Project Title

Advancing in Bronchiectasis Knowledge and Management.

Project Lead and Members

Project lead: A/Prof. Lim Yick Hou Albert.

Project members: (1) A/Prof. John Abisheganaden, and (2) A/Prof. Sanjay Chotirmall.

Organisation(s) Involved

(1) Tan Tock Seng Hospital

(2) Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Healthcare Family Group(s) Involved in this Project

Medical

Applicable Specialty or Discipline

Respiratory and critical care medicine

Project Period

Start date: 2016

Completed date: 2021

Aims

The aims of this multi-dimensional research programme are to study endo-phenotyping disease, the translational application of microbiomics into patient care, fungal infections, clinical outcomes improvement, non-tuberculous mycobacteria (NTM) infections and innovation.

Project Details

Globally, there are more than 57 million people suffering from bronchiectasis. The difficulty in removing sputum from the damaged airways results in persistent cough, shortness of breath, wheezing and recurrent chest infections. In addition to living with persistent symptoms, these

patients experience lifelong clinical, economic and social consequences from recurrent hospitalizations, work days lost, and poor quality of life. In Singapore, the health care cost exceeds USD 7,000 per hospitalised bronchiectasis patient annually. In USA, the healthcare burdens exceeds USD 30 billion.

Having been awarded >SGD$10 million in competitive funding to further research into bronchiectasis, we have published >130 papers (>65% in Tier 1 journals) including the *Lancet Respiratory Medicine, the American Journal of Respiratory and Critical Care Medicine (AJRCCM), the European Respiratory Journal (ERJ)*. We have established *national, international, and interdisciplinary collaborations*. *Nationally*, 'The Academic Respiratory Initiative for Pulmonary Health (TARIPH)' at LKCMedicine was launched in 2018 that aligns strategic academic expertise across Singapore to benefit Singaporeans with lung disease through research. Since its establishment, TARIPH has garnered >500 active members, formed collaborative networks with national (14 institutions: 15 grant applications, 12 publications and 14 legal agreements) and international bodies (20 institutions: 6 publications and 5 legal agreements) including *interdisciplinary* collaborations. *Internationally,* 'The Singapore – Initiative Respiratory Consortium (SIRC)' has been formed, a strategic, self-funded, international partnership between 7 international universities with leading respiratory research programs to facilitate student exchange and project collaborations. A fundamental aspect of our research impact and success is the emphasis on *teamwork*, *collaboration*, and the *mentorship* of young scientists. The success and regularity of our staff and students in attaining prizes and recognitions (national and international) exemplifies the quality of our work. In the last three years alone, the team has been awarded >10 accolades including those to undergraduate and PhD students, research assistants and post-doctoral fellows. *Interdisciplinary* research is central to adequately addressing complex clinical problems such as bronchiectasis and work with colleagues in the mathematical, chemical, environmental, and material science engineering fields. Our growing *international reputation for innovative, impactful, and influential science* in bronchiectasis is best summarised into endo-phenotyping disease, the translational application of microbiomics into patient care, fungal infections, clinical outcomes improvement, non-tuberculous mycobacteria (NTM) infections and innovation

Endo-phenotyping of pulmonary disease: We have made key advances in understanding novel, ethnic and geographically variable endophenotypes of pulmonary disease including bronchiectasis. We have identified clinically meaningful ethnic and geographically variable patient groups including the discovery of the geographic variation of *Aspergillus* in bronchiectasis and the first description of 'immuno-allertypes'. Collectively, these data are published in >15 high impact papers including the *Lancet Respiratory Medicine, AJRCCM, Lancet Global Health, Nature Reviews Disease Primers, ERJ, mBio* and *JACI*.

Translational application of microbiomics into patient care: To avail of the potential offered by next generation sequencing for precision medicine, we have employed bronchiectasis as an exemplar condition. We have demonstrated the importance of microbial stratification in

bronchiectasis including work that identified a novel (Asian) bronchiectasis pathogen, *Neisseria subflava,* for which an NMRC-CS-IRG grant was secured. The scientific and clinical implications of this discovery are significant: it identifies a unique and treatable feature of Asian respiratory disease uncovering new mechanisms of pulmonary damage. In addition, we have successfully linked air microbiomes to allergic clinical phenotypes in bronchiectasis and, led the first study applying lung metagenomics to the antimicrobial airway resistome (Fig 4) [1]. In this latter work, inhalers were identified as key reservoirs of antimicrobial resistance. Most recently, we completed a 'paradigm shifting' study describing the lung's microbial 'interactome': a novel inter-kingdom interaction network that drives infection. To further the latter, we have developed, with mathematicians, an open science online bioinformatics webtool (https://integrative-microbiomics.ntu.edu.sg/) to allow a mathematical integration of inter-kingdom microbiomes (bacteriomes, viromes and mycobiomes), reframing our understanding of pulmonary infection, a key feature of bronchiectasis(Fig2) [1]. Collectively, these findings are published in >10 high impact papers including *Nature Medicine, Lancet Respiratory Medicine, AJRCCM* and the *ERJ*.

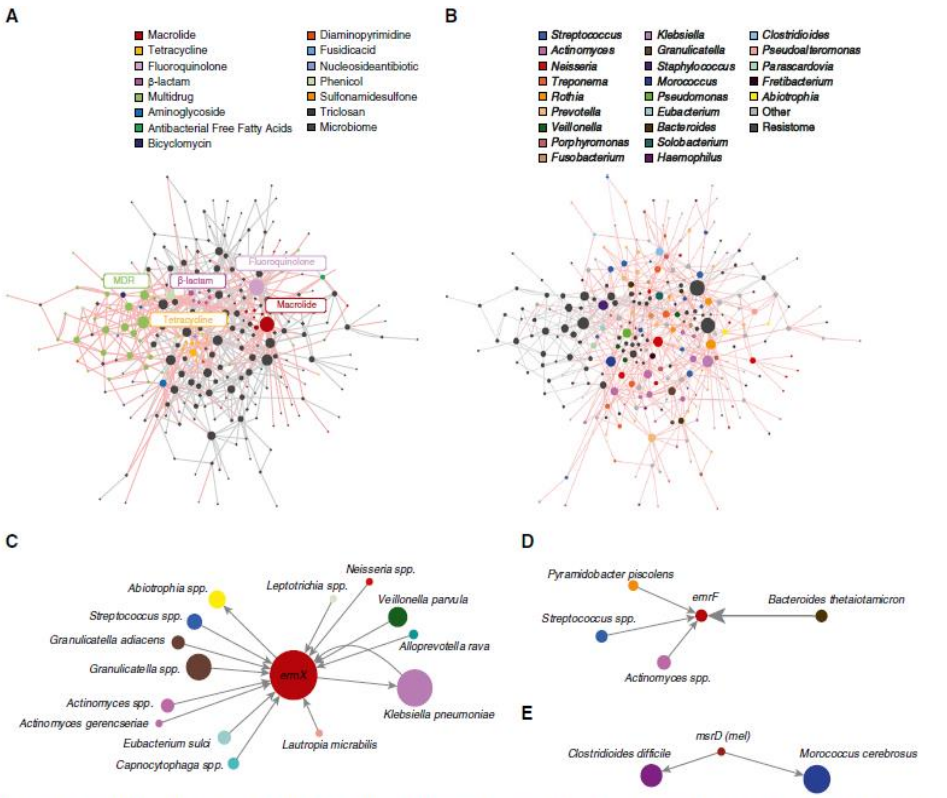

Figure 4. Network inference through co-occurrence analysis reveals gene–microbe associations of the core macrolide resistome. (*A*) Antibiotic resistance genes within the co-occurrence network are color-coded with respect to antibiotic class, whereas microbes are colored black. Gray lines denote interactions between nodes (representing both microbes and resistance genes), with line thickness reflecting their observed interaction strength. Interactions between resistance genes are highlighted by red lines. (*B*) Microbes within the co-occurrence network are color-coded with respect to their species, whereas antibiotic resistance genes are colored black. Gray lines denote interactions between nodes (microbes or resistance genes), with thickness reflecting interaction strength. Interactions between species are highlighted by red lines. (*C–E*) Identified nodes of the macrolide resistome are highlighted, indicating the specific microbes (by species) that associate with *ermX* (*C*), *emrF* (*D*), and *msrD* (*E*). Line thickness reflects the observed interaction strength between microbial nodes and the central resistance gene, whereas arrows depict directionality of the co-occurrence prediction.

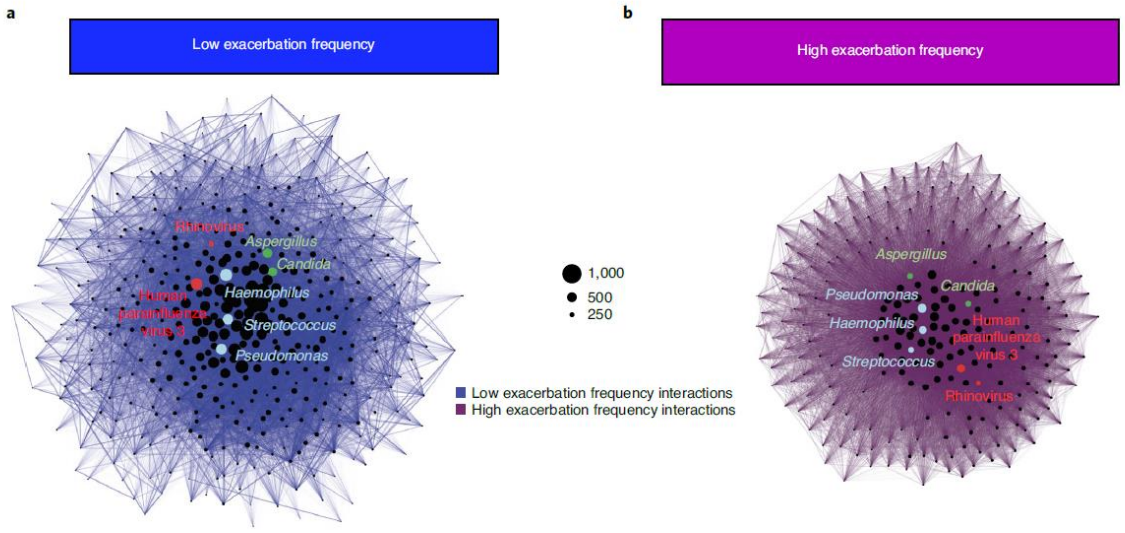

Fig. 2 | Co-occurrence analysis of the multi-biome network in high-frequency exacerbators. a,b, Co-occurrence network maps of low and high exacerbation frequency clusters illustrating identified microbial interactions. Interactions between microbes (nodes) are represented by connecting lines (edges), and the number of interactions for each microbe is reflected by node size (see scale bar). Selected bacterial (light blue), fungal (green) and viral (red) taxa of clinical relevance are indicated by node coloration.

Respiratory fungal infections in bronchiectasis: Infectious lung disease are classically associated with bacteria and/or viruses; however, an increasing recognition of fungi, largely due to our work in bronchiectasis has emerged. We have performed influential work characterising the clinical, immunological, and inflammatory effects of *Aspergillus spp.* in chronic respiratory disease including the composition, importance, and clinical relevance of the airway mycobiome (the fungal microbiome) in bronchiectasis. This work, for the first time, demonstrates the key relevance of 'unculturable' fungi in the airway, an unrecognised cause of lung damage and potential microbial 'biomarker' in 'high-risk' patients. We have resolved methodological challenges in the field including optimal primer use and, the lack of fungal genomes for reliable reference database curation. Collectively, these advances are published in >20 high impact papers including the *AJRCCM, ERJ* and *JACI*.

Clinical outcomes improvement in bronchiectasis: Bronchiectasis is characterised by excessive sputum production, cough, breathlessness, and recurrent lung infections. The damaged lung subjects to intense airway inflammation and opportunistic infections. We have set up a multidisciplinary bronchiectasis one-stop service, providing a joint care on medical, nursing, and physiotherapy on one-visit, one-site, and one-team of specialists. This pioneer service demonstrates significant impact on clinical outcomes and a positive change of patients' attitude towards long term treatment of this devastating disease. Hospitalisation and emergency department attendance rate due to bronchiectasis has reduced >50%. We have positively changed the patients' attitude on self-treatment with daily chest physiotherapy, truly achieving patient centred treatment with empowerment of self-management.

Non-tuberculous mycobacteria (NTM) infection in bronchiectasis: This opportunistic infection is the greatest threat for bronchiectasis patients due to its multi-drug resistance with genetic

variation, severe drug related toxicities and side effects, a dismal treatment outcome and rapid deterioration of lung function. We have identified the NTM profile for the Singapore population. A new concept of treatment for NTM infection has been designed and will be tested ex-vivo. We are researching the novel therapy by targeting new pathway, and generating new compound for NTM treatment.

Innovation- Wearable Intra-Nasal Device (WIND): We have invented WIND, an independent airway clearance solution for home-based management. This novel solution is a hands-free mucus reliever that is safe, effortlessly provides consistent quality therapy and easily integrates into the patient's daily routine. A smart solution, the WIND Airway Clearance System is comprised of a wearable electronics system and an integrated mobile application for chronic therapy management. WIND is capable of producing fully-automated percussion in the respiratory system. As a result, mucus is loosened from the entire airways and mobilised proximally towards to mouth for expectoration. WIND uses the principle of acoustic technology coupled with dynamic air flow in the respiratory system to achieve a safe and efficacious airway clearance outcome. This portable and lightweight smart wearable solution removes the need for user participation in airway clearance therapy and encourages patients to continue their daily routine without therapy interference. Achieving integration with their lifestyles and their chronic care pathway, WIND ultimately aims to achieve an easy and effective airway clearance outcome with significant patient satisfaction.

Lessons Learnt

Bronchiectasis is a complex lung disease with extensive heterogeneity. Understanding the uniqueness of the disease such as the microbiomes, endo-phenotype, and intervening at the appropriate part of the disease vicious cycle are the keys for the successful treatment of this disease. The successful of this research programme is due to excellent collaborations between institutions and team members.

Conclusion

We have identified clinically meaningful ethnic and geographically variable patient groups including the discovery of the geographic variation of *Aspergillus* in bronchiectasis and the first description of 'immuno-allertypes'. We have discovered a unique and treatable feature of Asian respiratory disease uncovering new mechanisms of pulmonary damage. We have described the interaction of bacteria, viruses and fungi in the lung. This led to the development of an open science online bioinformatics webtool (https://integrative-microbiomics.ntu.edu.sg/).We have demonstrated the key relevance of 'unculturable' fungi in the airway, an unrecognised cause of lung damage and potential microbial 'biomarker' in 'high-risk' patients. Our bronchiectasis one-stop out-patient service reduces acute health care utilization and cost. We have identified a unique NTM profile in our population. A new concept of NTM treatment has been designed and development of novel therapy. A wearable

intranasal device (WIND) airway clearance system has been developed for independent airway clearance solution for home-based management.

References

1. Metagenomics Reveals a Core Macrolide Resistome Related to Microbiota in Chronic Respiratory Disease. AM J Respir Crit Care Med. 202 Aug 1;202(3):433-447. https://DOI: 10.1164/rccm.201911-2202OC
2. Integrative microbiomics in bronchiectasis exacerbations. Nat Med 2021. 27,688-699. https://doi.org/10.1038/s41591-021-01289-7

Additional Information

NHG Research Award 2021: NHG Research Impact Award

Project Category

Applied/ Translational Research

Mixed-methods

Keywords

Bronchiectasis, Microbiomes, Endo-Phenotype

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