# Distribution and diversity of pulmonary and extrapulmonary *Mycobacterium abscessus* infections

***Kimberley Smith[1,6], Steven Taylor[2], Geraint Rogers[2], Lu Zheng[2], Chris Coulter[3], Sushil Pandey[3], Jennifer Robson[4], Nicola Scorer[4] , Rachel Thomson[1,6,7], and Scott Bell[1,5,8]***

*[1]Child Health Research Centre, The University of Queensland, Brisbane, Queensland , [2]Respiratory Health Group, Microbiome and Host Health, South Australian Health Medical Research Institute, Adelaide, South Australia, [3]Queensland Mycobacterium Reference Laboratory (QMRL), Pathology Queensland, Brisbane, Queensland, [4]Department of Microbiology, Sullivan Nicolaides Pathology (SNP), Brisbane, Queensland, [5]Adult Cystic Fibrosis Centre, The Prince Charles Hospital, Brisbane, Queensland, [6]Gallipoli Medical Research Foundation, Brisbane, Queensland, [7]Greenslopes Clinical Unit, The University of Queensland, Brisbane, Queensland , [8]Translational Research Institute, Brisbane, Queensland*

## BACKGROUND

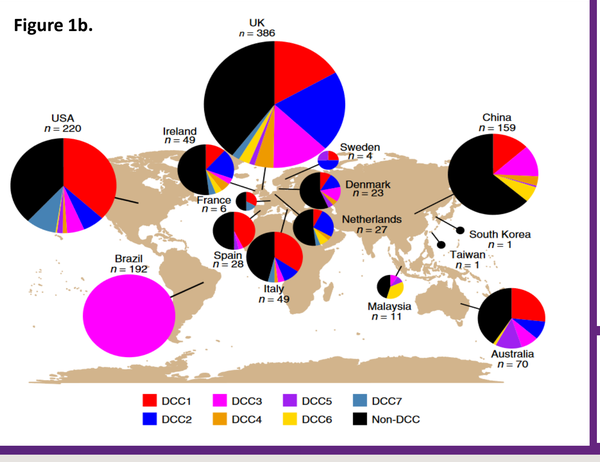
***Mycobacterium abscessus* group *(Mabs)***

- Rapidly growing nontuberculous mycobacteria (NTM), commonly found in a variety of water sources
- Three subspecies including *abscessus, massiliense and bolletii*
- Opportunistic pathogens causing pulmonary and extrapulmonary infections
- Third most common species of NTM reported in Qld in between 2001 and 2016[1]
- Dominant circulating clones (DCCs) of *Mabs* have been recently identified[2]
- The global prevalence and distribution of DCCs in cystic fibrosis (CF) and non-CF populations are highlighted in Figure 1

**KNOWLEDGE GAP**
**Limited information on the prevalence and distribution of DCCs in non-CF infections**

## AIMS

1. Determine the genomic relationships between *Mabs* isolates from non-CF pulmonary and extrapulmonary infections in QLD between 1999 to 2021
2. Report the prevalence of DCCs in non-CF pulmonary and extrapulmonary infections



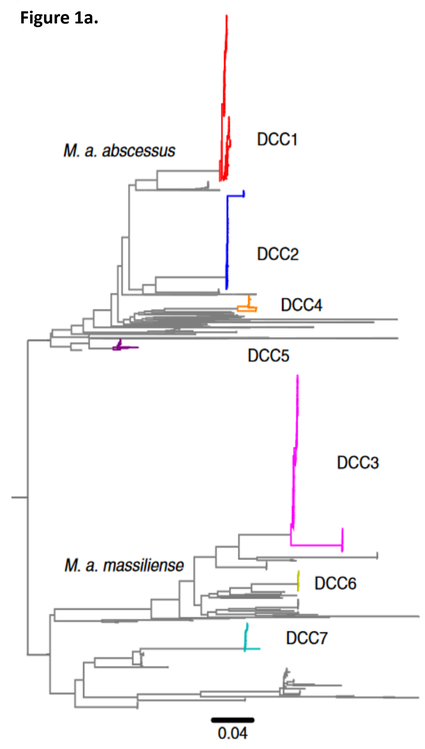




**Figure 1. Global dissemination of DCCs in CF and non-CF populations[3]**
a. *Mabs* phylogenetic tree
b. Global proportion of DCCs

## METHODS

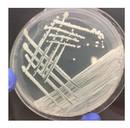
Two hundred and fifty-four *Mabs* isolates from 228 patients were supplied from two Queensland laboratories, QMRL and SNP (Table 1). Inclusion was limited to a single isolate per patient unless mixed colony morphology was evident.

**Table 1. *Mabs* isolate sampling**

| Sampling Characteristics | QMRL (n=144* patients) | SNP (n=84* patients) |
|---|---|---|
| **Isolate selection** | Systematic sampling# | Convenience sampling^ |
| **Years** | 2000-2020 | 2018-2021 |
| **Sample collection sites** | | |
| **Pulmonary** | Sputum, washings, BAL, tissue | Sputum, washings, BAL |
| **Extrapulmonary** | Skin, soft tissue, fluid, blood | Soft tissue |
| **Number of isolates** | | |
| **Pulmonary** | 84 | 95 |
| **Extrapulmonary** | 73 | 2 |

# If available the first *Mabs* isolate of the month, every 5 years spanning 20 years
* 26 patients had >1 mixed colony morphology (rough or smooth)
^ *Mabs* isolates made available through another study

### Workflow



gDNA from a single colony extracted using Qiagen DNeasy ultraclean microbial kit

WGS & Bioinformatic analysis at SAHMRI

Phylogenetic tree generation (FastTree)

## RESULTS

- Genetic relatedness of *Mabs* subspecies to published strains[3]
- This study 254 isolates from 228 patients (Figure 2)

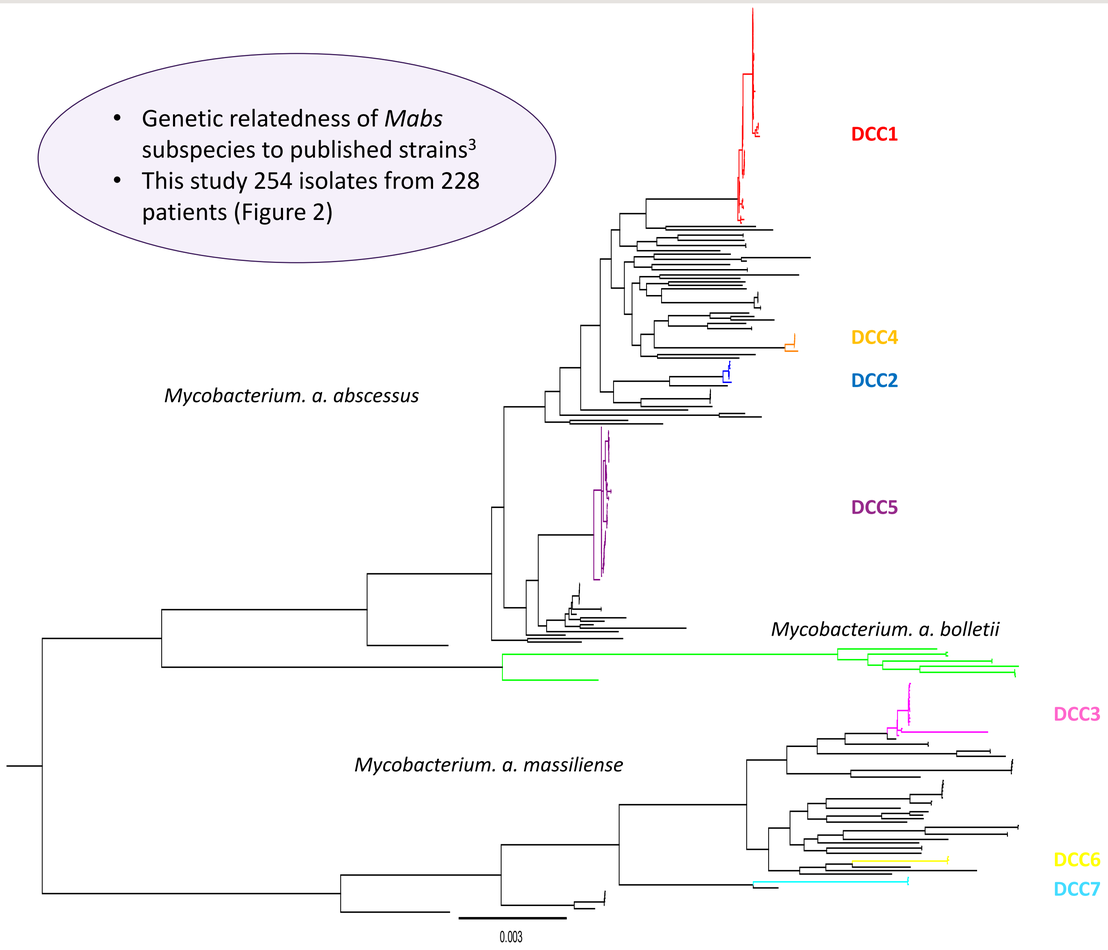



**Figure 2. Phylogenetic tree of *Mabs* from non-CF pulmonary and extrapulmonary origin (reference strains from Ruis 2021[3])**

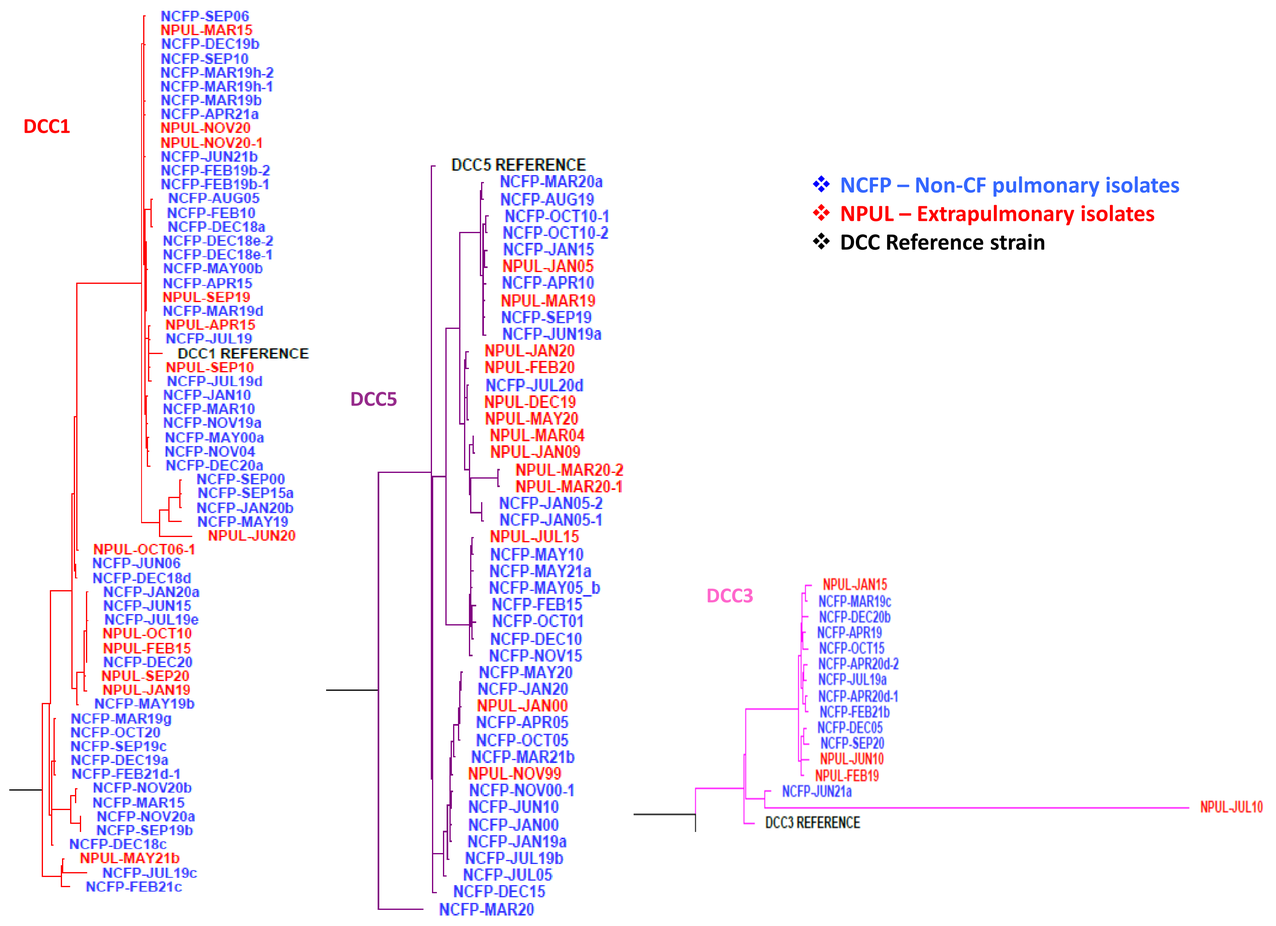



**Figure 3. Highlighted predominant DCC branches from Figure 2 (reference strains from Ruis 2021[3])**

**Table 2. *Mabs* subspecies identification in patient cohorts†**

| *Mabs* subspecies | Non-CF^ n= 158 n (%) | CF‡ n=71 n (%) | p-value* | Combined Pulmonary n= 229 n (%) | Extrapulmonary^ n= 70 n (%) | p-value* |
|---|---|---|---|---|---|---|
| *M. a. abscessus* | 112 (70.9) | 52 (73.3) | 0.75 | 164 (71.7) | 48 (68.6) | 0.65 |
| *M. a. massiliense* | 42 (26.6) | 15 (21.1) | 0.41 | 57 (24.9) | 14 (20.0) | 0.43 |
| *M. a. bolletii* | 3 (1.9) | 1 (1.4) | >0.99 | 4 (1.7) | 6 (8.6) | **0.013** |
| >1 *Mabs* subspecies | 1 (0.6) | 3 (4.2) | 0.09 | 4 (1.7) | 2 (2.8) | 0.63 |

†For footnotes see Table 3

***Mabs* subspecies identified in patient cohorts (Table 2)**

- Pulmonary non-CF and CF patient isolates have similar distribution of subspecies (p>0.05)
- *M. a. bolletii* was significantly greater in extrapulmonary compared with combined pulmonary isolates (p=0.013)
- No significant difference was noted when subspecies identification from SNP and QMRL cohorts were compared (p>0.05, data not shown)

**DCCs identified in patient cohorts (Table 3; Figure 2 and 3)**

- DCC1 was significantly greater from combined pulmonary compared with extrapulmonary isolates (p=0.02)
- The proportion of DCC5 was:
  - Significantly greater in non-CF compared with CF individuals (p=0.003)
  - Significantly greater in QMRL (21 years) compared with SNP cohort (3 years)(p=0.006, data not shown)

**Table 3. Dominant circulating clones identified in patient cohorts**

| *Mabs* DCC | Non-CF^ n= 158 n (%) | CF‡ n=71 n (%) | p-value* | Combined Pulmonary n= 229 n (%) | Extrapulmonary^ n= 70 n (%) | p-value* |
|---|---|---|---|---|---|---|
| *M. a. abscessus* | | | | | | |
| DCC1 | 44 (27.9) | 23 (33.4) | 0.53 | 67 (29.3) | 11 (15.7) | **0.02** |
| DCC2 | 3 (1.9) | 2 (2.8) | 0.65 | 5 (2.2) | 3 (4.3) | 0.39 |
| DCC4 | 3 (1.9) | 1 (1.4) | >0.99 | 4 (1.7) | 1 (1.4) | >0.99 |
| DCC5 | 29 (18.4) | 3 (4.2) | **0.003** | 32 (14.0) | 12 (17.2) | 0.56 |
| *M. a. massiliense* | | | | | | |
| DCC3 | 10 (6.3) | 4 (5.6) | >0.99 | 14 (6.1) | 4 (5.7) | >0.99 |
| DCC6 | 1 (0.6) | 1 (1.4) | 0.52 | 2 (0.9) | 0 (0.0) | >0.99 |
| DCC7 | 1 (0.6) | 0 (0.0) | >0.99 | 1 (0.4) | 1 (1.4) | 0.41 |
| >1 *Mabs* clone$ | 1 (0.6) | 3 (4.2) | 0.09 | 4 (1.7) | 2 (2.9) | 0.62 |
| Unclustered | 66 (41.8) | 34 (47.9) | 0.39 | 100 (43.7) | 36 (51.4) | 0.27 |

^Non-CF pulmonary and extrapulmonary patients from this study cohort as described in Table 1
‡CF pulmonary patients (159 isolates) obtained from unpublished work, personal communication, Stockwell RE, 2022
*Fisher's exact test (two-tail) used for comparison of proportions, p-value <0.05 was significant
$DCC and/or unclustered mixed infections. Pulmonary non-CF 1 patient: DCC1 and unclustered. CF 3 patients: DCC1, DCC3 and unclustered; DCC1 and unclustered; and DCC6 and unclustered. Extrapulmonary 2 patients: DCC1 and DCC6; unclustered (subspecies *abscessus* and *massiliense*)

## SUMMARY

- The results from this study have contributed to our understanding of the diversity and clonal dispersion of DCCs within non-CF clinical patient isolates in QLD
- *Mycobacterium a. abscessus* is the predominant subspecies in the non-CF isolates
- These results demonstrate that DCCs can be isolated from a range of clinical infections and are not limited to people with CF
- Limited understanding of acquisition pathways and antibiotic resistance

kim.smith@uq.edu.au
Kimberley Smith
uq.edu.au

Faculty of Medicine

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