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NTM Australian Research Network

Symposium PROGRAM

Thursday 16 and Friday 17 November, 2023
Mayne Events, UQ Medical School, Herston, Brisbane, QLD

NTM Symposium 2023 Program

Thursday 16 November 2023 – Clinical Focus

ES Meyers Lecture Theatre, Level 4 Mayne Medical School

8:00am	REGISTRATION	
8:30am	Welcome and Patient Perspective	Rachel Thomson
8:45am	Natural History of NTM Infections	Michael Holt
9:05am	Radiology of NTM Lung Disease – Latest Developments	Taryn Reddy
9:25am	Laboratory Essentials and New Developments	Chris Coulter
9:45am	Q&A Panel Discussion	Malcolm Wilson
10:05am	MORNING TEA	
10:35am	Host Susceptibility to NTM Pulmonary Disease	Ken Olivier
10:55am	NTM in Cystic Fibrosis	Ieuan Evans
11:15am	Environment and Reducing Exposure	Rachel Thomson
11:35am	Assessing QoL with NTM Treatment/PROs	Tim Baird
11:55am	Q&A Panel Discussion	Scott Bell
12:15am	LUNCH	
1:15pm	ARISE/ENCORE Clinical Program Design and Progress	Marie-Laure Nevoret
1:25pm	Update on Management of *M. avium complex* Infection	Malcolm Wilson
1:45pm	Update on Management of *M. abscessus* Infection	Andrew Burke
2:05pm	Approach to Less Common NTM	Ted Marras
2:25pm	Monitoring and Management of Drug Toxicity	Vicky Grey
2:45pm	Q&A Panel Discussion	Claire Wainwright
3:00pm	AFTERNOON TEA & POSTER VIEWING (#1 – 5)	
3:30pm	Epetraborole in NTM Lung Disease	David Clarke
3:40pm	Case Discussions	Tim Baird
5:00pm	NETWORKING AND DRINKS	
6:30pm-10:00pm	DINNER AT VICTORIA PARK, ALABASTER ROOM Fireside Chat "NTM disease – where have we come from and where are we going to?"	MC: Scott Bell

Notes

Thursday 16 November 2023 – Patient Forum (Satellite Session)

0887-113 Seminar Room, Public Health Building

9:00am	REGISTRATION	
9:15am	Welcome	Justine Gibson
9:30am	To Treat or not to Treat? Natural History & Long Term Outcomes	Justine Gibson
10:00am	Physiotherapy Management 101 of NTM & Bronchiectasis	Kathleen Hall, Libby Yap
10:30am	Nutritional Considerations for NTM	Brianna McCarthy
11:00am	MORNING TEA & SUBMIT ANONYMOUS QS	
11:30am	Coping with Chronic Illness	Vanessa Smith
12:00pm	Should I Enrol in a Clinical Trial?	Preethi Guru
12:20pm	Current Clinical Trials	Andrew Burke
12:40pm	Reducing Exposure	Rachel Thomson
1:00pm	Q&A Panel Discussion	Kathleen Hall
1:30pm	LUNCH	

Notes

Friday 17 November 2023 – Research Focus

ES Meyers Lecture Theatre, Level 4 Mayne Medical School

8:00am	REGISTRATION	
8:20am	Welcome	Andrew Burke
	EPIDEMIOLOGY	
8:30am	*M. avium* Evolution and Transmission	Ted Marras
8:50am	*M. abscessus* Evolution and Transmission	Rachel Thomson
9:10am	*M. abscessus* Outbreak in QLD Prisons	Chris Coulter
9:30am	*Selected abstracts:* Mycobacteria Acquisition from Potable Water	Kay Ramsay
9:50am	*Selected abstracts:* NTM in Australians with Cystic Fibrosis: A National Study	Christine Duplancic
10:00am	Q&A Panel Discussion	Rachel Thomson
10:15am	MORNING TEA & POSTER VIEWING (#6 – 9)	
	MICROBIOLOGY & MICROBIOME, BIOMARKERS & DIAGNOSTICS	
10:45am	Microbiome in NTM	Steven Taylor
11:05am	Investigation of the Gut-Lung in TB – Translations for NTM	Antje Blumenthal
11:25am	Biomarkers and Novel Diagnostics	Julia Bashford
11:45am	*Selected abstracts:* Assessing the Efficacy and Mechanisms of Disinfectants on *M. abscessus*	Casey Huang
11:55am	*Selected abstracts:* Laboratory Evolution of Imipenem Resistance in a *M. abscessus* Water Isolate	Kirby Fahy
12:05pm	*Selected abstracts:* Oral-bait BCG Vaccination of Possums May Address the Worsening Victorian Epidemic of Buruli Ulcer	Dan O'Brien
12:15pm	Q&A Panel Discussion	Geoffrey Eather
12:30pm	LUNCH	
	HOST SUSCEPTIBILITY & NOVEL THERAPIES	
1:30pm	Host Directed Therapies	Ken Olivier
1:50pm	Emerging Antimicrobials	Andrew Burke
2:10pm	Phage Therapy for *M. abscessus*	Ameneh Khatami
2:30pm	*Selected abstracts:* Concurrent Treatment of *M. kansasii* Pulmonary Disease and PD-L1 Immunotherapy for Metastatic Non-Small Cell Lung Cancer	Richard Turner
2:40pm	Q&A Panel Discussion	Ieuan Evans
3:00pm	AFTERNOON TEA	
	NTM PHARMACOLOGY	
3:30pm	Pharmacokinetics and the Importance of Dosing Considerations	Andrew Burke
3:40pm	Amikacin Dose Optimisation in NTM	Amy Legg
4:00pm	Linezolid Dose Optimisation in NTM	Cindy Lau
4:20pm	Home Intravenous Therapy for NTM Infection	Zack Klyza
4:40pm	Discussion and Closing Remarks	Andrew Burke

Poster Abstracts

#	Title	Author
1	Case Report: The Turtle's Accomplice – Collaborative Management of *M. chelonae* Skin Infection in an Elderly Immunosuppressed Patient with Rheumatoid Arthritis on Baricitinib	Helen Baxter
2	Recurrent Disseminated *M. avium complex* Infection in a Patient with Anti-Interferon-Gamma Autoantibody Syndrome	Chris Wong
3	Disseminated NTM infection in the context of a new diagnosis of Interferon-Gamma Autoantibody Syndrome	Victoria Jordan
4	A Method for the Isolation of Environmental Mycobacterium Species from Soil and House Dust	Robyn Carter
5	The Comparison of Culture-Based Methodologies for the Isolation of NTM from Potable Water Samples	Kay Ramsay
6	The Mycobacterial Disease Biobank	Felicia Goh
7	The Isolation of *M. intracellulare* from Soils and Household Dust	Robyn Carter
8	Disseminated *M. abscessus* Infection. An Unexpected Complication Following Trauma Laparotomy	Sebastian Primrose
9	*M. abscessus* Infection Complicating Coronary Stent Causing Massive Coronary Pseudoaneurysm in a 39-Year-Old Male of Nepalese Background	Nicky Betts

NTM Forum Organising Committee

Professor Rachel Thomson

Respiratory Research Unit Head

Professor Rachel Thomson is a Thoracic Physician and clinical researcher working at Greenslopes Private Hospital and the Gallipoli Medical Research Foundation. Prof Thomson has an international reputation for her research into lung disease due to NTM. She is at the forefront of research and treatment of patients with NTM infection.

Dr Andrew Burke

Respiratory and infectious diseases physician at The Prince Charles Hospital, Brisbane.

He is an investigator on multiple NTM drug trials and is undertaking research at the University of Queensland Centre for Clinical Research with a focus on pharmacokinetics and novel treatment strategies in NTM lung disease.

Felicia Goh, BSc, PhD

Senior Research Officer, UQ, Brisbane, Australia

Dr Felicia Goh is a Senior Research Officer with the Greenslopes Clinical Unit and Gallipoli Medical Research Foundation based at Greenslopes Private Hospital, Brisbane. She obtained her PhD in Immunology at the Institute for Molecular Biosciences, University of Queensland and CSIRO and has over 10 years' experience in managing human biobanks. Currently, she is the biobank coordinator for the Mycobacterial Biobank, a multi-site collaboration between the University of Queensland and various Health Services in Brisbane, and a project manager for the FORMaT clinical trial.

Alice Sawka, MBBS, MPH, FRACP

Respiratory Specialist, Royal Adelaide Hospital, Adelaide, Australia

Dr Alice Sawka currently works at the Royal Adelaide Hospital as a respiratory specialist with particular interest in NTM pulmonary disease, and a tuberculosis consultant with the SA Tuberculosis Service. In 2021, she undertook a clinical fellowship in the diagnosis and management of mycobacterial disease at the Princess Alexandra Hospital in Brisbane. She is an associate investigator with current clinical trials that seek to improve treatment of patients with NTM-pulmonary disease including the ENCORE, ARISE and FORMaT studies. Dr Sawka is an associate clinical lecturer at the University of Adelaide and holds a Master of Public Health from Monash University.

Scott Bell, MBBS, MD, FRACP

Professor and Thoracic Physician, The Prince Charles Hospital, Brisbane, Australia

Scott Bell is the Chief Executive of the Translational Research Institute and a Senior Physician of the Adult Cystic Fibrosis Centre at The Prince Charles Hospital where he has worked since 1996 in Brisbane. As a clinician scientist, he leads the Lung Bacteria Laboratory at UQ. Scott has >290 peer reviewed publications and has received grant support >$24 million. His research interests include acquisition and transmission pathways for human infection and his multi-disciplinary research has resulted in significant changes to clinical practice and policy implementation globally. He has been principal investigator on numerous pivotal CFTR modulator trials. He was the Editor of the Journal of Cystic Fibrosis from 2013 until 2020.

Geoffrey Eather, MBBS, FRACP

Respiratory Physician, Princess Alexandra Hospital and Acting Deputy Director, Metro South Clinical Tuberculosis Service, Brisbane, Australia

Dr Geoff Eather is a respiratory, sleep and mycobacterial diseases physician based at the Princess Alexandra Hospital Department of Respiratory Medicine and is the current acting Deputy Director of the Metro South Clinical Tuberculosis Service. His main research interest includes understanding transmission dynamics of TB focussing on developing a better understanding of diagnostic testing in the immune compromised.

Malcolm Wilson, BSc, MBBS, FRACP

Respiratory Specialist, Metro South Clinical Tuberculosis Service and Logan Hospital, Brisbane, Australia

Dr Malcolm Wilson is a specialist in Respiratory & Sleep Medicine practising between the Metro South Clinical Tuberculosis Service and Logan Hospital in Southeast Queensland, Australia. He completed a Fellowship in Respiratory Mycobacterial Disease in 2018 and remains actively involved in clinical research focused on improving diagnostic tools and therapeutic options in mycobacterial disease.

Michael Holt, BSc, MBBS, FRACP

Respiratory Physician, Royal Brisbane and Women's Hospital and Greenslopes Private Hospital, Brisbane, Australia

Michael is a respiratory and sleep physician, with subspecialty interest in bronchiectasis and mycobacterial lung disease. He was awarded Fellowship of the RACP in 2014, after completing respiratory training at The Prince Charles Hospital and a sleep medicine fellowship at Royal Brisbane and Women's Hospital. In 2015, Michael commenced his sub-specialty training with the inaugural Mycobacterial Fellowship at Princess Alexandra Hospital. He subsequently undertook further training as the Lowerre Fellow at National Jewish Health in Denver.

Tim Baird, BMSc, MBChB, MRCP(UK), PhD

Respiratory Physician, Sunshine Coast University Private Hospital, Australia

Tim graduated from the University of Queensland, completed a Diploma of Tropical Medicine in the United Kingdom and volunteered as a medical officer in rural Papua New Guinea. He then underwent an international fellowship at the Royal Papworth Hospital in Cambridge, UK, where he developed further skills in complex lung infections, bronchiectasis and cystic fibrosis. Tim holds a staff specialist position at the Sunshine Coast University Hospital and consults privately at the Sunshine Coast University Private Hospital and Buderim Private Hospital. He remains actively involved in research and is a current member of the Thoracic Society of Australia and New Zealand, The European Respiratory Society, the Australasian Sleep Association, and the Lung Foundation of Australia.

Ieuan Evans, MRCP, PhD

Specialist Trainee in Respiratory Medicine, Royal Papworth Hospital, UK

Ieuan is a Respiratory and Cystic Fibrosis physician, with a subspeciality interest in NTM and complex lung infection. He completed his fellowship at the Centre for Complex Lung Infection (CCLI), Royal Papworth Hospital, UK between 2017-2020, before obtaining his PhD in Medicine from the University of Cambridge titled 'Developing novel therapies for *M. abscessus*' in August 2021. Research from his PhD has led to the launch of a Phase II clinical trial – RESP301 (nebulised nitric oxide generating solution) in patients with treatment naïve or refractory *M. abscessus* infection current running at the Royal Papworth Hospital. Ieuan holds a consultant position at The Prince Charles Hospital, Brisbane and remains actively involved in research in Cystic Fibrosis and NTM disease.

Claire Wainwright, MBBS, MRCP, FRACP, MD

Paediatric Respiratory Physician and co-lead for Cystic Fibrosis Services, Queensland Children's Hospital, Brisbane, Australia

Professor Claire Wainwright is a paediatric respiratory physician and co-lead for cystic fibrosis services at the Queensland Children's Hospital, and a professor of paediatrics and child health at The University of Queensland. She started her medical and paediatric training in London and completed her training in paediatric respiratory medicine and doctoral studies at the royal children's hospitals in Brisbane and Melbourne. Her research focuses on airway microbiology, paediatric respiratory infections and early lung disease in cystic fibrosis, and ataxia telangiectasia and the clinical management of bronchiolitis and asthma. In 2018, Professor Wainwright was awarded a Member of the Order of Australia (AM) for her significant service to medicine as a respiratory clinician, and for leadership into the study of cystic fibrosis.

International Keynote Speakers

Theodore K. Marras, MD, FRCPC, MSc

Associate Professor of Medicine, University of Toronto, Respirology Consultant and Director, NTM Disease Program, Toronto Western Hospital, University Health Network, Canada

Dr Marras received his M.D. at Queen's University (Kingston, Canada), clinical training in Internal Medicine and Respirology (FRCPC) at University of Toronto, and M.Sc. in Clinical Epidemiology at University of Toronto. He took advanced training in mycobacterial diseases at University of California, San Francisco, with electives at National Jewish Health, Denver Colorado, and Stanford University, Palo Alto, California. Much of his research has focused on population-based epidemiological aspects of NTM lung disease. He is a co-author of the American Thoracic Society, Infectious Diseases Society of America, European Respiratory Society, European Society of Clinical Microbiology and Infectious Disease NTM treatment guidelines.

Kenneth N. Olivier, MD, MPH

Professor of Medicine and Director, Bronchiectasis/NTM Care and Research Center, University of North Carolina, USA

Dr Olivier is the Michael E Hatcher Professor of Medicine and Director of the UNC Bronchiectasis/NTM Care and Research Center at the University of North Carolina at Chapel Hill. His research is focused on pathogenesis and population characteristics of bronchiectasis and chronic airway infection such as NTM and he has been actively engaged in the therapeutics development in these areas.

Speakers

Christopher Coulter, MBBS, FRACP, FRCPA

Director of Queensland Mycobacterium Reference Laboratory, Pathology Queensland Central Laboratory, Brisbane, Australia

Dr Chris Coulter is medical microbiologist and infectious diseases physician. He is Director of the Qld Mycobacterium Reference Laboratory, (Pathology Queensland) a World Health Organization (WHO) Collaborating Centre and SupraNational Reference Laboratory. He is medical lead for the Qld TB programme (Communicable Diseases branch, Dept of Health) and maintains clinical work in the area of mycobacterial diseases. He has published on both TB and NTM diseases and has served on various WHO expert groups regarding molecular diagnosis of TB, the setting of critical concentrations for new and repurposed anti-TB drugs and PkPd assessment of second line agents.

Steven Taylor, PhD

Group Head, South Australian Health and Medical Research Institute, Adelaide, Australia, Adelaide, Australia

Steven is an NHMRC Emerging Leader Investigator Fellow and heads the Respiratory Health Group within the Microbiome and Host Health Programme at SAHMRI. His background combines mucosal immunology, microbiology, and bioinformatics, which allows him to explore the contribution of airway microbiology to disease onset, progression and treatment efficacy in people with chronic airway disease.

Ameneh Khatami, MBChB, MD, FRACP

Senior Lecturer and NHMRC EL1 Research Fellow, University of Sydney, and Paediatric ID Specialist, The Children's Hospital at Westmead, Sydney, Australia

Ameneh's research background is in clinical vaccine trials with the Oxford Vaccine Group, but her current interests include novel and precision therapeutic options for difficult to treat infections, including for patients with cystic fibrosis, in particular phage therapy. Ameneh is Deputy Director (Clinical) of Phage Australia, and she is the Phage Therapy Content Expert for the Sydney Children's Hospitals Network (SCHN) Advanced Therapeutics Steering Committee. Ameneh has overseen phage treatment in 8 children in NSW for a variety of conditions including NTM infections in patients with cystic fibrosis and immune compromise.

Cindy Lau

Antimicrobial Stewardship Pharmacist at St Vincent's Hospital, Sydney, Australia

Cindy is the AMS pharmacist at St Vincent's Hospital, Sydney. Her interests include clinical implementation of antimicrobial therapeutic drug monitoring, and infections in immunocompromised hosts.

Julia Bashford, MBBS, FRACP

Sunshine Coast University Hospital, Brisbane, Australia

Julia Bashford has previously published on the role of serology in the diagnosis of NTM pulmonary disease. She is a Sunshine Coast-based respiratory physician with a clinical and research interest in bronchiectasis and chronic pulmonary infection.

Taryn Reddy, MBChB, FRANZCR

Medical Imaging Department, The Prince Charles Hospital and Senior Lecturer, University of Queensland, Brisbane, Australia

Dr Reddy is a dual trained thoracic radiologist who has completed Cardiothoracic Imaging fellowships in Christchurch, NZ and Vancouver, Canada. She has been working as a thoracic radiologist at The Prince Charles Hospital since 2013.

Victoria Grey, MBChB, MPH

Infectious Diseases Advanced Trainee, Princess Alexandra Hospital and Metro South Tuberculosis Service, Brisbane, Australia

Victoria is in her final year of infectious diseases advanced training and is currently undertaking a fellowship in mycobacterial disease at the Princess Alexandra Hospital and the Prince Charles Hospital. She received her medical degree from the University of Birmingham and holds a Master in Public Health from Griffith University.

Antje Blumenthal, Dr rer. nat., Dipl-Biol

Frazer Institute, University of Queensland, Brisbane, Australia

Antje Blumenthal is an ARC Future Fellow and leads the Infection & Inflammation Group at The University of Queensland Frazer Institute. Leveraging her expertise in microbiology and immunology, Professor Blumenthal's goal is to improve treatment options for severe bacterial infections, with a specific focus on tuberculosis and sepsis. Professor Blumenthal has been recognised for her contributions to the scientific community through leadership roles within the University and professional societies. She is a Fellow of the Australian Society for Microbiology and twice elected member of the national council of the Australian and New Zealand Society for Immunology.

Amy Legg

Senior AMS pharmacist, Brisbane, Australia

Amy Legg is an experienced clinical pharmacist in infectious diseases and antimicrobial stewardship. She is current undertaking a PhD with Menzies School Of Health Research. Her research interests include urinary biomarkers for diagnosis of AKI, and antimicrobial therapeutic drug monitoring to ensure efficacy and safety.

Zackary Klyza

Infectious Diseases Pharmacist Team Leader, Princess Alexandra Hospital, Brisbane, Australia

Zack is a senior pharmacist at the Princess Alexandra Hospital (PAH). His role involves: clinical management of Infectious Diseases patients, the Metro South antimicrobial prescribing guidelines, the PAH antimicrobial stewardship program and aseptic manufacturing of outpatient parenteral antimicrobial therapy for patients across the Metro South district.

Kathleen Hall

Respiratory Physiotherapy Specialist, Brisbane, Australia

Kathleen Hall has over 25 years' experience as a cardiorespiratory physiotherapist, with a special interest in the management of Bronchiectasis. She runs a bespoke private practice for clients with NTM & other Bronchiectasis conditions, is the lecturer in charge - Cardiorespiratory physiotherapy programme at ACU & works in the Adult CF/Thoracic Medicine units at PCH.

Libby Yap

Cardiorespiratory Physiotherapist, Pulmedica, Brisbane, Australia

Libby Yap is a Cardiorespiratory Physiotherapist who has had a long-term interest and involvement in the care and support for people with Respiratory conditions. She completed her Bachelor of Physiotherapy (UQ) in 1980 and Certificate in Chronic Disease Self-Management (Flinders Uni) in 2009. Libby has a special interest in teaching evidence-based airway clearance techniques and other appropriate measures that will ensure a thorough understanding by the patients (and their carers, as appropriate) to perform ongoing supported self-management.

PHOTO NOT PROVIDED

Brianna McCarthy, APD

A/Senior Dietitian, The Prince Charles Hospital, Brisbane, Australia

Brianna McCarthy is an Accredited Practicing Dietitian at The Prince Charles Hospital with 5 years of clinical experience. She has a keen interest in the nutrition care of patients with respiratory conditions and is currently working in the Adult Cystic Fibrosis Centre and Queensland Lung Transplant Service.

Preethi Guru, BSc, MSc – Medical Microbiology

Clinical Trial Coordinator, Gallipoli Medical Research Foundation, Brisbane, Australia

Preethi is a clinical trial coordinator at GMRF's Clinical Trials Unit, specialising in Respiratory trials. She has 11 years of clinical trial experience, working in Phase I – III/IV trials. Her current interest is in NTM infection and other Bronchiectasis conditions.

Vanessa Smith, BA (Hons), MPsych (Clin)

Clinical Psychologist, Mater Hospital, Brisbane, Australia

Vanessa Smith is a Clinical Psychologist who works within the adults Psychology Service at the Mater Hospital. She works with patients experiencing a range of chronic health conditions and recognises that this can impact people in differing and significant ways. She provides holistic patient-centred care to support the emotional, physical and functional impacts of living with a chronic illness in order to improve coping and enhance quality of life.

Speakers – Selected Oral Abstracts

EPIDEMIOLOGY

Nontuberculous mycobacteria (NTM) colonisation of hospital water sources

Ramsay, KA[1,2], Smith, KJ[1,2], Carter, R[1,2], Huang, CK[3,4], Smith, W[5], Ahmed, W[5], Clayton, M[1,2], Guo, J[3], Thomson, RM[2,6], Bell, SC[1,7,8]

1Child Health Research Centre, Faculty of Medicine, The University of Queensland, 2Respiratory Research Unit, Gallipoli Medical Research Foundation,
3Australian Centre for Water and Environmental Biotechnology, The University of Queensland,
4Queensland Alliance for Environmental Health Sciences, The University of Queensland,
5CSIRO Environment, Ecosciences Precinct
6Greenslopes Clinical Unit, The University of Queensland,
7Adult Cystic Fibrosis Centre, The Prince Charles Hospital,
8Translational Research Institute, Australia

Background:
NTM are environmental bacteria which can be readily isolated from water sources, as such acquisition from water could be an infection risk to vulnerable individuals. This work highlights the diversity and degree to which NTM colonises potable water within the hospital setting, leading to question if hospitals are a source of nosocomially acquired infections. Individual hospitals are now investing in in-house water decontamination to reduce bacterial colonisation within their plumbing systems.
Methods:
Water was collected and cultured from three hospitals (Hospital 1: adult; Hospital 2: paediatric; Hospital 3: adult) over a two-year period. Unlike Hospitals 1 and 3, Hospital 2 has a water management plan, providing in-house chlorination of the plumbing system when required. Samples were collected from taps and showers in respiratory, infectious disease, oncology and coronary care wards every six months. One litre water collections were aliquoted into two samples, neat and decontaminated. Each sample was inoculated onto a range of solid and liquid media and incubated aerobically at 35°C for up to 12 weeks. Acid fast bacilli were identified by Ziehl Nielsen staining and species determined by MALDI-TOF mass spectrometry. In addition to culturing, samples for biofilm and water were collected during the final collection at each hospital for molecular biology analyses. Biofilms were collected by swabbing the insides of each tap and the surface of the showerheads. DNA was extracted from the water and biofilm swabs samples for qPCR with the *Mycobacterium* genus and *M. abscessus* as targets.
Results:
A total of 200 water samples were cultured and screened for the presence of NTM. Over the four collection time points, NTM were isolated from every tap at least once, from Hospital 1 and 3 and 83% of the samples from Hospital 2. The most prevalent species identified were *M. gordonae* (37.2%) and *M. mucogenicum* (18.2%). *M. abscessus* was isolated from six, two and five taps from Hospital 1, 2 and 3, respectively. The two adult hospitals (Hospital 1 and 3) had the greatest diversity of Mycobacteria species, with six and seven species respectively. This was in contrast to Hospital 2 with only three species identified. However, this site was predominantly colonised with *M. gordonae* and *M. kansasii*. The qPCR analyses of the water and biofilm swab DNA samples for the final collection are ongoing and results are pending.
Conclusion:
In-house chlorine dosing when concentrations fall below recommended levels by Hospital 2 may contribute to reduced prevalence and species diversity seen in their water samples. NTM species, both those considered to be environmental and pathogenic, were isolated from taps within these hospitals. This work highlights the need for awareness and the implementation of mitigation techniques to reduce NTM exposure.

Mycobacteria acquisition from potable water (MAP) study: home water collections

Ramsay, KA[1,2], Smith, KJ[1,2], Carter, R[1,2], Clayton, M[1,2], Thomson, RM[2,3], Bell, SC[1,4,5]

1Child Health Research Centre, Faculty of Medicine, The University of Queensland
2Respiratory Research Unit, Gallipoli Medical Research Foundation
3Greenslopes Clinical Unit, The University of Queensland
4Adult Cystic Fibrosis Centre, The Prince Charles Hospital
5Translational Research Institute, Australia

Background:
Nontuberculous mycobacteria (NTM) are common environmental organisms and have been isolated from potable water sources. Studies have suggested that the water may be a source of infections. The MAP study aims to explore the relationship between clinical and water derived *Mycobacteria abscessus* (Mabs) isolates to better understand the role of exposure from common water sources in the disease process.
Materials and Methods:
Between July 2020 and September 2023 water samples have been collected from the homes of 50 participants with existing Mabs airway infection. To date water sampling and culture have been completed from 44 participants residing in 47 homes. One litre water collections were aliquoted into two samples, one decontaminated with 0.005% cetylpyridinium chloride, and one neat sample. This was then concentrated by filtration and used to inoculate solid agar (7H11 Middlebrook agar) and liquid growth media tubes (Mycobacteria Growth Indicator Tube*;* MGIT). Both MGITs were inoculated with a growth supplement to promote NTM growth and one was further treated with an antimicrobial cocktail to prevent bacterial and fungal overgrowth. The six different cultures, for each water sample, were incubated aerobically at 35°C for up to 12 weeks. Acid fast bacilli were identified by Ziehl Nielsen staining and species determined by Matrix-assisted laser desorption/ionization time of flight mass spectrometry. Whole genome sequencing (WGS) of the Mabs isolated from the water samples will be compared to those from clinical samples and bioinformatic analysis undertaken to assess relatedness.
Results:
Fifty participants have been enrolled to date: 43 adults and seven children recruited from the FOR*Ma*T trial (n=33), National NTM study (n=1) and non-trial participants (n=16). From each participant home included in the analysis (n=47), water was sampled from the kitchen sink (n=48), showerhead (n=48) and the bathroom handbasin (n=52). Additional sites from within and around the house also sampled include filtered water sources (n=24), bathtubs (n=4), plumbed-in-fridges (n=4), pools (n=5), laundry (n=2), kettle (n=1), CPAP (n=1) and outdoor water sources (n=11). Mabs was isolated from 24 homes, from different water sources including shower (n=11), handbasin (n=9), kitchen sink (n=8), water filters (n=10), pools (n=5), rainwater tank (n=2), and plumbed-in-fridges (n=2). Other species identified from the home water samples include *M. gordonae* (31 homes), *M. mucogenicum* (23), *M. lentiflavum* (21), *M. kansasii* (11), *M. chelonae* (10), *M. fortuitum* (4), *M. szulgai* (3) *M. intracellulare* (2), *M. simiae* (1) *and M. immunogenum* (1). WGS of both clinical and environmental Mabs isolates are currently underway.
Discussion:
Mabs has been isolated from a significant number of patient homes (51%) to date. WGS comparison of water and clinical Mabs isolates will enhance understanding of acquisition routes. Currently, mitigation techniques to reduce NTM exposure within the home should be explored as a means of infection/reinfection prevention.

Prevalence and diversity of NTM within two drinking water distribution systems (DWDS)

Ramsay, KA[1,2], Smith, KS[1,2], Carter, R[1,2], Clayton, M[1,2], Thomson, RM[2,3], Bell, SC[1,4,5]

1Child Health Research Centre, Faculty of Medicine, The University of Queensland
2Respiratory Research Unit, Gallipoli Medical Research Foundation
3Greenslopes Clinical Unit, The University of Queensland
4Adult Cystic Fibrosis Centre, The Prince Charles Hospital
5Translational Research Institute, Australia

Background:
Water within the DWDS is chlorinated to deter the growth of contaminating, disease-causing organisms from the environment. Here, we investigate if the current water treatment is sufficient to prevent the growth of nontuberculous mycobacteria (NTM). Water from two utility companies was tested - water from a subtropical region with high prevalence of NTM infection (DWDS1) and water from a temperate environment with a low prevalence of NTM infection (DWDS2). This approach was undertaken to determine if rates and diversity of environmental NTM mirrors that of disease prevalence.
Methods:
One litre water samples were collected during summer and winter months over a two-year period. DWDS1 and DWDS2 had 14 and 28 sample points screened at each collection, respectively. One litre water collection were aliquoted into two samples, one decontaminated with 0.005% cetylpyridinium chloride (CPC+), and one neat sample (CPC-). This was then concentrated by filtration and used to inoculate solid agar (7H11 Middlebrook agar) and liquid media (Mycobacteria Growth Indicator Tubes; MGIT). Both MGITs were supplemented with Oleic Albumin Dextrose Catalase (OADC) to encourage NTM growth and one was further supplemented with an antimicrobial cocktail of polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, and azlocillin (PANTA) to prevent bacterial and fungal overgrowth. The six different cultures, for each water sample, were incubated aerobically at 35°C for up to 12 weeks. Acid fast bacilli were identified by Ziehl Nielsen staining of suspected colonies and species determined by Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF).
Results:
NTM were isolated from both DWDS. A median of 3 different NTM species were isolated from sampling points within DWDS1, compared to a median single species from DWDS2. When the prevalence of species was compared between DWDS there was no difference noted for *M. abscessus* (DWDS1 12.0%, DWDS2 13.6%, p=0.755), however there were significant differences observed for *M. gordonae* (DWDS1 2.7%, DWDS2 25.0% p=<0.05), *M. kansasii* (DWDS1 13.3%, DWDS2 3.4%, p=0.019) and *M. mucogenicum* (DWDS1 n=0, DWDS2 14.7%). Of the 14 sampling points from DWDS1, only one was NTM-free at each of the sampling points. For DWDS2, five of the 28 sampling points were NTM-free at each collection.
Conclusion:
DWDS1 had a greater number of samples positive for NTM and greater species diversity compared to DWDS2. There was a marked difference in the number of commonly isolated environmental NTM (*M. kansasii*, *M. gordonae*, *M. mucogenicum*) between the sites, while there was no difference in the rates of the pathogenic species, *M. abscessus*, between the systems. These results demonstrate that NTM is part of the microbial flora within our drinking water system and therefore could be a potential reservoir for disease.

Nontuberculous mycobacteria (NTM) in Australians with cystic fibrosis (CF): A national study

Christine M. Duplancic[1], Claire E. Wainwright[1,2], Rachel Thomson[3,4], Geraint B Rogers[5], Archie C A Clements[6], R Andres Floto[7], Scott C. Bell[1,8,9] and the NTM in CF Study Investigator Group

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Background: There is an emerging threat to the survival of people with CF (pwCF) survival; chronic nontuberculous mycobacterial (NTM) infection, in particular species from the *Mycobacterium abscessus* group (Mabs). This study aims to understand the aetiology and epidemiology of NTM in Australians with CF.

Methods: Nationally, 18 CF services prospectively collected respiratory samples from pwCF for acid fast bacilli (AFB) culture at regular clinic appointments (Baseline, 6 months, 1 year, then annual to 4 years); associated clinical, microbiological, geographic and social data was supplied by the clinic and supplemented by linkage with Australian CF Data Registry. NTM infection was classified as persistent with ≥3 positive sputum cultures, or transient with <3 positive sputum cultures. Spontaneous clearance of a persistent infection was defined as at least 3 negative cultures over >12 months since the last positive culture. Clearance of NTM infection following treatment was defined as ≥3 negative cultures during or subsequent to treatment with no subsequent positive cultures in 12 months. An infection was defined as recurrent if clearance was achieved and the NTM species was cultured again after clearance.

Results: Cumulatively, 268 of 1321 study participants cultured NTM (NTM+) from at least one AFB culture during the study (20.3%). Of NTM+ recruits more than half are incident cases (57.5%) (fig. 1). Species from the *Mycobacterium avium* complex (MAC) and Mabs occurred at a similar rate in recruits (~8%; fig. 1). Adolescents and young adults had the highest prevalence of NTM ($p<0.01$ to $p<0.0001$) (fig. 2) and MAC ($p<0.05$ to $p<0.01$). Participants <25 years had higher prevalence of Mab*s* than older participants ($p<0.01$ to $p<0.0001$). (fig. 2). Infection was persistent in 71.0% of Mabs+ participants, 56.6% of MAC+ participants and 19.5% of participants infected with other NTM species. NTM-PD was diagnosed in 47.2% Mabs+ participants and 20.5% of MAC+ participants (fig. 3). Eradication therapy was administered in 80.4% of participants with Mabs-PD and 69.5% of participants with MAC-PD. Treatment led to clearance of infection in 43.2% of Mabs+ and 60% of MAC+ cases, respectively. Treatment efficacy could not be determined for 5 participants due to insufficient samples in follow-up/r insufficient follow-up period. Spontaneous clearance of infection occurred in 40% of participants with a persistent infection that had not progressed to NTM-PD (irrespective of the infecting species).

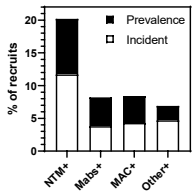

Figure 1: NTM isolation in the national cohort. Prevalence cases are those who have a history of NTM+ cultures at recruitment, incident cases had first NTM+ culture while participating. Other NTM include all non-Mabs and non-MAC NTM species.

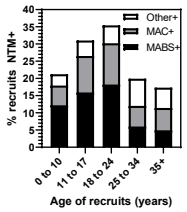

Figure 2: NTM occurrence in age segregated cohort.

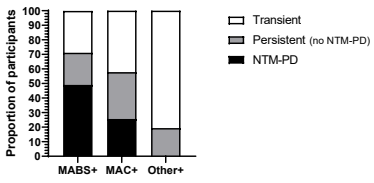

Figure 3: Proportion of the Mabs+, MAC+ and other NTM+ cohorts with NTM-PD, a persistent infection, or a transient infection.

Conclusion: In Australia, pwCF <25 years of age were more likely to be infected with Mabs than older pwCF, progression to PD was more common with Mabs infection and eradication less successful.

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Assessing the efficacy and mechanisms of disinfectants on *Mycobacterium abscessus*

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Background

Opportunistic pathogens (OPs) in drinking water distribution systems are an emerging health concern; however, there is limited understanding of the factors influencing their colonisation and the control strategies to effectively manage them in plumbing networks. OPs such as nontuberculous mycobacteria (NTM) and *Legionella* are leading causes of waterborne disease outbreaks in developed countries, and of major concern to hospitals and healthcare facilities (HCF). Of the many pathogenic NTM species, *M. abscessus* causes serious pulmonary infections, leading to severe health and economic burdens in Australia and internationally. Recent studies have found NTM, including *M. abscessus* and *M. avium* in drinking water and plumbing biofilms, where persistent biofilms have been identified as the primary infectious source of OPs, supporting the complex physiology, ecology, and transmission of OPs to vulnerable individuals. Although existing controls of microorganisms in drinking water systems rely on residual levels of disinfectants such as chlorine being monitored and maintained, they are at levels that do not remove persistent biofilms, and OPs such as NTM can persist in plumbing for many years. Thus, there is an urgent need to evaluate various treatment strategies that target plumbing biofilms and remove OPs.

Methods and materials

Various disinfectants were tested on planktonic cultures and biofilms of *M. abscessus* isolates. The efficacy of a range of conventional and novel disinfectants were evaluated, including chlorine (in the form of sodium hypochlorite), copper-silver ions, ozone, free nitrous acid (FNA), hydrogen peroxide, potassium peroxymonosulfate (PMS), peroxydisulfate (PDS), sulfur-modified zero-valent ions (S-nZVI). Disinfection was performed on planktonic cultures and biofilm models of *M. abscessus* at various concentrations. The efficacy of the disinfectants on both the planktonic cells and biofilms of *M. abscessus* were evaluated through culture-based methods, live/dead staining with flow cytometry, and confocal microscopy. Whole genome proteomics was used to assess the metabolic and physiological responses of *M. abscessus* under exposure to increasing concentrations of selected disinfectants.

Results and discussion

Chlorine (at 100 parts per million (ppm)), S-nZVI (at 500 and 1000 ppm) and PMS (at 10 mM and 20 mM) were the disinfectants and concentrations that had the best performances to inactivate the planktonic cells of *M. abscessus* (8 log reduction), whereas the *M. abscessus* biofilms were highly resistant to the same concentrations of disinfectants applied (2 log reduction). This illustrated that the *M. abscessus* and NTM in the form of biofilms are highly resistant to conventional and novel disinfectants in comparison to their planktonic counterpart. The response of *M. abscessus* against various concentrations of disinfections by proteomics are currently underway to gain insights for the development and application of biofilm and OP mitigation strategies.

Laboratory evolution of imipenem resistance in a *Mycobacterium abscessus* water isolate

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Background
Mycobacterium abscessus (Mabs) has been described as an "antibiotic nightmare" due to its extensive innate and acquired antibiotic resistance. Treatment guidelines for Mabs disease recommend antibiotic susceptibility testing (AST) to guide antibiotic selection. Broth microdilution is the gold standard method for AST of Mabs and has significant limitations. Whole genome sequencing (WGS) and other molecular techniques have increasingly been used to predict antibiotic susceptibility of Mabs however the genetic mechanisms of resistance are unknown for many of the commonly used antibiotics. Imipenem is a beta-lactam that has been associated with Mabs disease treatment success where the genetic mechanism of resistance is unknown.

This project aimed to determine 1) if exposure to sub-inhibitory concentrations of imipenem in a laboratory evolution experiment would result in imipenem resistant Mabs, 2) if the beta-lactamase inhibitor relebactam would restore susceptibility and 3) potential genetic mechanisms of imipenem resistance.

Method
A single Mabs isolate was passaged over 6 weeks with imipenem 2mg/l exposure and a no-antibiotic control conducted in triplicate. AST by broth microdilution and WGS were conducted at baseline, 2, 4 and 6 weeks. DNA was extracted from colony sweeps and sequenced using Illumina NextSeq (150 bp paired end) at a depth allowing >120 times coverage. The Breseq tool was used to determine structural variants of passaged cultures using default parameters and the original isolate as the reference strain. Only assigned, predicted structural variants were reported.

Results
Imipenem minimum inhibitory concentrations (MICs) increased by 2-fold at 2 weeks, 16-fold at 4 weeks and >16-fold by 6 weeks. The imipenem resistant Mabs had a longer incubation time required before visible growth to measure the MIC by broth microdilution. It did not develop cross resistance to other antibiotics tested and the beta-lactamase inhibitor relebactam did not restore imipenem susceptibility. WGS at week 6 revealed a 10bp deletion in the penicillin binding protein ponA1 for one of the three lines, with no genetic changes detected at 2 or 4 weeks or for the other two replicates.

Conclusion
Imipenem resistance in Mabs can occur within four weeks of passaging at a clinically relevant concentration and relebactam does not restore susceptibility suggesting beta-lactamase production is not the mechanism of resistance. WGS revealed no genetic changes at week 2 or 4, and two out of three triplicates with imipenem exposure had no genetic changes at week 6 which suggests that imipenem resistance is not genetically encoded. A limitation of the methods was the need to extract DNA from colony sweeps as single resistant colonies could not be isolated due to the instability of imipenem. Mechanisms of resistance not genetically encoded could include changes in expression of efflux pumps or of penicillin binding proteins. WGS or other DNA-based methods are unlikely to be able to predict imipenem susceptibility in Mabs and alternative methods to improve AST for Mabs should be investigated.

Oral-bait BCG vaccination of possums may address the worsening Victorian epidemic of Buruli ulcer

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Background
Buruli ulcer (BU), caused by *Mycobacterium ulcerans,* is endemic in Victoria, Australia, with an increasing incidence, severity and spread of the disease. It is a potentially severe disease often leading to serious long-term consequences and resulting in significant community costs and concern. Evidence suggests that possums are a zoonotic reservoir for *M. ulcerans.* Currently there are no proven public health interventions to address this worsening disease.

Methods

We will provide the evidence, rationale and research plan for a novel proposal to vaccinate possums in the wild with oral-bait BCG.

Results

A *M. ulcerans* infection model of ringtail possums has recently been developed at the CSIRO research facility in Geelong. Next steps include possum vaccination with BCG before *M. ulcerans* challenge to assess the level and durability of protection by comparing vaccine with control groups. This could utilise tools developed previously by a BCG-based oral-bait vaccination scheme for brushtail possums against *M. bovis* in New Zealand. In the proposed study, blood samples would be collected to measure the immune responses to BCG vaccination, which would be correlated with immune protection. If effective, attempts would be made to optimize the palatability and feasibility of oral-bait BCG delivery. Finally, testing the effectiveness of oral-bait BCG vaccine against *M. ulcerans* in ringtail possums would be performed in laboratory and real-life field settings.

Conclusion

Vaccination of possums in the wild with oral-bait BCG provides hope for an acceptable, safe and feasible intervention benefiting human and possum populations by reducing the transmission of *M. ulcerans*.

Concurrent treatment of *Mycobacterium kansasii* pulmonary disease and PD-L1 immunotherapy for metastatic non-small cell lung cancer

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Background/ Aims Immune checkpoint inhibitors (ICIs) are now an essential part of treatment against advanced cancers. Associated with altered host T-cell function, cases of non-tuberculous pulmonary disease (NTM-PD) during ICI treatment are increasingly recognised. Outcomes in such patients appear mixed, and the balance of risks and benefit of continuing ICI treatment unclear. Most published cases appear to concern *de novo* NTM-PD diagnosed during ICI treatment, rather than the identification of NTM infection simultaneous to the diagnosis of malignancy. We present the case of a patient coincidentally diagnosed with *Mycobacterium kansasii* pulmonary disease and stage 4 lung cancer, treated concurrently with an ICI and anti-mycobacterial medication.
Method Case records were retrospectively reviewed with the assent of the patient's representative. Online literature databases were reviewed for similar cases.
Results A 67-year-old male heavy smoker presented with a 3-week history of breathlessness and haemoptysis, and 3-kg weight loss over 3 months with occasional night sweats. He had schizophrenia controlled on risperidone but no other co-morbidities, HIV testing was negative and performance status good. Chest imaging revealed emphysema, spiculated nodules in the right middle and lower lobes, cavities in both lungs, right hilar soft tissue enlargement and a large necrotic subcarinal node. Endobronchial thoracic lymph node sampling confirmed the diagnosis of stage 4 lung adenocarcinoma (T1b, N2, M1b) with programmed death ligand 1 (PD-L1) expression <1%. Sputum and bronchial washings revealed 2+ acid-fast bacilli on microscopy, and positive cultures for *M. kansasii* at 15 and 21 days, respectively, resistant to ethambutol only.
A literature search revealed no similar cases. Following multidisciplinary discussion including oncology, radiology and respiratory physicians, the patient was commenced on the standard guideline-based anticancer regimen pembrolizumab (an ICI), pemetrexed and carboplatin, and then 17 days later rifampicin, isoniazid and ethambutol. At 3 months into NTM treatment sputum was smear and culture negative, cough had improved, and imaging suggested a regression of the NTM-PD, with resolution of nodular consolidation, but progression of cancer with an increase in the size of the right hilar mass and new liver metastases. Liver function tests deteriorated at 6½ months into treatment and NTM treatment was ceased. Anticancer treatment was suspended, but then, following clinical improvement, a second line non-ICI regimen introduced (docetaxel and nintendanib). Multiple sputum samples four months later remained culture negative. Shortly after this time there was a physical and radiological decline in keeping with progression of metastatic lung cancer. Anticancer treatment was ceased after four months of the second regimen, and the patient died several months afterwards.
Discussion *M. kansasii* pulmonary disease can be treated during concurrent lung cancer treatment with pembrolizumab. However, the incidence of drug-related adverse effects is likely to be increased, and the immunological interplay between immune checkpoint inhibition, cancer and mycobacterial infection remains poorly understood.

Speakers – Selected Oral Abstracts

Case Report: The Turtle's Accomplice – Collaborative Management of *Mycobacteroides chelonae* skin infection in an elderly immunosuppressed patient with Rheumatoid Arthritis on Baricitinib

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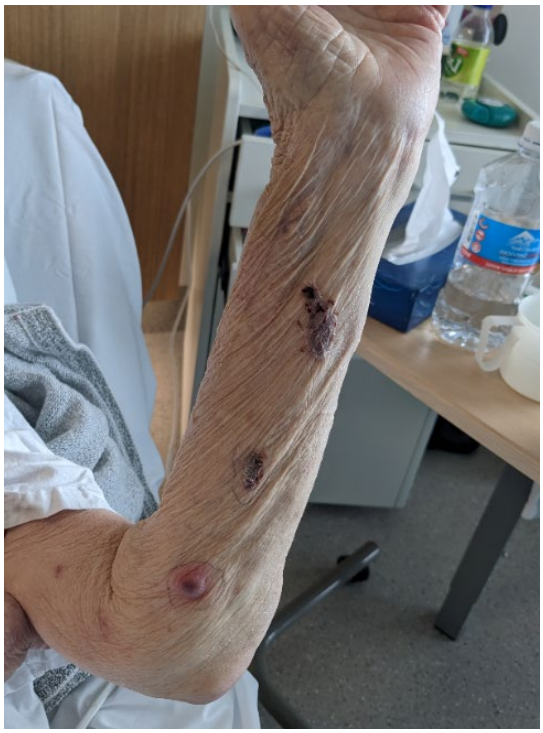
1. University of NSW

A case study of an elderly multimorbid immunosuppressed woman with Rheumatoid arthritis diagnosed with *Mycobacteroides chelonae* multifocal skin infection with possible lung involvement, and the challenging treatment.



An 83-year-old woman was admitted with a history of well controlled Rheumatoid arthritis who developed fluctuant lumps of the right upper arm and right forearm in November 2022 which progressed to involve the dorsum of her hand. She had an extensive past medical history including Rheumatoid Arthritis on long term prednisone 5 mg and Baricitinib 4mg daily with sulfamethoxazole/ trimethoprim *Pneumocystis jirovecii* prophylaxis, Crohn's Disease with an ileostomy and extra-intestinal manifestations included previous healed left leg pyoderma gangrenosum, macrocytic anaemia subsequently diagnosed with transfusion dependent myelodysplastic syndrome with low-risk 5q deletion with a plan for supportive transfusions via a right sided port-a-cath, chronic cardiac failure with preserved ejection fraction, adrenal insufficiency on context of long term steroid use, atrial fibrillation, gastric angiodysplasia, and sick euthyroid on thyroxine replacement. She had a historical penicillin allergy.

Initial clinical impression and differentials included erythema nodosum, erythema induratum of Bazim, Sweets syndrome, blastomycosis or a fungal infection. Dermatology review and biopsy of her right elbow lesion demonstrated granulomatous panniculitis, Acid-Fast Bacilli were detected and *Mycobacterium tuberculosis* was not detected on polymerase chain reaction. Further workup for disseminated non-tuberculous mycobacterial infection including mycobacterial blood cultures, sputum cultures, and bronchoalveolar lavage cultures which were negative for mycobacteria. High-resolution CT of her chest demonstrated mixed ground glass and solid opacities within the lungs, small apical nodules with a tree in bud appearance and left sided rib lesions of unclear aetiology.

Discussion

Managing *M.chelonae* infection in multimorbid often elderly individuals with rheumatoid arthritis on immunosuppressive therapy requires a comprehensive approach. Here are some important considerations which I will elaborate upon in the case report:

1. Consultation with Rheumatologist and Infectious Diseases Specialist
2. Assessment of Immunosuppression
3. Multiple Antimycobacterial Agents
4. Treatment Duration
5. Regular monitoring

The case report reinforces that the management of *M.chelonae* infection in a patient with rheumatoid arthritis requires a tailored approach that takes into account the individual's clinical condition, the extent of immunosuppression, and the specific characteristics and sensitivities and extent of the infection.

Recurrent disseminated mycobacterium avium complex infection in a patient with anti-interferon-gamma autoantibody syndrome

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Royal Melbourne Hospital[1]

Case Presentation: A 65-year-old Vietnamese man presented with recrudescence of severe constitutional symptoms and small volume haemoptysis, on a background of pulmonary *mycobacterium szulgai* infection 5 years prior. CT chest, abdomen and pelvis was performed showing left lower lung consolidation, multi-lobar solid nodules, mediastinal lymphadenopathy and a T12 vertebral lytic lesion. Sputum smears demonstrated positive acid-fast bacilli and cultures subsequently isolated *mycobacterium avium complex* (MAC), also grown on a core biopsy of the vertebral lesion. The patient was commenced on oral rifampicin 300 mg twice a day, clarithromycin 500 mg twice a day and ethambutol 800 mg/day. Immunology were involved and testing for anti-interferon-gamma (anti-IFN-y) auto-antibody was performed, showing partial signalling of serum in a standard concentration (1:1000) of exogenous interferon-gamma. This confirmed a diagnosis of anti-IFN-y autoantibody syndrome and 1g of Rituximab, a CD-20 depleting agent was administered on day 0 and 15. The patient developed an epidural abscess 2 months later requiring neurosurgical decompression and evacuation, further complicated by wound dehiscence and post operative collection, still isolating MAC on operative cultures on both occasions. **Discussion:** Anti-interferon gamma autoantibody syndrome is an adult-onset immunodeficiency predominantly in South-East Asians, which predisposes to recurrent non-tuberculous mycobacterium. Diagnosis can be confirmed by measurement of neutralising interferon antibodies. The mainstay of treatment is antibiotic therapy, but immunosuppression targeting circulating anti-IFN-y auto-antibodies such as rituximab, bortezomib and daratumumab have been used on an individual basis. People from different countries with anti-IFN-y antibody syndrome have distinct demographic and clinical features. Further research that evaluates these characteristics in an Australian context are warranted. **Conclusion:** Awareness of this rare, but debilitating immunological syndrome is important in patients with disseminated recurrent non-tuberculous mycobacterium infections.

Disseminated non-tuberculous mycobacterial infection in the context of a new diagnosis of interferon-gamma autoantibody syndrome

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Introduction

Interferon-gamma (IFN-γ) autoantibody syndrome is an emerging clinical entity that has been associated with disseminated non-tuberculous mycobacterial infection (dNTM) particularly in previously healthy young people, a population not previously thought to be at increased risk of infection with these organisms. This case presents the diagnostic dilemma of a previously well young man with a febrile illness in whom a delayed diagnosis of dNTM was ultimately made, and the investigation of underlying predisposing factors leading to a diagnosis of IFN-γ autoantibodies.

Case

A 29-year-old immunocompetent South-East Asian man presented with several weeks of fever, cough, and lymphadenopathy developing while working on an international cargo ship. The differential diagnoses were tuberculosis and lymphoma, however initial investigations including nodal biopsy were inconclusive, and his clinical status deteriorated significantly. Eventually cultures revealed a diagnosis of dNTM with two species. This prompted investigation of underlying immune defects and the association with IFN-γ autoantibodies in this population was found, which he was confirmed to have. He was commenced on a combination anti-mycobacterial regimen and rituximab, after which he significantly improved and was able to be transferred to a hospital in his home country.

Learning points

This case highlights some of the difficulties faced by patients with dNTM in the context of IFN-γ autoantibodies, particularly delayed diagnosis and lack of long-term evidence surrounding best management. Further research into long-term outcomes and treatment is required as well as increased awareness among clinicians.

A method for the isolation of environmental *Mycobacterium* species from soil and house dust

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Background: Inhalation of aerosolised environmental nontuberculous mycobacteria (NTM) from soil and water is a recognised mode of infection acquisition. *M. intracellulare* is the most frequently isolated respiratory NTM in Queensland, Australia, where it has been isolated from soil and house dust. Environmental samples such as soil and dust contain a wide diversity of microbes and these will overgrow the slowly growing mycobacterial species, making isolation of NTM problematic. A review of methods for the isolation of mycobacteria from soil and dust failed to identify a "gold standard". To evaluate soil and dust as sources of infection, this study investigated a range of methods, in order to maximise recovery of diverse species with the least overgrowth and contamination. **Method**: Garden soil and household dust samples were incubated in Trypticase Soy Broth (TSB) to encourage spore germination, then aliquoted into equal volumes and treated with different decontamination agents, including varying concentrations of sodium hydroxide (NaOH) +/- malachite green (MG) and 5% oxalic acid (OA). Liquid media (BD Bactec™ MGIT + Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin (PANTA) at a double concentration) and solid media including M7H10 + Oleic acid, Albumin, Dextrose Catalase (OADC), M7H11 agar and Rapid Grower Media (RGM) media were included. Solid media were incubated at 30ºC and 35ºC and the MGIT vials were incubated at 37ºC. Plates were read daily for the first week and then weekly. Any colonies resembling mycobacteria were confirmed with a Zeihl-Neelsen (ZN) stain, sub-cultured and stored at -80ºC for later identification. **Results:** 43/147 individual culture plates showed fungal and bacterial overgrowth which prevented mycobacteria being isolated. 5% oxalic acid used for 5 min showed the least contamination with 4/33 plates discarded. Doubling the concentration PANTA did not prevent fungal and bacterial overgrowth in liquid media, and MG interfered with fluorescent indicator in the Bactec™ MGIT™ vial. The least contamination was seen from the bioMerieux NTM Elite agar plates. Both rapid grower (RG) and slow grower (SG) mycobacterial species were identified using 16S rRNA sequencing. Rapid grower media (RGM) such as bioMerieux NTM Elite and Phigenics MYChrOme™ media were useful in the recovery of mycobacteria. **Conclusions:** Decontamination with 5% OA for 5 minutes appeared to produce the best results for with a low discard rate as well as the recovery SG mycobacteria and RG mycobacteria as a single method. 4% NaOH for 15 minutes resulted in the greatest yield of SG mycobacteria, including *M. intracellulare*. The use of RGM increased the recovery of NTM species.

The comparison of culture-based methodologies for the isolation of nontuberculous mycobacteria (NTM) from potable water samples

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Background:
Surveillance studies are required to determine the diversity and prevalence of NTM in the environment, however culturing of NTM is difficult for several reasons and isolation focuses around two main factors. Firstly, reducing or eliminating contamination from non-NTM organisms and secondly, enhancing the growth and therefore the recovery of NTM. The standard method for optimal recovery of NTM from water samples uses a combination of decontamination techniques and the inoculation of selective media. Here we compare selected solid and liquid based media, and decontamination reagents, to determine which culture technique has the highest NTM yield.
Materials and Methods:
A total of 112 water samples collected between October 2022 and June 2023 from homes, hospitals and a drinking water distribution system were included. One litre water collection were divided into two samples, one decontaminated with 0.005% cetylpyridinium chloride (CPC+), and one neat sample (CPC-). This was then concentrated by filtration and used to inoculate solid (7H11 Middlebrook agar) and liquid media (Mycobacteria Growth Indicator Tube; MGIT). Both MGITs were supplemented with Oleic Albumin Dextrose Catalase (OADC) to promote NTM growth and one was further supplemented with an antimicrobial cocktail of polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, and azlocillin (PANTA) to prevent bacterial and fungal overgrowth. The six different cultures, for each water sample, were incubated aerobically at 35°C for up to 12 weeks. The neat sample was also inoculated onto Biomerieux NTM Elite and Phigenics MyChrome agars. An additional MyChrOme plate was inoculated with water treated with the Phigenic decontamination reagent MyCOn. These media were incubated aerobically at 30°C for up to 12 weeks. Acid fast bacilli were identified by Ziehl Nielsen staining of suspected colonies and species determined by Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF).
Results:
The highest rates of contamination were observed in MGITs without CPC, where 90% of these cultures were discarded. Of the solid media without decontamination reagents, 7H11 CPC- had the highest rates of contamination (58%), compared to MYChrOme (36%) and NTM Elite (1%). The use of CPC reduced contamination rates of both the solid and liquid media ($p<0.05$), whereas the addition of the MYCOn reagent did not change the rate of contamination observed on the MYChOme media ($p=0.363$). Twelve different NTM species were identified. The environmental species *M. gordonae* and *M. mucogenicum* were most frequently isolated. This was followed by the pathogenic species *M. abscessus*, *M. chelonae* and *M. kansasii*. Use of the NTM Elite media resulted in the highest overall yield for NTM (all species) (38.3%). Greater species diversity was also observed with 11 species were recovered. *M. abscessus*, *M. chelonae*, *M. gordonae* and *M. kansasii* were isolated in greatest number from this media.
Conclusion:
NTM Elite media had the highest recovery of NTM and the lowest contamination rates suggesting that for the recovery of NTM from potable water, this was the most effective media to use.

The Mycobacterial Disease Biobank

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Introduction: Nontuberculous mycobacteria (NTM) are bacterial species found throughout the environment that usually do not cause illness. However, in a small subset of people (most commonly those with an underlying lung disease), these organisms can cause chronic respiratory disease with varying symptoms. The aim of the Mycobacterial Disease Biobank is to establish a long-term repository for biological specimens and associated clinical data of NTM patients to drive collaborative investigative research into this disease.
Methods: Previous specimen collection efforts were consolidated into the current Biobank, established in 2021 at The University of Queensland's Greenslopes Clinical Unit, which contains specimens from 459 blood collections, 53 stool collections and 193 collections during bronchoscopies from 1999 to 2023. 3 out of 6 secondary collection sites were opened in late 2022, but no samples have been collected as of mid-2023 from these sites. Blood samples were stored as whole blood, serum, plasma, buffy coat, PAXGene RNA and peripheral blood mononuclear cell (PBMC) fractions, and bronchoalveolar lavage (BAL) and associated control samples (mouth wash, saline control and scope flush) were separated into cell and supernatant components or left as a whole (neat) sample. Stool samples were collected and stored in preservative. All samples were cryo-stored at -80C and liquid nitrogen (see Table 1).
Results: BAL specimens were collected from a total of 189 participants from multiple lobes, with 25.4% participants having multiple (two or more) collections. Blood samples were collected from a total of 297 participants, with 19.2% participants having multiple collections. Stool samples were collected from a total of 22 participants, with 68.2% of participants having multiple collections. Delays in secondary collection site activation has significantly impacted on sample collection rates. However, 98 BAL samples from 32 unique participants and 220 unique whole blood samples have been used in projects in the past year.
Conclusions: A significant number of cryopreserved samples are available for use in novel NTM research, including some potential for longitudinal studies. Current work focusses on assessing specimen quality and suitability for projects, particularly for the older samples, and establishing a linked clinical data repository.

Table 1:

Lung BAL Lobe	# Specimen Collections
Right Upper	85
Right Middle	126
Right Lower	35
Left Upper	32
Left Lower	19
Lingula	73
Mouth Wash Saline Control	182
Saline Control	3
Scope Flush Control	3
Blood Component	**# Specimen Collections**
Whole blood	340
Serum	311
Plasma	320
Buffy coat	292
PBMC	177
Stool Collection Tubes	**# Specimen Collections**
OM-200 preservative	54

The Isolation of *M. intracellulare* from soils and household dust

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Background. *M. intracellulare* is the most frequently isolated nontuberculous mycobacteria species in QLD. The incidence of these infections is increasing both in QLD and world-wide. In some patients, *M. intracellulare* can cause a chronic debilitating infection which is challenging to treat. The disease is more commonly found in older patients particularly women who have underlying co-morbidities such as bronchiectasis. The source of the infection is unclear. An early study in SEQ based on soil and dust isolated *Mycobacterium avium* complex (MAC) species where the common serotype found was the same as that for patients. A later study looking at the SEQ water distribution failed to isolate this species. One factor which has complicated studies in the past has been the changing nomenclature for *M. intracellulare*. These changes are not always reflected in different studies with the level to which studies speciated MAC. **Method.** Due to the lack of a standard method for decontaminating soil and dust samples, a preliminary study was conducted to evaluate three decontamination methods. 5% oxalic acid (OA), 1.5% sodium hydroxide (NaOH) + malachite green (MG) and 4% NaOH. Several variables were used to assess the decontaminants including the culture media, the decontamination time and incubating temperature. Three samples collected from the same location were used for this preliminary study. These samples were soil, a sample of vacuum dust and a sample of vacuum cleaner dust which was sieved to remove large particulate material. The initial identification of all mycobacterial isolates was performed using 16S rRNA sequencing. To further speciate the MAC group, internally transcribed spacer (ITS) sequencing was undertaken. **Results.** From this single location, *M. intracellulare* was isolated using two different decontaminants from the soil sample. However, there were isolates which were not differentiated using 16S rRNA such as *M. colombiense* and *M. marseillence.* The results from ITS sequencing provide a useful means of differentiating the MAC group. **Conclusion.** This study was undertaken from a single site. However, the results show that *M. intracellulare* is able to be isolated using culture and the identification confirmed using ITS sequencing. These results provide the basis for a wider study of environmental isolates.

Disseminated *Mycobacterium abscessus* Infection. An unexpected complication following trauma Laparotomy

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Mycobacterium abscessus complex is a group of non-tuberculosis mycobacteria differentiated into 3 subspecies: *M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii.* The complex is responsible for a wide spectrum of disease including skin and soft tissue infection, bacteraemia, ocular and central nervous system infections. Ubiquitous in soil and water, *Mycobacterium abscessus* is often rapidly growing, multidrug resistant and difficult to treat. Previously recognised to cause community-acquired skin and soft tissue infection, *M. abscessus* is emerging as the cause of increasing numbers of hospital acquired infections. We describe a case in a 79 year old male who underwent emergency laparotomy, small bowel and colonic resection with colostomy, following a high-speed motor vehicle accident, without penetrating injury. The post-operative course was complicated by a clinically aggressive multifocal surgical site infection with superficial dehiscence (Fig 1.). *M. abscessus* subsp. *bolletii* infection was cultured. Initial debridement was followed by intravenous imipenem, tigecycline and amikacin but recurrent wound dehiscence with new bilateral axillary and inguinal lymphadenopathy was noted at four weeks. FDG PET scan confirmed disseminated disease (Fig 2). Delayed excision was undertaken of the laparotomy wound, and involved lymph nodes in both axillae and left groin. The patient remains on intravenous antimicrobial therapy with a good response to treatment. This case highlights the difficulty in treating *M. abscessus* infection, the indication for surgical debridement and debulking and the importance of a multi-disciplinary infectious diseases and surgical team in treating complex *M. abscessus* infection.

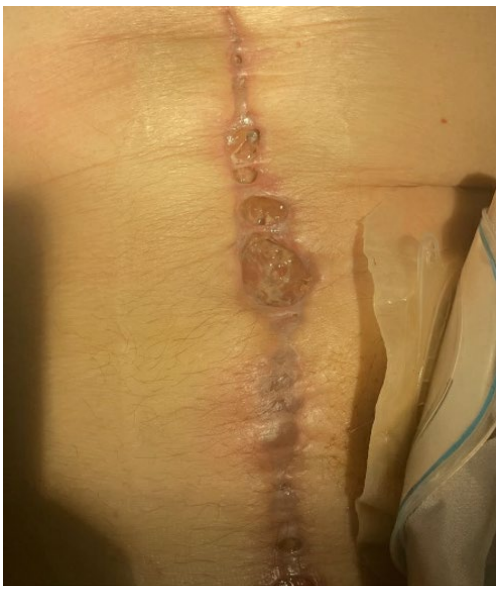

Figure 1: Midline laparotomy wound with superficial dehiscence secondary to *M. abscessus* infection

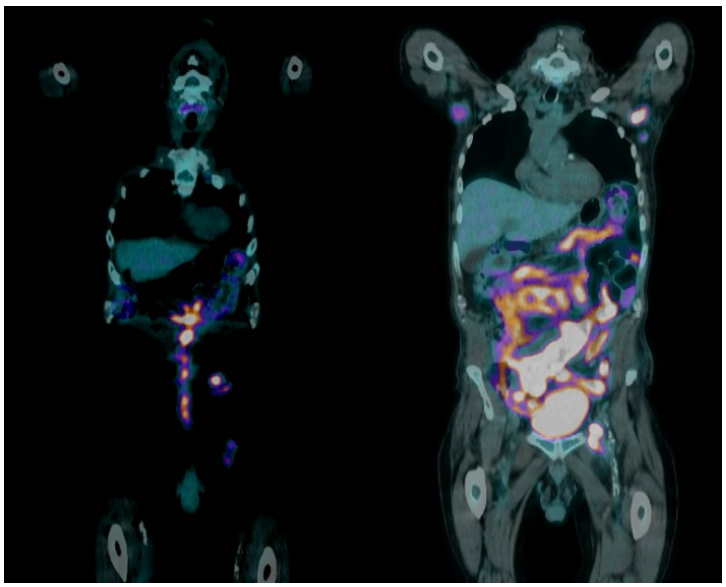

Figure 2: FDG PET scan demonstrating midline laparotomy wound uptake with bilateral axillary and left groin avidity

Mycobacterium abscessus infection complicating coronary stent causing massive coronary pseudoaneurysm in a 39-year-old male of Nepalese background

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Introduction: *Mycobacterium abscessus* is a rapid-growing mycobacterium which most commonly causes pulmonary infections but can also cause disseminated infections with high mortality, particularly in the setting of macrolide resistance.
Case study: A 39 year old male, born in Nepal but living in Australia and New Zealand since 2008, was admitted to a major tertiary hospital in Sydney after presenting with a three-day history of haemoptysis and subjective fevers. His past medical history was significant for an anterior ST-elevation myocardial infarction in December of 2022, which occurred whilst he was visiting Nepal. This was managed with percutaneous intervention; stenting his left anterior descending (LAD) artery.
CT chest demonstrated a large left-sided coronary aneurysm with mass effect, which was further investigated with CT coronary angiogram. This demonstrated a 90 x 75 x 61 mm pseudoaneurysm arising from the proximal LAD causing extrinsic compression of both the coronary and pulmonary circulation. Blood cultures then grew a gram-positive bacilli concerning for non-tuberculous mycobacteria, which was later identified to be *Mycobacterium abscessus*. Infectious diseases consultation occurred and the patient was commenced on empirical amikacin 20 mg/kg stat dose, azithromycin 500 mg IV daily and moxifloxacin 400 mg IV daily. He then underwent median sternotomy, cardiopulmonary bypass and repair of coronary aneurysm, requiring veno-arterial extracorporeal membrane oxygenation (VA ECMO) due to poor cardiac output despite high-dose inotropes. Intraoperatively, his pre-existing coronary stent was noted to be protruding through the anterior wall of the LAD, giving rise to the pseudoaneurysm. The stent was removed and the aneurysmal sac repaired.
Intraoperative cultures from aneurysmal tissue, thrombus and stent all grew *M. Abscessus*. He was admitted to ICU postoperatively on VA ECMO and weaned off this over six days. Sequencing confirmed the isolate subspecies with inducible erm gene suggestive of macrolide resistance, which was later confirmed. Antibiotics were rationalised to Amikacin 1g daily, Ampicillin 2g q6h, Ceftazidime-Avibactam 2g/500 mg q8h, Linezolid 600 mg IV q12h and Tigecycline 50 mg IV q12h. He was extubated and clinically improved, without further positive blood cultures. After transfer out of ICU, he began experiencing chest pain and suffered a cardiac arrest. After initial return of spontaneous circulation, he suffered a second arrest while undergoing urgent percutaneous coronary intervention and died. Reaccumulation of aneurysm was noted on angiography.
Conclusion: We report a case of likely iatrogenic cardiac stent-associated mycotic aneurysm with mortality despite aggressive surgical intervention and appropriate antimicrobial therapy.