



**QUEEN'S
UNIVERSITY
BELFAST**

Tropical Australia is a potential reservoir of non-tuberculous mycobacteria in cystic fibrosis

Sherrard, L., Tay, G. T., Butler, C. A., Wood, M. E., Ramsay, K. A., Reid, D. W., Moore, V. L., Kidd, T. J., & Bell, S. C. (2017). Tropical Australia is a potential reservoir of non-tuberculous mycobacteria in cystic fibrosis. *European Respiratory Journal*, 49(5), [1700046]. <https://doi.org/10.1183/13993003.00046-2017>

Published in:
European Respiratory Journal

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights
Copyright ©ERS 2017.
This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Tropical Australia is a potential reservoir of non-tuberculous mycobacteria in cystic fibrosis

Laura J. Sherrard^{1*†} George T. Tay^{2*}, Claire A. Butler², Michelle E. Wood¹⁻³, Stephanie Yerkovich³, Kay A. Ramsay^{1,3}, David W. Reid^{2,4}, Vanessa L. Moore², Timothy J. Kidd^{5,6}, Scott C. Bell¹⁻³

¹Lung Bacteria Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

²Adult Cystic Fibrosis Centre, The Prince Charles Hospital, Brisbane, Queensland, Australia

³School of Medicine, The University of Queensland, Brisbane, Queensland, Australia

⁴Lung Inflammation and Infection Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia.

⁵School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia

⁶Centre for Experimental Medicine, Queen's University Belfast, UK

*These authors contributed equally to this article.

†Address for correspondence: Laura J. Sherrard, QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, Brisbane, QLD 4006, Australia; phone: +61 7 3845 3886; e-mail: Laura.Sherrard@qimrberghofer.edu.au

Take-home message

Living in tropical Australia was associated with NTM acquisition whilst long-term azithromycin was protective in CF.

Word count, 1195

To the editor:

Improved survival rates and increased treatment intensity of people with cystic fibrosis (CF) has been accompanied by the rising incidence of multi-antibiotic resistant and difficult to treat respiratory pathogens, including non-tuberculous mycobacteria (NTM) [1]. NTM epidemiology in CF varies globally with a prevalence of >20% in some geographical locations [2]. In particular, there are concerns that active NTM disease from rapidly growing mycobacteria (*Mycobacterium abscessus* complex) may be increasing and causing accelerated pulmonary decline [3, 4]. NTM are found naturally in ecological niches such as soil and water and susceptible individuals may also acquire infection from potable water in their homes [5, 6]. Recent reports demonstrate person-to-person transmission [7, 8], which might occur *via* fomites and cough aerosols [8] and further emphasise the potential clinical importance of these organisms.

The principle aim of this observational study was to determine predictors of NTM acquisition in the adult CF patient cohort at The Prince Charles Hospital (TPCH) in Queensland, Australia. The study was approved by TPCH Human and Research Ethics Committee, Metro North Hospital and Health Service, Queensland, Australia (HREC/13/QPCH/51).

CF patients ($n=434$) receiving their care at TPCH between 2001-2013 were included. Patients were stratified into one of two cohorts depending on whether they were NTM

positive or negative (naïve) based on sputum culture results, which were available for 399/434 (92%) patients. The remaining patients ($n=35$) who were not screened for NTM during the study period were classed as NTM naïve. Mycobacterial culture and species identification methods are described previously [9]. The date of NTM acquisition was defined as the date of first positive culture identified by the Queensland Mycobacterial Reference Laboratory or the referring CF centre (if patient was transferred to TPCH). Active NTM disease was defined according to American Thoracic Society guidelines [10].

Clinical and demographic data (Table 1) were collected from medical records, the referring CF centre or the Australian CF Data Registry. For NTM positive patients, these data correlated with the date of NTM acquisition. In NTM naïve patients, data corresponded to the calendar year prior to 1st January 2013 or year prior to death ($n=43$) or lung transplantation ($n=72$). Data could not be retrieved for NTM naïve patients, who had not attended the clinic during 2012 (previously moved interstate/overseas or lost to follow-up).

TPCH is uniquely situated geographically with a catchment area of 1.8 million km² including zones north (tropical) and south (sub-tropical) of the Tropic of Capricorn (Latitude 23.5° south of the Equator). Therefore, residential location for each person was defined as tropical or sub-tropical according to residence in the five years prior to (i) the date of NTM acquisition or (ii) 1st January 2013, death ($n=43$), or lung transplantation ($n=72$) if NTM naïve. Patients who resided in both zones during these five years were excluded from further analyses ($n=9$).

Categorical data were analysed using a chi-square test with Yates continuity correction or Fisher's exact test, as appropriate. Univariable and multivariable logistic regression analysis was performed to identify factors associated with NTM acquisition based on complete data from 375 patients. Variables with $P < 0.1$ were included in the multivariable model. Cox regression survival analysis was used to assess the association between NTM acquisition and active NTM disease (both modelled as time dependent covariates) and time to death or lung transplantation (based on the entire CF cohort with all data censored to December 2013). Data were analysed using Stata v14 (StataCorp) or SPSS v22 (IBM).

Spatial clustering of NTM has been reported in the United States with western and southeastern states designated high-risk areas and associated with high surface water and atmospheric moisture availability [11]. In our study, NTM was detected in 54/375 (14%) patients with CF. In a multivariable logistic regression, the odds of NTM acquisition were 2.5 (95% confidence interval, 1.2-5.4) times higher (Table 1) in those who resided in the tropical ($n=19/70$, 27%) compared to the sub-tropical zone ($n=35/305$, 11%).

Factors such as heightened clinician awareness, increased surveillance practices, and improved culture procedures have likely contributed to increased detection and identification of NTM [4, 12]. At TPCH, the percentage of sputum samples screened for NTM increased by >60% between 2001-2013. The mean number of sputum

samples per person that underwent NTM screening was similar between the tropical (2.8 sputum/person/year) and sub-tropical (2.5 sputum/person/year) patients.

Mycobacterial species identification was available for 72/73 (99%) isolates and a quarter of NTM positive patients acquired more than one NTM species during the study period (tropical patients: $n=6/70$, 9%; sub-tropical patients: $n=7/305$, 2%; $P=0.02$) with a range of 1-4 species identified per person, potentially adding to the complexity when selecting treatment. The most commonly identified NTM species were *M. abscessus* complex ($n=33/73$, 45%) and the slowly growing species, *Mycobacterium intracellulare* ($n=23/73$, 32%). Previously, spatial clusters of these NTM species were found in Queensland (*M. abscessus* complex, coastal Whitsunday region, tropical zone; *M. intracellulare*, agricultural Darling Downs region, subtropical zone) [9]. When the primary NTM species acquired was considered for each CF patient, an association between *M. abscessus* complex and geographical zone was observed ($P=0.02$) with a higher recovery from tropical patients ($n=10/70$, 14%) than sub-tropical patients ($n=17/305$, 6%). Conversely, there was no association between *M. intracellulare* acquisition and geographical zone (tropical patients, $n=6/70$, 9%; sub-tropical patients, $n=10/305$, 3%; $P=0.09$). Further studies are required to determine the specific regions within each zone, which are high risk for acquisition of a particular NTM species in CF. Other rapidly growing species identified were *Mycobacterium chelonae* ($n=2$) and *Mycobacterium fortuitum* ($n=3$) and the remaining slowly growing species included *Mycobacterium avium* ($n=6$), *Mycobacterium scrofulaceum* ($n=2$), *Mycobacterium lentiflavum* ($n=1$), *Mycobacterium simiae* ($n=1$) and *Mycobacterium shimoidei* ($n=1$).

NTM lung disease was reported to occur in 69% of *M. abscessus* infected CF patients [4]. Here, active NTM disease was diagnosed in 14/70 (20%) tropical patients and 11/305 (4%) sub-tropical patients ($P<0.001$) and overall, 23/25 (92%) people diagnosed with lung disease were positive for *M. abscessus* complex.

Markers for severe CF disease (e.g. pancreatic insufficiency, minimal CFTR function, *P. aeruginosa* infection) have also been reported to coincide with higher rates of NTM infection [4]. Whilst we did not find that such markers were associated with NTM acquisition (Table 1) after adjustment for other variables, we found that macrolide treatment for at least 6 months in the prior year was associated with reduced NTM acquisition. The adjusted odds of NTM acquisition was 15.4 (95% confidence interval, 7.8-30.5) times higher in those who were not prescribed azithromycin compared to those that were (Table 1). The role of macrolide antibiotic use in NTM infection remains controversial with one study suggesting enhanced risk of NTM and others refuting this claim [13-15].

The impact of NTM infection and patient survival is currently unclear. One study observed that a quarter of patients with *M. abscessus* complex died or were lung transplanted [12]. Here, neither being NTM positive (hazards ratio, 0.6; 95% confidence interval, 0.2-1.4; $P=0.2$) nor being diagnosed with active NTM disease (hazards ratio, 0.6; 95% confidence interval, 0.2-2.6; $P=0.5$) were associated with an increased risk of death/lung transplantation.

Although this study is limited by its retrospective cross-sectional design, we found that living in the tropical zone of Australia was independently associated with NTM

acquisition whilst long-term azithromycin treatment was protective. Tropical patients were more likely to acquire more than one NTM species and have a diagnosis of active NTM disease. Australian population-based studies are required to determine specific host, socio-ecological, economic, climatic and environmental factors that affect acquisition and development of active NTM disease in CF.

References

1. Cystic Fibrosis Foundation Patient Registry, 2014 Annual Data Report, Bethesda, Maryland. ©2015 Cystic Fibrosis Foundation.
2. Adjemian J, Olivier KN, Prevots DR. Nontuberculous mycobacteria among patients with cystic fibrosis in the United States: screening practices and environmental risk. *Am J Respir Crit Care Med* 2014; 190: 581-586.
3. Esther CR, Jr., Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. *J Cyst Fibros* 2010; 9: 117-123.
4. Bar-On O, Mussaffi H, Mei-Zahav M, Prais D, Steuer G, Staffler P, Hananya S, Blau H. Increasing nontuberculous mycobacteria infection in cystic fibrosis. *J Cyst Fibros* 2015; 14: 53-62.
5. Thomson R, Tolson C, Carter R, Coulter C, Huygens F, Hargreaves M. Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM. *J Clin Microbiol* 2013; 51(9): 3006-3011.
6. Prevots DR, Adjemian J, Fernandez AG, Knowles MR, Olivier KN. Environmental risks for nontuberculous mycobacteria. Individual exposures and

climatic factors in the cystic fibrosis population. *Ann Am Thorac Soc* 2014; 11: 1032-1038.

7. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, Inns T, Reacher M, Haworth CS, Curran MD, Harris SR, Peacock SJ, Parkhill J, Floto RA. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 2013; 381: 1551-1560.

8. Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, Verma D, Hill E, Drijkoningen J, Gilligan P, Esther CR, Noone PG, Giddings O, Bell SC, Thomson R, Wainwright CE, Coulter C, Pandey S, Wood ME, Stockwell RE, Ramsay KA, Sherrard LJ, Kidd TJ, Jabbour N, Johnson GR, Knibbs LD, Morawska L, Sly PD, Jones A, Bilton D, Laurenson I, Ruddy M, Bourke S, Bowler IC, Chapman SJ, Clayton A, Cullen M, Dempsey O, Denton M, Desai M, Drew RJ, Edenborough F, Evans J, Folb J, Daniels T, Humphrey H, Isalska B, Jensen-Fangel S, Jonsson B, Jones AM, Katzenstein TL, Lillebaek T, MacGregor G, Mayell S, Millar M, Modha D, Nash EF, O'Brien C, O'Brien D, Ohri C, Pao CS, Peckham D, Perrin F, Perry A, Pressler T, Prtak L, Qvist T, Robb A, Rodgers H, Schaffer K, Shafi N, van Ingen J, Walshaw M, Watson D, West N, Whitehouse J, Haworth CS, Harris SR, Ordway D, Parkhill J, Floto RA. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 2016; 354: 751-757.

9. Chou MP, Clements AC, Thomson RM. A spatial epidemiological analysis of nontuberculous mycobacterial infections in Queensland, Australia. *BMC Infect Dis* 2014; 14: 279.

10. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von

Reyn CF, Wallace RJ, Jr., Winthrop K, Subcommittee ATSM, American Thoracic S, Infectious Disease Society of A. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; 175: 367-416.

11. Adjemian J, Olivier KN, Seitz AE, Falkinham JO, 3rd, Holland SM, Prevots DR. Spatial clusters of nontuberculous mycobacterial lung disease in the United States. *Am J Respir Crit Care Med* 2012; 186: 553-558.

12. Qvist T, Gilljam M, Jonsson B, Taylor-Robinson D, Jensen-Fangel S, Wang M, Svahn A, Kotz K, Hansson L, Hollsing A, Hansen CR, Finstad PL, Pressler T, Hoiby N, Katzenstein TL, Scandinavian Cystic Fibrosis Study C. Epidemiology of nontuberculous mycobacteria among patients with cystic fibrosis in Scandinavia. *J Cyst Fibros* 2015; 14: 46-52.

13. Renna M, Schaffner C, Brown K, Shang S, Tamayo MH, Hegyi K, Grimsey NJ, Cusens D, Coulter S, Cooper J, Bowden AR, Newton SM, Kampmann B, Helm J, Jones A, Haworth CS, Basaraba RJ, DeGroote MA, Ordway DJ, Rubinsztein DC, Floto RA. Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection. *J Clin Invest* 2011; 121: 3554-3563.

14. Coolen N, Morand P, Martin C, Hubert D, Kanaan R, Chapron J, Honore I, Dusser D, Audureau E, Veziris N, Burgel PR. Reduced risk of nontuberculous mycobacteria in cystic fibrosis adults receiving long-term azithromycin. *J Cyst Fibros* 2015; 14: 594-599.

15. Binder AM, Adjemian J, Olivier KN, Prevots DR. Epidemiology of nontuberculous mycobacterial infections and associated chronic macrolide use among persons with cystic fibrosis. *Am J Respir Crit Care Med* 2013; 188: 807-812.

TABLE 1. Logistic regression analysis of predictor variables associated with NTM acquisition ($n=375$).

Univariable model	Odds ratio (95% CI)	P-value
Age (per 10 years)	0.7 (0.5-0.9)	0.02
Gender	1.0 (0.6-1.8)	1.0
<i>CFTR function</i> [‡]		
- Residual versus minimal	1.3 (0.5-3.8)	0.6
- Non-classified versus minimal	0.6 (0.3-1.2)	0.2
Tropical residence in the previous 5 years	2.9 (1.5-5.4)	0.001
Chronic <i>Pseudomonas aeruginosa</i> infection	0.6 (0.3-1.4)	0.2
FEV ₁ % predicted (per 10% increase)	1.2 (1.1-1.4)	<0.001
Pancreatic sufficiency status	0.5 (0.2-1.0)	0.04
Not prescribed azithromycin >6 months in the previous year	15.7 (8.1-30.6)	<0.001
Hospital admissions in the previous year (per 5 admissions)	0.3 (0.1-0.7)	0.003
Hospital days in the previous year (per 10 hospital days)	0.8 (0.7-0.9)	0.002
Multivariable model*	Odds ratio (95% CI)	P-value
Age (per 10 years)	0.7 (0.5-1.0)	0.03
Tropical residence in the previous 5 years	2.5 (1.2-5.4)	0.02
Not prescribed azithromycin for >6 months in the previous year	15.4 (7.8-30.5)	<0.001

CFTR, cystic fibrosis transmembrane conductance regulator; FEV₁ % predicted, forced expiratory volume in the first second percentage predicted; CI, confidence interval.

* Final model shows predictor variables that retained a significant association with NTM acquisition after adjustment for all other factors.

[‡] Definitions of CFTR function: Residual function, harbouring ≥ 1 allele with Class IV-V mutations; Minimal function, harbouring two alleles with Class I-III mutations; Non-classified, harbouring two alleles with mutations of unknown function.