568 41. Laham, M.N. and J.L. Carpenter, *Aspergillus terreus, a pathogen capable of causing*  
569 *infective endocarditis, Pulmonary mycetoma, and allergic bronchopulmonary*  
570 *aspergillosis*. Am Rev Respir Dis, 1982. **125**(6): p. 769-72.
- 571 42. Dunne, K., A.R. Prior, K. Murphy, N. Wall, G. Leen, T.R. Rogers, B. Elnazir, P.  
572 Greally, J. Renwick, and P. Murphy, *Emergence of persistent Aspergillus terreus*  
573 *colonisation in a child with cystic fibrosis*. Med Mycol Case Rep, 2015. **9**: p. 26-30.
- 574 43. Lass-Flörl, C., K. Griff, A. Mayr, A. Petzer, G. Gastl, H. Bonatti, M. Freund, G.  
575 Kropshofer, M.P. Dierich, and D. Nachbaur, *Epidemiology and outcome of infections*  
576 *due to Aspergillus terreus: 10-year single centre experience*. Br J Haematol, 2005.  
577 **131**(2): p. 201-7.
- 578 44. Walsh, T.J., V. Petraitis, R. Petraitiene, A. Field-Ridley, D. Sutton, M. Ghannoum, T.  
579 Sein, R. Schaufele, J. Peter, J. Bacher, H. Casler, D. Armstrong, A. Espinel-Ingroff,  
580 M.G. Rinaldi, and C.A. Lyman, *Experimental pulmonary aspergillosis due to*  
581 *Aspergillus terreus: pathogenesis and treatment of an emerging fungal pathogen*  
582 *resistant to amphotericin B*. J Infect Dis, 2003. **188**(2): p. 305-19.
- 583 45. Slesiona, S., M. Gressler, M. Mihlan, C. Zaehle, M. Schaller, D. Barz, B. Hube, I.D.  
584 Jacobsen, and M. Brock, *Persistence versus escape: Aspergillus terreus and*  
585 *Aspergillus fumigatus employ different strategies during interactions with*  
586 *macrophages*. PLoS One, 2012. **7**(2): p. e31223.
- 587 46. Hill, A.T., C.S. Haworth, S. Aliberti, A. Barker, F. Blasi, W. Boersma, J.D. Chalmers,  
588 A. De Soyza, K. Dimakou, J.S. Elborn, C. Feldman, P. Flume, P.C. Goeminne, M.R.  
589 Loebinger, R. Menendez, L. Morgan, M. Murriss, E. Polverino, A. Quittner, F.C.

590 Ringshausen, G. Tino, A. Torres, M. Vendrell, T. Welte, R. Wilson, C. Wong, A.  
591 O'Donnell, T. Aksamit, and E.B.d.w. group, *Pulmonary exacerbation in adults with*  
592 *bronchiectasis: a consensus definition for clinical research*. Eur Respir J, 2017. **49**(6).  
593  
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**Table 1.** Demographics of the study population including non-diseased (healthy) controls and stable bronchiectasis patients comprising Asian and European matched cohorts.

Characteristic	Non-diseased controls (n=10)	Bronchiectasis patients (n=238)	Bronchiectasis patients SG-KL (n=138)	Matched cohorts	
				SG-KL (n=100)	DD (n=100)
Age : median (IQR)	37 (30-49)	68 (64-71)	65 (58-73)	65 (58-74)	69 (64-76)
Gender : n (%)					
Female	4 (40%)	130 (55%)	77 (55%)	59 (59%)	53 (53%)
Male	6 (60%)	108 (45%)	61 (45%)	41 (41%)	47 (47%)
Aetiology n (%)					
Idiopathic	-	145 (61%)	85 (62%)	63 (63%)	60 (60%)
Post-infection	-	56 (23.5%)	43 (31%)	27 (27%)	27 (27%)
Other	-	37 (15.5%)	10 (7%)	10 (10%)	13 (13%)
Smoking status n (%)					
Never	-	170 (70%)	108 (78%)	80 (80%)	62 (62%)
Current	-	11 (5%)	7 (5%)	4 (4%)	4 (4%)
Past	-	57 (25%)	23 (17%)	16 (16%)	34 (34%)
BSI status : n (%)					
Severe	-	147 (62%)	84 (61%)	63 (63%)	63 (63%)
Moderate	-	71 (30%)	45 (33%)	26 (26%)	26 (26%)
Mild	-	20 (8%)	9 (6%)	11 (11%)	11 (11%)
BSI score : median (IQR)	-	9 (6-13)	10 (7-14)	10 (7-14)	9 (6-12)
BMI (kg/m <sup>2</sup> ) : median (IQR)	24(22-24)	21 (18-27)	19 (17-22)	19 (17-22)	27 (22-31)
MRC dyspnea score : n (%)					
1-3	-	200 (84%)	121 (88%)	90 (90%)	79 (79%)
4	-	26 (11%)	10 (7%)	6 (6%)	16 (16%)
5	-	12 (5%)	7 (5%)	4 (4%)	5 (5%)
FEV <sub>1</sub> % predicted : median (IQR)	-	74 (54-87)	69 (51-84)	69 (52-84)	76 (57-96)
Radiological severity : n (%)					
1-2 lobes involved	-	106 (45%)	62 (45%)	43 (43%)	44 (44%)
3 or more lobes involved	-	132 (55%)	76 (55%)	57 (57%)	56 (56%)
No. of exacerbations in previous year : n (%)					
0	-	84 (35%)	69 (50%)	44 (44%)	15 (15%)
1-2	-	82 (35%)	51 (37%)	41 (41%)	31 (31%)
3 or more	-	72 (30%)	18 (13%)	15 (15%)	54 (54%)
Hospital admissions before study : n (%)					
Yes	-	88 (37%)	63 (46%)	43 (43%)	25 (25%)
No	-	150 (63%)	75 (54%)	57 (57%)	75 (75%)
Colonization with other organisms : n (%)					
Yes	-	127 (53%)	60 (43%)	44 (44%)	67 (67%)
No	-	111 (47%)	78 (57%)	56 (56%)	33 (33%)
<i>Pseudomonas</i> colonisation : n (%)					
Yes	-	23 (10%)	18 (13%)	15 (15%)	5 (5%)
No	-	215 (90%)	120 (87%)	85 (85%)	95 (95%)
Bronchodilator use					
Yes	-	107 (45%)	58 (42%)	39 (39%)	49 (49%)
No	-	131 (55%)	80 (58%)	61 (61%)	51 (51%)
Inhaled corticosteroids					
Yes	-	80 (34%)	21 (15%)	14 (14%)	59 (59%)
No	-	158 (66%)	117 (85%)	86 (86%)	41 (41%)
Mucolytics					
Yes	-	118 (50%)	60 (44%)	45 (45%)	13 (13%)
No	-	120 (50%)	78 (56%)	55 (55%)	87 (87%)
Long-term antibiotics					
Yes	-	48 (20%)	22 (16%)	14 (14%)	26 (26%)
No	-	190 (80%)	116 (84%)	86 (86%)	74 (74%)

598 Data are presented as median (interquartile range; IQR) or n (percentage; %). Patients in the  
599 matched cohorts were matched on age, gender and disease severity according to the  
600 Bronchiectasis Severity Index (BSI). The variables defining composite BSI score including  
601 Body Mass Index (BMI), shortness of breath (MRC) dyspnoea score, Forced expiratory  
602 volume in the 1<sup>st</sup> second (FEV<sub>1</sub>) % predicted values, Radiological severity, number of  
603 exacerbations in the preceding year defined by established consensus [46], hospitalisations in  
604 the preceding year, microbial colonisation with other organisms and colonisation by *P.*  
605 *aeruginosa* are also reported.  
606

607 **FIGURE LEGENDS**

608 **Figure 1.** The pulmonary mycobiome in stable bronchiectasis (a) Mirrored Sankey plots  
609 illustrating the relative abundance of read classification by taxonomic rank from phylum  
610 (centre; blue) to genus level (margins; red). Non-diseased (healthy) controls (n=10) are  
611 compared to patients with stable bronchiectasis (n=238). Central coloured bars demonstrate  
612 phylum-level abundance of Ascomycota (yellow) versus Basidiomycota (blue) between the  
613 cohorts. Cohort-specific fungal genera are indicated in bold. (b) Individual patient phylum-  
614 level classification of the pulmonary mycobiome in stable bronchiectasis (n=238) illustrating  
615 the relative abundance of Ascomycota (yellow) versus Basidiomycota (blue) for individual  
616 patients (c) Percent prevalence of fungal genera (present at >1% relative abundance) in non-  
617 diseased (healthy) controls (n=10) and patients with stable bronchiectasis (n=238). The  
618 prevalence of the top genera (observed at >1% relative abundance) are illustrated across non-  
619 diseased controls and patients with stable bronchiectasis. Coloured bars indicate membership  
620 to either Ascomycota (yellow) or Basidiomycota (blue) phyla. Filled dots (●) indicate genera  
621 found in bronchiectasis and open dots (○) genera in non-diseased (healthy) controls.

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623 **Figure 2.** The pulmonary mycobiome differs between Singapore/Kuala Lumpur (SG-KL)  
624 and Dundee (DD) cohorts of stable bronchiectasis (a) Genus level classification of the  
625 pulmonary mycobiome in non-diseased (ND, n=10), stable bronchiectasis (BR, n=238) and  
626 matched SG-KL (n = 100) and DD (n = 100) cohorts. The relative abundance of identified  
627 taxa is colour-illustrated. (b) Percent prevalence of observed fungal genera (present at >1%  
628 relative abundance) in matched bronchiectasis cohorts from SG-KL (red, n=100) and DD  
629 (blue, n=100). Filled squares (■) indicate genera found only in the DD cohort while dots (●)  
630 indicate genera detected only in the SG-KL cohort. Significant differences in prevalence  
631 between cohorts are indicated; \*\* p <0.01, \*\*\* p<0.001.

632 **Figure 3.** Identification of specific airway *Aspergillus* species and their association with  
633 exacerbations in bronchiectasis (a) Quantitative polymerase chain reaction (qPCR)-based  
634 screening for the presence of the major specific *Aspergillus* species: *A. fumigatus*, *A. terreus*,  
635 *A. flavus* and *A. niger* in non-diseased (healthy) controls (ND, n=10) and stable  
636 bronchiectasis (BR, n = 238). Bar colouration indicates the relative proportion of positive  
637 Singapore/Kuala Lumpur (SG-KL) (red) and Dundee (DD) (blue) patients respectively  
638 according to *Aspergillus* species. (b) Exacerbations for the year preceding study recruitment  
639 in patients with qPCR detectable *A. fumigatus* (left) and *A. terreus* (right). (c) Classification  
640 of qPCR detectable *Aspergillus* species by percentage (%) of the total bronchiectasis patient  
641 cohort (n=238). Patients are classified as having no detectable species (none, n=39), *A.*  
642 *fumigatus* only (AF, n=26), *A. terreus* only (AT, n=78) or both *A. fumigatus* and *A. terreus*  
643 (both, n=95). Bar colouration indicates the relative proportion of positive patients from SG-  
644 KL (red) and DD (blue) respectively by classification. (d) Exacerbations for the year  
645 preceding study recruitment in patients with no detectable species (None), *A. fumigatus* only  
646 (AF), *A. terreus* only (AT) or both *A. fumigatus* and *A. terreus* (Both). Dot colouration  
647 indicates patient origin: SG-KL (red) and DD (blue). Median number of exacerbations per  
648 group is indicated. ns – non-significant, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

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656 **Figure 4.** Quantification of airway *Aspergillus* conidial burden and its association with  
657 exacerbations in bronchiectasis (n =238). (a) Conidial burden per gram (g) of sputum was  
658 quantified for *A. fumigatus* and *A. terreus* respectively and classified according to load as  
659 Low (<500 conidia / g sputum), Intermediate (500-2000 conidia / g sputum) or High (>2000  
660 conidia / g sputum). Conidial load categories are illustrated according to fungal airway status  
661 as *A. fumigatus* only (AF), *A. terreus* only (AT) or the presence of both species (Both). Dot  
662 colouration indicates patient origin: Singapore/Kuala Lumpur (SG-KL) (red) and Dundee  
663 (DD) (blue). (b) Scatter plot of *A. fumigatus* (x-axis) and *A. terreus* (y-axis) conidial load in  
664 stable bronchiectasis. Patients with single (●) and both (●) species are indicated. Patients  
665 with both species are classified by their relative *A. fumigatus* and *A. terreus* burdens  
666 respectively into low burden of both (LL), low burden of *A. fumigatus* and high burden of *A.*  
667 *terreus* (LH), high burden of *A. fumigatus* and low burden of *A. terreus* (HL) and high burden  
668 of both (HH). Dotted lines indicate cut-off levels for a high conidial burden (>2000 conidia /  
669 g sputum). Dot colouration indicates patient origin: SG-KL (red) and DD (blue). (c)  
670 Exacerbations for the year preceding study recruitment in bronchiectasis patients with  
671 detectable conidial burdens of both *A. fumigatus* and *A. terreus* classified as LL, LH, HL and  
672 HH respectively. Dot colouration indicates patient origin: SG-KL (red) and DD (blue).  
673 Median number of exacerbations per group is indicated and Benjamini–Hochberg-adjusted p-  
674 values for all groups compared to ‘None’ are shown and significance indicated as \*p=0.01  
675 and \*\*p=0.006 respectively. .

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681 **Figure 5.** Immunologic classification reveals high frequencies of Aspergillosis in stable  
682 bronchiectasis and an association of serological allergic bronchopulmonary aspergillosis  
683 (sABPA) with greater exacerbations, poorer pulmonary function and more severe disease.  
684 Measured levels of specific IgE (sIgE) responses to (a) *A. fumigatus* and (b) *A. terreus*  
685 respectively where Aspergillosis is classified immunologically as non-diseased (ND, n = 4);  
686 *Aspergillus*-colonized (AC, n=8); *Aspergillus*-sensitized (AS, n = 182); serological allergic  
687 bronchopulmonary aspergillosis (sABPA, n = 43) and suspected chronic pulmonary  
688 aspergillosis (sCPA, n =1) (Supplementary table 4). Thymus and activation regulated  
689 chemokine (TARC); a proposed ABPA-marker in cystic fibrosis [23] was assessed according  
690 to the same classification. Broken lines indicate respective cut-offs indicating a positive test  
691 for each marker (Specific IgE ; 0.35 kU/L, TARC ; 386 pg/mL) (d), Exacerbations for the  
692 year preceding study recruitment (e), Pulmonary function (as percent predicted FEV<sub>1</sub>) and (f)  
693 disease severity (as BSI) was assessed according to immunologic aspergillosis class. Dot  
694 colouration indicates patient origin: SG-KL (red) and DD (blue). Mean values are indicated  
695 except for exacerbations and BSI where median values are shown. \* p<0.05, \*\* p <0.01, \*\*\*  
696 p<0.001. FEV<sub>1</sub>: Forced Expiratory Volume in the first second; BSI: Bronchiectasis Severity  
697 Index.

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705 **Figure 6.** Pulmonary Mycobiome profiles illustrate specific taxa-associated patterns  
706 according to immunological classification of Aspergillosis in stable bronchiectasis. The  
707 Mycobiome profiles of (a) Non-diseased (ND, n = 4); (b) *Aspergillus*-colonized (AC, n=8);  
708 (c) *Aspergillus*-sensitized (AS, n = 182) and (d) serological allergic bronchopulmonary  
709 aspergillosis (sABPA, n = 43) illustrate the different composition by relative abundance of  
710 reads classified to genus level, with the (b) AC, (c) AS and (d) sABPA states exhibiting  
711 increased exacerbations and higher conidial load. Only a single patient had suspected chronic  
712 pulmonary aspergillosis and therefore data is not shown. Mycobiome profiles are represented  
713 by pie-charts with colour coding according to the taxonomic legend. Adjacent colour  
714 illustrated log-scaled bar charts detail the observed taxa patterns in each immunological  
715 patient class (Formal statistical assessment is provided in Table E5). Exacerbations in the  
716 preceding year (x-axis) are plotted against *Aspergillus* conidial load (y-axis) and colour  
717 coded according to qPCR detection status of *Aspergillus* species into *A. fumigatus* alone  
718 (green), *A. terreus* alone (orange), presence of Both (purple) or None (grey). . Patients are  
719 further classified according to their individual conidial load as Negative (‘Neg’ ; no detected  
720 conidia / g sputum), Low (<500 conidia / g sputum), Intermediate ( ‘Int’ ; 500-2000 conidia /  
721 g sputum) or High (>2000 conidia / g sputum).

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