

WORLD CONGRESS ON RHEUMATIC HEART DISEASE

2-4 November 2023 • Abu Dhabi



Submission Id: 235

Title: Targeted profiling of the oropharyngeal microbiota in patients with severe Rheumatic Heart Disease: a case-control study

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Background & Aims: Acute rheumatic fever (ARF) and its chronic sequel, rheumatic heart disease (RHD), is an autoimmune condition that affects millions annually, disproportionately inflicting detrimental health on indigent persons globally. Although it is known that cross-reactive to host proteins following infection with group A Streptococcus (GAS) is the primary trigger, multiple knowledge gaps exist in the subsequent progression to RHD disease. The determinants of differential clinical presentations and progression rates are unknown. These may explain inconsistencies in responses to secondary prevention in different settings. Given the link between pharyngitis and RHD, we profiled the pharyngeal microbiota of patients with RHD.

Methods: Pharyngeal swabs were taken from a pilot cohort of 15 patient with moderate to severe RHD before their scheduled heart valve replacement surgery at the Groote Schuur hospital Cardiothoracic surgical unit. These were matched with 15 age-, gender-, and ethnicity matched controls without RHD. A multiplexed real time polymerase chain reaction (PCR) amplification test targeting 33 Respiratory Pathogens was used to diagnose the presence of these microbial pathogens in pharyngeal swab of the 15 RHD patients, compared to the matched healthy controls. Subsequently, the swab samples were sub-cultured on specific culture media for identification of live bacteria.

Results: Multiplex PCR found *K. pneumoniae*, *S aureus* *M. catarrhalis* and *H influenzae* in swabs from RHD compared to the healthy controls. No viral pathogens were detected in the cases, while *H influenzae* was also detected in 2 of the 15 control participants. Culture based method found abundant growth of gram-negative bacteria (GNB) in 2 of the 15 cases and abundant growth of other bacteria in most of the RHD samples, needing further identification. Further metagenomic sequencing is on going to identify these bacteria.

Conclusions: It is not known if these bacteria shed antigenic proteins that may stimulate further cross reactive immune response, driving progression of RHD. Metagenomic sequencing will be necessary to identify and differentiate the GNB and the other bacteria growth found in the study participants and their potential role in RHD. These may help inform if these bacteria contribute to subsequent immune activation and progression of RHD and guide future management of RHD. Identification and treatment of other microbiota may improve outcome of RHD management.