Cape Higher Education Consortium (CHEC) and City of Cape Town (CCT)
CHEC – CCT RESEARCH PROGRAMME

Do non-conventional types of respiratory control (Queen Charlotte, buff, bandana) reduce the infectiousness of patients with tuberculosis?

Progress Report for period ending February 2020

Professor Grant Theron
Clinical Mycobacteriology & Epidemiology (CLIME) Group
Division of Molecular Biology and Human Genetics
Faculty of Medicine and Health Sciences (FMHS)
Tygerberg Campus, Stellenbosch University, Cape Town, South Africa

E-mail: gtheron@sun.ac.za

Telephone: 021 938 9693

1. ABSTRACT

The abstract should highlight the value of the project to knowledge generation in universities and its linkages to the priorities of the CCT.

Tuberculosis (TB) is an airborne disease spread through person-to-person transmission. To reduce TB burden, transmission needs to be interrupted. This is especially true for extensively- and multi-drug resistant TB. The City of Cape Town aims to reduce TB transmission (and other airborne diseases) by promoting the use of alternative forms of respiratory protection (RP) that are more acceptable by communities outside health facilities, however, need data on if these RPs reduce infectiousness. This study was initiated pre-COVID-19.

We aimed to test the degree to which different RP reduce respirable infectious *Mycobacterium tuberculosis* (*Mtb*) particles in aerosols made naturally by TB patients. We did this using a Human Aerosol Chamber that simultaneously captures infectious aerosols on solid media using a Anderson Cascade Impactor and in liquid media using SKC Biosamplers. These can be cultured (MGIT 960 culture) or quantified using molecular tests like Xpert MTB/RIF Ultra. Digital data such as particle counts, humidity and CO_2 were also captured. We did this for both tidal breathing and coughing for TB-positive pre-treatment patients (n=28) who were allocated to wear a form of RP (surgical mask=8, queen charlotte=8, cyclist buff=8, bandana=4) and compared the values obtained on an adjacent day when no RP was worn.

We found that 6/28 and 5/28 (p=0.736) patients were positive (by either ACI, MGIT and/or Ultra) for tidal breathing, 12/28 and 7/28 (p=0.158) for spontaneous cough and 15/28 and 7/28 (p=0.028) for forced cough with NRP and RP respectively. Particle counts (median interquartile range) for tidal breathing with NRP and RP were 115 (27.2-21500) vs IQR: 8260 (19.3-32600; p=0.610) respectively for tidal breathing, 65.2 (15.3-11600) vs. 27.3 (13-11300; p=0.461) for spontaneous cough and 65.2 (17.9-28075) vs 20.3 (13-18775; p = 0. 001) for forced cough, respectively.

Our results show that that non-conventional RPs might be effective at reducing culturable respiratory particles in TB patients, however, larger sample sizes are needed to accurately determine effectiveness, especially across different respiratory manoeuvres.

2. INTRODUCTION AND PROJECT AIMS / QUESTIONS

The TB pandemic is driven by person-to-person transmission [1]. Transmission requires inhalation of aerosolised droplets containing *Mycobacterium tuberculosis* (*Mtb)* from a TB case by a susceptible contact [2]. While cough has been thought the main mechanism of TB transmission, a recent study showed TB patients generate respirable aerosols during normal (tidal) breathing [3]. We need to "turn off the tap" and stop the spread of TB, however, both the tools available to TB programmes to stop transmission and our understanding of the infectiousness of TB patients are limited. One major reason for this knowledge deficit is that research on the actual infectious aerosol from patients is technically challenging (as opposed to surrogate markers like patients' sputum, which have well-known limitations [4]). As a result, international funding and policy agencies have issued calls for new methods to understand the infectiousness of TB patients and study their aerosol specifically [5-7].

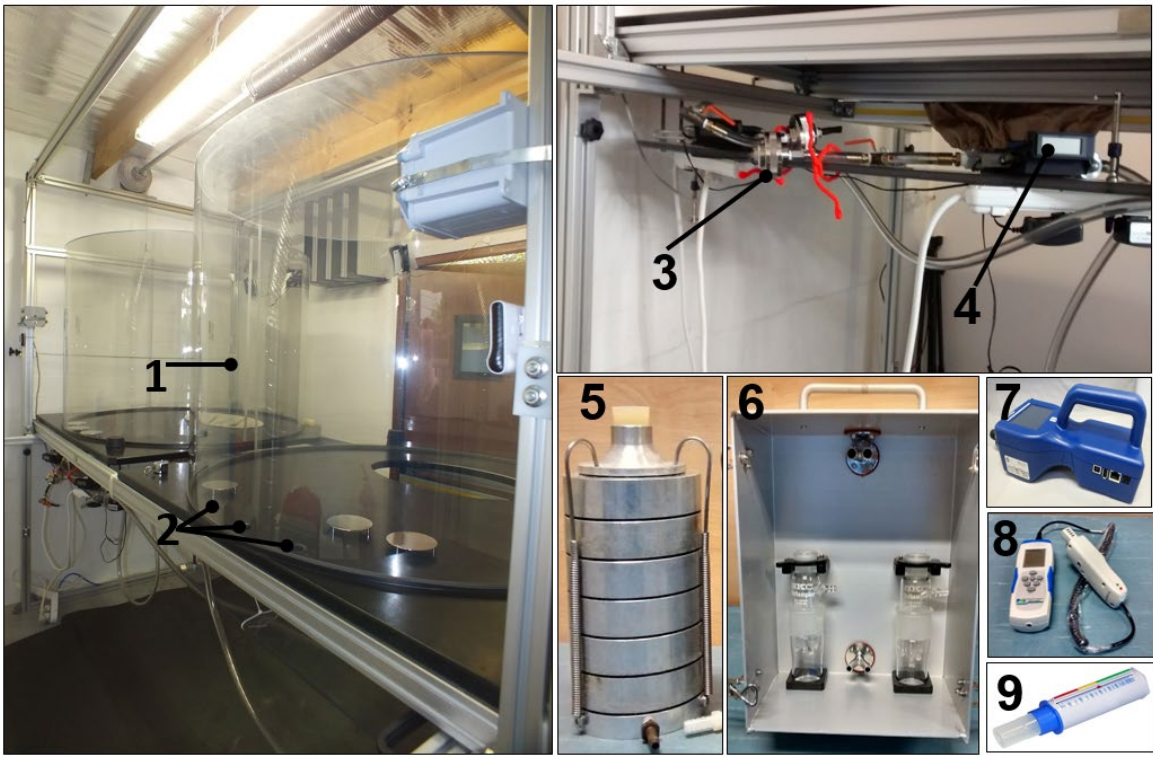

Figure 1: The human aerosol chamber (HAC), comprised of 1) 950L transparent polycarbonate cylinders in which patients sit, 2) Ports for aerosol sampling, 3) Knob to operate aerosol accumulation and collection, 4) Air-flow regulator, 5) Six-stage Andersen Cascade Impactors containing agar plates (plates 3-6 collect particles that deposit in the lower airways), 6) SKC-Biosamplers for the capture of aerosol for growth in culture and for Xpert MTB/RIF Ultra , 7) Particle counter (TSI 9306), 8) Monitor for CO2, humidity and temperature, and 9) Peak-flow meter. These platform, partly enabled through this award, enables a comprehensive approach to measure the infectiousness of TB cases, with or without respiratory protection.

Partly in response to this call, we have designed a Human Aerosol Chamber (HAC) that consists of a polycarbonate chamber for accumulation and sampling of TB patient's aerosols for TB diagnosis (**Figure 1**). HAC includes use of an Anderson Cascade Impactor (ACI) which contains solid agar plates to capture aerosols, a case containing two SKC Biosamplers to capture aerosols in liquid as well as digital monitoring tools such as a particle counter and CO_2 monitor.

Figure 2: Types of included respiratory control. 1) Queen Charlotte face mask, 2) Surgical mask, 3) buff 4) bandana.

In addition to the aforementioned knowledge gaps, there are surprisingly few data on the efficacy of surgical masks on reducing the infectiousness of TB cases: one study showed that transmission to guinea pigs exposed to air exhausted from a ward was reduced by 56% when patients wore masks [8, 9]. Although surgical masks are widely recommended for routine infection control, many TB programmes in low resource settings cannot afford to provide them, especially to patients who are suspected of TB but not yet confirmed. Versions of respiratory protection (RP) that are alternatives to surgical masks are orders of magnitude cheaper (e.g., paper "Queen Charlotte" masks) and are used routinely in some settings, especially in China, however, there is no evidence they are effective, especially when patients cough forcefully. Importantly, many patients report not wearing a surgical mask

outside of TB clinics (even when masks are freely available), primarily for reasons of stigma [9, 10]. One way to approach this is to evaluate the effectiveness of alternative, potentially less-stigmatizing forms of respiratory control (e.g., buffs). The City of Cape Town, Department of Health plans to distribute these at clinics and community centres as part of community-wide efforts to promote destigmatizing forms of infection control for TB. The aim is thus to use HAC to test the degree to which different types of RPs (**Figure 2**; surgical mask, Queen Charlotte, bandana and cyclist buff) can reduce the spread of respirable infectious *Mtb* in aerosols of TB patients using different microbiological and digital readouts during tidal breathing and coughing (both spontaneous and forced). This finding will provide evidence to support the roll-out of alternative forms of respiratory control.

3. RESEARCH APPROACH AND METHODS:

A short summary of the approach and methods used should be included, but detailed descriptions of highly technical processes may be omitted.

Patients were eligible for the study if they had a positive Xpert MTB/RIF Ultra test with a semi-quantitation ≥ medium, were ≥ 18 years, had no TB treatment and signed informed consent. The patients were asked to enter HAC on 2 consecutive days, one while wearing RP and the other wearing no RP (NRP) to use as a baseline reading of infectiousness. Both the type of RP worn (surgical mask, Queen Charlotte, cyclist buff or bandana) and the day on which the RP was worn (day 1 or 2) was randomised. Aerosols captured on solid media using the ACI were incubated and monitored for colony forming units (CFUs). Aerosols captured in liquid using the SKC Biosamplers were used for liquid culture using the BACTEC MGIT 960 system as well as for molecular testing using the Xpert MTB/RIF Ultra test. Digital data (particle counts, CO_2 readouts, humidity, temperature and counts of coughs etc. were also collected. Patients were sampled in 20 min sessions on each day for tidal breathing (Phase 1) spontaneous cough (Phase 2) and forced cough (Phase 3) and had a 40 min rest period between each phase. At the end of the reporting period, we had recruited n=28 TB-positive patients (surgical mask=8, queen charlotte=8, cyclist buff=8 and bandana=4).

4. CONCLUSIONS AND RECOMMENDATIONS FOR FOLLOW-UP ACTION

This is the most critical aspect of the report in that it needs to synthesise the findings, draw conclusions and suggest follow-up actions, e.g. for further research, policy development, programme implementation etc.

Microbiological testing on aerosol

Overall (respiratory protection and phases combined)

Of the 28 participants, 8/28 (29%) were tested for surgical mask, queen charlotte and cyclist buff and 4/28 (14%) were tested with the use of a bandana. It is to be noted that the bandana was excluded from further testing as it was shown to be less effective at an early stage. We found that 6/28 and 5/28 (p=0.736) were positive (using either ACI, MGIT and/or Ultra) for tidal breathing, 12/28 and 7/28 (p=0.158) for spontaneous cough and 15/28 and 7/28 (p=0.028) for forced cough with NRP and RP respectively (**Table 1**). Overall (all RP and phases combined), we found a significant reduction in particle counts overall when comparing NRP to RP [median IQR: 186 (43-58250) vs 73 (27-62825); p=0.004 (**Table 1**)]. Furthermore, we found a trend towards reduction in aerosols using NRPs compared to RP when looking at CFUs on ACI (15/84 vs 8/84; p=0.171, and Ultra on aerosols trapped in liquid (29/84 vs. 22/84;

p=0.240, **Table 1**). MGIT 960 liquid culture had 5/84 vs 4/84 (p=0.732) for NRP vs RP respectively.

Comparison of aerosols across different respiratory maneuvers when using respiratory protection (all RP combined)

There was a significant reduction in particle counts when using RP vs NRP for forced cough (**Figure 1a**; p=0.001) and a similar trend was seen for spontaneous cough (**Table 1**). Similarly there was a trend towards reduction in CFU and Ultra testing on aerosol trapped in liquid when using RP vs NRP for both spontaneous and forced cough, though numbers were too small to show significance (**Table 1**; **Figure 1b, c and d**). Numbers of positive MGIT 960 liquid culture were too low to conclude anything (**Figure 1e**).

Comparison of aerosols when using various types of respiratory protection (all phases combined)

When looking at RPs individually it was found that when using surgical masks (SM), a significant reduction when wearing RP was seen for forced cough and a similar trend was seen for spontaneous cough. Overall (all phases combined) aerosol positive cases with and without SM detected by CFUs on ACI [6/24 vs. 4/24; p=0.540], Ultra [9/24 vs. 7/24; p=0.540] and MGIT960 [3/24 vs. 1/24; p=0.300] were found to be similar with a trend of reduction after using SM (**Table 2**). Interestingly the same proportion of positive cases were detected using Ultra for both NRP and RP during forced cough when using SM. Similar results were seen for Cyclist Buff (CB; Table 3). However, after using queen charlotte (QC), aerosol positive cases with and without QC were found to be similar (Table 4). Bandana (BD) was compared on only four study participants, aerosol positive cases with and without and were found to be similar after using BD and was excluded at an early stage (Table 5).

Conclusion

While our data suggests that certain RPs (SM, CB) may be useful at reducing *Mtb* positive aerosol compare to NRP, further investigation is needed, and we have now provided the preliminary data to justify such investigations. This will be important to inform important safety strategies that garner less stigma, not only for the current TB pandemic but also for any other pandemics that are reliant on the spread of aerosols.

Table 1: The effectiveness of using a respiratory control by TB patients that were *Mtb* aerosol positive with and without respiratory protection (RP) during different respiratory manoeuvres (tidal breathing, spontaneous cough and forced cough) was compared to a baseline NRP (no respiratory protection).

OVERALL	**Overall (all phases combined)**		**Tidal breathing**		**Spontaneous cough**		**Forced cough**	
	NRP	RP	NRP	RP	NRP	RP	NRP	RP
Particle counts ×10^6	26/26	26/26	19/26	19/26	26/26	26/26	26/26	26/26
median (IQR)	186 (43-58250)	73 (27-62825) ***0.004***	115 (27.2-1500)	8260 (19.3-32600) *0.609*	65.2 (15.3-11600)	27.3 (13-11300) *0.426*	65.2 (17.9-28075)	20.3 (13-18775) ***0.001***
ACI aerosol positive	15/84	8/84 *0.116*	3/28	3/28 *1.000*	5/28	1/28 *0.084*	7/28	4/28 *0.313*
CFU median (IQR)	5 (2 – 37.5)	4.5 (1 – 9.8) *0.171*	5 (3 – 18.3)	2 (1 – 9) *0.400*	5 (2 – 56.4)	7 (7 – 7) -	9 (2 – 75)	5.5 (1 - 13.2) *0.410*
Ultra-positive	29/84	22/84 *0.240*	6/28	6/28 *1.000*	12/28	7/28 *0.158*	11/28	9/28 *0.577*
IS6110/1081 C_{Tmin} median (IQR)	26.7 (25.2 – 29.5)	26.4 (23.3– 27.7) *0.210*	27.4 (26.7 – 37.0)	26.8 (25.7 -32.7) *0.132*	26.6 (23.7 – 32.7)	25.1 (18.5 – 29.0) *0.385*	24.4 (26.5 – 28.0)	26.1 (21.2 – 27.8) *0.565*
rpoB C_{Tmin} median (IQR)	33.2 (30.1 – 35.3)	30 (26.6 - 33.4) *0.154*	-	34.6 (34.6 - 34.6)	33.9 (28.8 -36)	26.2 (25.5 – 26.9) *0.071*	31.6 (31.1 – 34.5)	30.7 (29.2 – 33.0) *0.400*
MGIT aerosol positive	5/84	4/84 *0.732*	2/28	1/28 *0.553*	1/28	3/28 *0.299*	2/28	0/28 *0.150*
TTP median (IQR)	18.2 (14.6 - 21.4)	19.3 (17.5 – 21.5) *0.730*	18.4 (14 – 22.8)	19.5 (19.5 – 19.5) -	20.0 (20.0 – 20.0)	19.1 (16.9 - 22.1) -	16.7 (15.1 – 18.2)	- -
Aerosol positive by either CFU, Ultra and/or MGIT	20/28	16/28 *0.4990*	6/28	5/28 *0.736*	12/28	7/28 *0.158*	15/28	7/28 ***0.028****

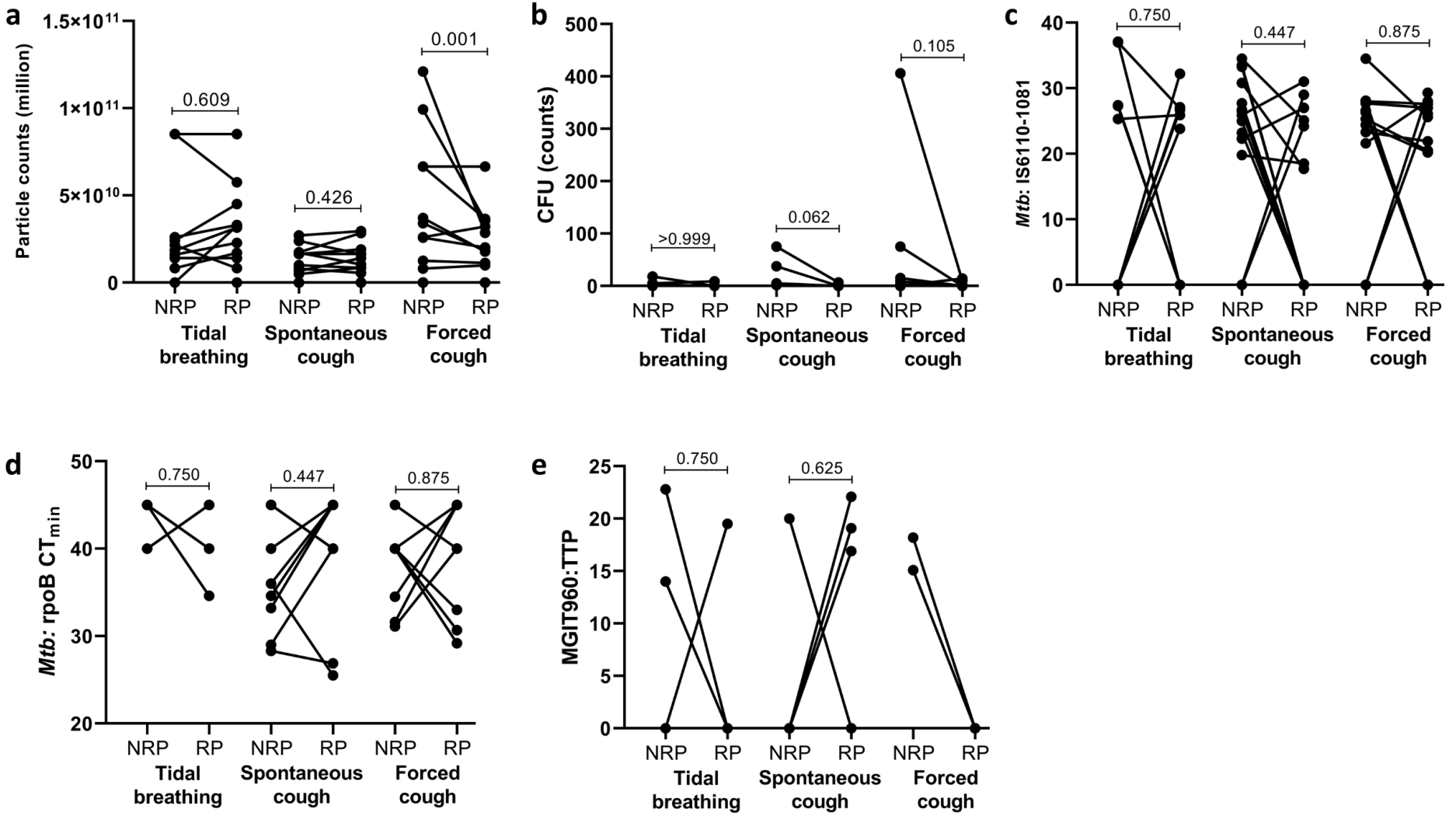

Figure 1: The effectiveness of using a respiratory control during any respiratory manoeuvres was compared to a baseline NRP (no respiratory protection). Different methods were used to test for aerosol infectiousness such as particle counts (a), CFU (b), MTB/RIF Ultra (c & d) and MGIT/TTP (e).

5. BUDGET

Please reflect the actual spend against the budget included in the original proposal. Please indicate the amount of any unspent monies. A table format would be appreciated.

Details of budget spent (2019-2020)		
	Detailed description of budget line item	**Amount spent**
a.	**Community health worker** *(to assist with recruitment, consent, and sampling; "soft funded")*	**15 000**
b.	**Participant compensation**	**7 500**
c.	**Transport costs**	**2 500**
d.	**Specimen microbiology costs: culture and Xpert Ultra of captured aerosol and sputum**	**7 500**
e.	**Stipend for PhD student**	**7 500**
f.	**Upkeep and single use consumables for Human Aerosol Chamber**	**5 000**
g.	**Publication and dissemination costs (hosting a talk and workshop)**	**5 000**
TOTAL		**50 000**

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