Bio-aerosol production by patients with tuberculosis during normal tidal breathing: implications for transmission risk

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What is the key question?

Does the presence of active tuberculosis increase bio-aerosol production during normal tidal breathing in a particle size range that could plausibly transport *Mycobacterium tuberculosis*?

What is the bottom line?

Our study provides the first evidence that intra-thoracic tuberculosis increases bio-aerosol particle production in a particle size range that could plausibly transport *M. tuberculosis* and that there is substantial variation in production within tuberculosis patients that may plausibly relate to the degree of infectivity.

Why read on?

Our findings suggest that measures of bio-aerosol production may contribute to assessments of infectiousness and TB transmission risk.

ABSTRACT

Background: The size and concentration of exhaled bio-aerosols may influence tuberculosis transmission risk. This study piloted bio-aerosol measurement in patients with tuberculosis and assessed variability in bio-aerosol production during normal tidal breathing. Understanding this may provide a tool for assessing heterogeneity in infectivity and may inform mathematical models of tuberculosis control practices and policies.

Methods: Optical particle counter technology was used to measure aerosol size and concentration in exhaled air (range 0.3-20µm in diameter) during 15 tidal breaths across four groups over time: healthy/uninfected, healthy/M.tuberculosis-infected, patients with extra-thoracic tuberculosis and patients with intra-thoracic tuberculosis. High-particle production was defined as any 1-5µm-sized bio-aerosol count above the median count among all participants (median count=2 counts/L).

Results: Data from 188 participants were obtained pre-treatment (baseline). Bio-aerosol production varied considerably between individuals. Multivariable analysis showed intra-thoracic tuberculosis was associated with a three-and-a-half-fold increase in odds of high production of 1-5µm bio-aerosols (adjusted OR: 3.5; 95%CI: 1.6-7.8; p=0.002) compared to healthy/uninfected individuals.

Conclusions: We provide the first evidence that intra-thoracic tuberculosis increases bio-aerosol production in a particle size range that could plausibly transport M. tuberculosis. There is substantial variation in production within tuberculosis patients that may conceivably relate to the degree of infectivity. Further data is needed to determine if high bio-aerosol production during tidal breathing is associated with infectiousness.

[WORD COUNT: 215 WORDS]

INTRODUCTION

An estimated 9.1 million tuberculosis cases occurred worldwide in 2013 and global incidence is declining at less than 2% per year [1]. There are a reported 450,000 new cases of multi-drug-resistant (MDR) tuberculosis (defined as Mycobacterium tuberculosis resistant to at least rifampicin and isoniazid) worldwide who remain infectious for longer periods of time[1]. This highlights the importance of identifying better markers of infectivity which would particularly relevant for informing control. Molecular typing studies have shown some individuals generate high numbers of secondary cases [2]. This may be due to: behavioural factors; increased transmissibility of infecting strains; or host biological/clinical factors affecting how many infectious particles each patient generates. Aside from sputum-smear positivity, presence of cough, pulmonary cavitatory disease and laryngeal disease, which are known to be strongly associated with infectivity, other determinants of tuberculosis infectivity are poorly defined.

The size and concentration of exhaled particles may influence respiratory infection transmission risk [3]. Defining a particle diameter cut-off at which aerodynamic particle behaviour changes is not possible, although the WHO uses 5µm to distinguish between droplet and airborne transmission [4]. Bio-aerosols (<5µm) are responsible for airborne transmission. These smaller bio-aerosols once expelled remain suspended in air for long periods of time exposing a greater number of contacts at greater distances to potential infection [3 5-7]. They are formed in the lower respiratory tract and contain lung mucus, surfactant and pathogens [8-10]. Whilst a single cough predominantly produces large droplets measuring greater than 5µm [7] it also produces large numbers of bio-aerosols [8 11 12]. However as normal breathing is continuous, the majority of bio-aerosols are in effect produced by normal breathing,[11-14] with some studies reporting up to 10,000 bio-aerosols per litre[15].

Evidence suggests bio-aerosols contribute to infectious disease transmission [10 15-19]. Bio-aerosols with a critical size range of 1-5µm, once inhaled, have a higher probability of reaching and depositing in alveolar regions than those greater than 5µm [20]. The *Mycobacterium tuberculosis* bacillus is 0.2-0.5µm wide and 2-4µm long and so it is bio-aerosols measuring 1-5µm that may plausibly serve as vehicles of this pathogen, permitting infection of alveolar macrophages in distal portions of lungs [8]. Given tuberculosis transmits via bio-aerosols and most bio-aerosols measuring less than 5µm are

produced during normal breathing, variations in bio-aerosol production during normal tidal breathing may be a determinant of infectivity.

Nicas *et al* [21] defined super-spreaders, "as those infrequently encountered persons with high cough and/or sneeze frequency, elevated pathogen concentration in respiratory fluid, and/or increased respirable aerosol volume per expiratory event such that their pathogen emission rate is much higher than average". Modelling studies suggest super-spreading is a normal feature of many infections and have demonstrated substantial heterogeneity in transmission for various respiratory infections [22 23]. Yet this feature had until recently been largely neglected in transmission models. The lack of data to inform parameters on individual variability in infectiousness over time has been highlighted [24]. Tuberculosis control is highly reliant on transmission models to inform national guidance. Such models may provide more accurate predictions if variations in infectiousness are explicitly considered.

Contact tracing decisions are often guided by perceived infectiousness of index cases and may be based on presence of sputum smear-positive disease and extent and duration of cough [25]. Additional criteria to identify particularly infectious cases could help target contact tracing activities.

Large-scale prospective studies of tuberculosis cases and their contacts are required to assess the importance of variations in bio-aerosol production on transmission. We have already shown the feasibility of measuring bio-aerosols in healthy volunteers using optical particle counter (OPC) technology that measures the size and concentration of particles in exhaled breath during normal tidal breathing [5]. This study makes an important next step by piloting OPC technology in patients with intra-thoracic, extra-thoracic, and latent tuberculosis compared to healthy controls.

METHODS FOR DATA COLLECTION

Bio-aerosol production measurements were obtained from four groups: healthy/un-infected volunteers recruited as a convenience sample of university personnel (group 1); healthy/*M.tuberculosis-infected* patients: non-infectious with no evidence of active tuberculosis as evidenced by a significant reaction (≥10 mm induration) to a Mantoux tuberculin skin test and/or a positive interferon-γ-release assay (IGRA) result (group 2); patients with active extra-thoracic tuberculosis (clinical, radiological and pathological evidence of granulomatous lesions external to

thoracic cavity and positive cultures for *M. tuberculosis* but with no clinical or radiological evidence of pulmonary or hilar or mediastinal involvement) (group 3); patients with active intra-thoracic tuberculosis (radiological and pathological evidence of lung cavitation and/or caseating and non-caseating granulomas, tubercles and fibro-caseous lesions in lung and/or hilar and/or mediastinal lymph nodes) confirmed by positive cultures and/or PCR for *M. tuberculosis*(group 4). We grouped those classified as pulmonary or intrathoracic as there was a high degree of overlap and it can be difficult to exclude pulmonary involvement in mediastinal disease.

Group 1 was recruited during the previous study measuring bio-aerosol production by healthy volunteers [5]. Groups 2-4 were recruited from University College London NHS Foundation Trust (UCLH) TB service. Active tuberculosis patients were enrolled at the start of anti-tuberculous treatment (at baseline) and followed-up every 4-8 weeks over the course of treatment. Bio-aerosol measurements were also obtained from active cases during the course of treatment and similarly followed up. Repeated cycles of bio-aerosol measurements were obtained from all participants (healthy volunteers and tuberculosis patients).

Data on age, smoking history, height, weight, immunological markers (C-reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR), HIV status, sputum-smear status, presence of prior BCG vaccination, anti-tuberculous treatment start and end dates and any chronic illness were extracted from patient notes. Presence or absence of latent tuberculosis infection (based on Mantoux testing and/or IGRA testing) in contacts was obtained through record review.

Bio-aerosol measurement procedure

The OPC device (Exhalair [model *102580-AK*], Pulmatrix Inc. Lexington, Massachusetts, USA), measured aerosol size and concentration using OPC coupled with respiratory flow rate and volume measurements.

Participants breathed into a disposable mouthpiece whilst wearing a nose clip to prevent nasal breathing. One-way valves and bacterial/viral High Efficiency Particulate Air (HEPA) filters in disposable tubing prevented inhalation of infectious particles from the environment. Exhaled particles (range of 0.3 to 20µm in diameter) over the course of 15 tidal breaths were measured (marked in green in Figure 1).

Three bio-aerosol measurement cycles were obtained at each participant session from the start of anti-tuberculous treatment (at baseline) and at repeated follow-up sessions every 4-8 weeks (during normal scheduled out-patient visits) over treatment course. Each measurement cycle commenced with an initial calibration to reduce measurement error. Following initial calibration, a washout period followed (which included three deep breaths to clear any ambient particles from the respiratory tract). Exhaled bio-aerosols were collected and arranged into four channels according to size ranges: ≥0.3 to ≤0.5µm; >0.5 to ≤1µm; >1 to ≤5µm; >5µm. Each cycle lasted up to two minutes to complete.

Ethical approval for the initial cohort study for group 1 participants was received by University College London Ethics Committee (reference number 1564/001). Ethical approval for groups 2-4 was secured from National Research Ethics Service (NRES) – City & East (REC study reference number 11/LO/1601) and R&D approval from UCLH/UCL/RF Joint Research Office (reference number 11/0256). All participants provided written informed consent.

STATISTICAL ANALYSIS STRATEGY

The dataset included up to 3 bio-aerosol measurement cycles per participant per session, each representing the average number of bio-aerosols exhaled over the course of 15 tidal breaths. Given the right-skewed distribution of the 1-5µm bio-aerosol count/L, the data were log transformed and normality was assessed using kernel density plots. The correlation between repeated measurement cycles (1st vs. 2^{nd}, 1^{st} vs. 3^{rd} and 2^{nd} vs. 3^{rd}) were calculated using linear regression models of log-transformed bio-aerosol count data using the Stata *regress command.* Since this indicated greater correlation between 2^{nd} and 3^{rd} readings than between first readings and subsequent readings we used the mean of the second and third readings as the main measure of bio-aerosol production for each session.

The distribution of 1-5µm bio-aerosol counts and log transformed counts across the four clinical groups were explored using histograms. The geometric mean counts and 95% confidence intervals for these groups were also compared. We focused on this particle distribution size as an *M.tuberculosis* is 0.2-0.5µm wide and 2-4µm long so this size of bio-aerosol particles would be plausibly expected to be involved in transmission of *M.tuberculosis* bacilli. Comparison of bio-aerosol counts in each of the four groups was explored further by comparing geometric means counts and 95% confidence intervals.

We explored the effect of demographic, clinical, and microbiological risk factors on 1-5µm and submicron bio-aerosol production patterns at baseline using stepwise selection for logistic regression models, using bio-aerosol counts as a binary outcome variable (above and below the median count) to control for confounders. The explanatory variables considered included gender, age, ethnicity, immigrant status (i.e. UK-born or not), current smoking status, BCG vaccination status, sputum smear status and environmental factors including season, indoor and outdoor temperature and humidity. Univariate models were initially built and variables which predicted the outcome and were associated with the main exposure of interest (as defined by the 4 clinical groups' disease category) at $p<0.05$ were retained for multivariable analysis if they appreciably altered the crude odds ratios.

Clustering between repeat measurement sessions within individuals was adjusted for in the final multivariable model using robust standard errors.

Based on our previous work showing that the first reading within a measurement session was poorly correlated with second and third readings in the session, we excluded the first reading from

subsequent analyses [5]) All analyses were performed using STATA 13.0, IC (College Station, Texas, USA).

RESULTS

Baseline characteristics

A total of 188 participants were analysed at baseline (Table 1), of which 86 (45.7%) were healthy/un-infected individuals (group 1), 27 (14.4%) were healthy/*M.tuberculosis*-infected patients (group 2), 11 (5.9%) were diagnosed with extra-thoracic tuberculosis (group 3) and 64 (34.0%) were diagnosed with intra-thoracic tuberculosis (group 4). More than half of the cohort, 108 (57.5%) were female. The median age of participants was 33 years old.

Table 1: Descriptive characteristics of 188 individuals at baseline

	All (%)	Healthy/uninfected	Healthy/*M.tuberculosis*-infected	Extra-thoracic tuberculosis	Intra-thoracic tuberculosis
	n=188 n (%)	n=86 n (%)	n=27 n (%)	n=11 n (%)	n =64 n (%)
Explanatory variable					
Sex					
Male	80 (42.6)	24 (27.9)	15 (55.6)	6 (54.6)	35 (54.7)
Female	108 (57.5)	62 (72.1)	12 (44.4)	5 (45.5)	29 (45.3)
Age group					
18-29	71 (37.8)	33 (38.4)	13 (48.2)	6 (54.6)	19 (29.7)
30-39	57 (30.3)	24 (27.9)	8 (29.6)	4 (36.4)	21 (32.81)
40-49	36 (19.2)	17 (19.8)	4 (14.8)	1 (9.1)	14 (21.9)
≥50	21 (11.2)	10 (11.6)	2 (7.4)	0 (0.0)	9 (14.1)
Missing	3 (1.6)	2 (2.3)	0 (0.0)	0 (0.0)	1 (1.6)
Ethnicity					
White	96 (51.1))	70 (81.4)	9 (33.3))	0 (0.0)	17 (26.6))
Non-white	87 (46.3)	16 (18.6))	17 (63.0))	11 (100.0)	43 (67.2))
Missing	5 (2.7)	0 (0.0)	1 (3.7)	0 (0.0)	4 (6.3)
Immigrant status[b]					
UK born	33 (17.6)	2 (2.33)	11 (40.7)	2 (18.2)	18 (28.1))
Non-UK born	65 (34.6)	6 (6.98)	15 (55.6)	6 (18.2)	38 (59.4))
Missing	90 (47.87)	78 (90.7)	1 (3.7)	3 (27.3)	8 (12.5)

	All	Healthy/uninfected	Healthy/*M.tuberculosis*-infected	Extra-thoracic tuberculosis	Intra-thoracic tuberculosis
	n=188 n (%)	n = 86 n (%)	n = 27 n (%)	n = 11 n (%)	n = 64 n (%)
Explanatory variable					
Current smoking status					
Non-smoker	109 (58.0)	36 (41.9)	18 (66.7)	8 (72.7)	47 (73.4)
Smoker	79 (42.0)	50 (58.1)	9 (33.3)	3 (27.3)	17 (26.6)
Previous BCG vaccination[b]					
No	41 (21.8)	NA	NA	3 (27.3)	38 (62.3)
Yes	30 (16.0)	NA	NA	7 (63.6)	23 (37.7)
Missing	117 (62.3)	86 (100.0)	27 (100.0)	1 (9.1)	3 (4.7)
Sputum smear status[a]					
Negative	31 (16.5)		NA	4 (36.4))	27 (42.19)
Positive	8 (4.3)	NA	NA	0 (100.0)	8 (12.5)
Missing	63 (33.5)	86 (100.0)	27 (100.0)	7 (63.6)	29 (45.3)
Comorbidity					
No	164 (87.2)	86 (100.0)	27 (100.0)	7 (63.6)	44 (68.8)
Yes	24 (12.8)	0 (0.0)	0 (0.0)	4 (36.4)	20 (31.3)
Season					
Winter, Dec-Feb	57 (30.3)	43 (50.0)	5 (18.5)	0 (0.0)	9 (14.1)
Spring, Mar-May	32 (17.0)	5 (5.8)	5 (18.5)	4 (36.4)	18 (28.1)
Summer, Jun-Aug	48 (25.5)	25 (29.1)	4 (14.8)	2 (18.2)	17 (26.6)
Autumn Sep-Nov	51 (27.1)	13 (15.1)	13 (48.2)	5 (45.5)	20 (31.3)

NA = data was not collected; [a]sputum smear status – this is only routinely obtained for suspected intra-thoracic patients; [b]data on immigrant status and previous BCG vaccination were not collected from convenience sample of healthy volunteers

Distribution of 1 to 5µm **diameter bio-aerosols in exhaled air**

Bio-aerosols in the 1-5µm diameter range expired during normal tidal breathing at baseline by all participants formed a highly right-skewed log-normal distribution. Correlation coefficients from linear regression models showed second and third bio-aerosol measurements from within a single session were most strongly correlated, where the correlation coefficient was 0.6 (95% CI: (0.5 - 0.7; p<0.001). Subsequent analyses focus on these measurements as the first measurements within a session appeared to be less consistent. The first measurements were excluded from subsequent analyses

Comparison of distributions of log transformed 1-5µm bio-aerosol counts across all clinical groups showed participants diagnosed with active tuberculosis (irrespective of intra-thoracic or extra-thoracic disease) had higher baseline bio-aerosol counts than those of healthy/*M.tuberculosis*-infected or healthy/uninfected participants (Figure 2). On comparison, the geometric mean for healthy/uninfected participants 36.3 counts/L (95% CI: 30.1 - 43.8); for healthy/*M.tuberculosis*-infected participants 29.7 counts/L (95% CI: 16.9 - 52.4); for active extra-thoracic cases was 52.6 counts/L (95% CI: 16.7 - 166.3); for active intra-thoracic cases was 67.6 counts/L (95% CI: 46.3 - 98.8).

Risk factors associated with 1 to 5µm diameter bio-aerosol production

High particle production was defined as any particle count above the median particle count among all study participants (median 1-5µm count = 2 counts/L). Table 2 shows the relationship between 1-5µm bio-aerosol count as a dichotomous outcome variable and risk factors. In multivariable analysis, intra-thoracic tuberculosis was strongly associated with substantially increased odds of being a high-particle producer (adjusted OR: 3.5; 95% CI: 1.6 - 7.8); p=0.002) compared to healthy uninfected controls. There was also a suggestion that those with extra-thoracic or latent infection had increased odds of high particle production, although these trends were not significant and larger studies would be needed to investigate this further. Age group also remained significantly associated in the final model with those aged 40-49 being most likely to have high bio-aerosol counts (adjusted OR: 6.0; 95% CI: 2.5 - 14.2; p<0.001). There was insufficient data to examine trends in bio-aerosol production over treatment course.

Table 2: Final multivariable model: Relationship between 1 to 5µm bio-aerosol production and baseline characteristics across all groups

Risk factor	Number of study participants	Low particle-producers n (%)	High particle-producers n (%)	Crude odds ratio (95% CI)	Crude odds ratio p-value	Adjusted odds ratio[a](95% CI)	Adjusted odds ratio p-value
Healthy/uninfected controls	83	59 (71.1)	24 (28.9)	1		1	
Healthy/*M.tuberculosis*-infected	27	13 (48.2)	14 (51.9)	2.6 (1.2 - 5.8)	0.021	2.5 (1.0 - 6.5)	0.062
Extra-thoracic tuberculosis	11	5 (45.5)	6 (54.6)	3.1 (0.9 - 11.3)	0.086	2.7 (0.6 - 12.0)	0.184
Intra-thoracic tuberculosis	63	19 (30.2)	44 (69.8)	4.5 (2.3 - 8.6)	<0.001	3.5 (1.6 - 7.8)	0.002
Sex							
Male	81	39 (48.2)	42 (51.9)	1			
Female	107	58 (54.2)	49 (45.8)	1.1 (0.6 - 1.9)	0.823		
Age group							
18-29	70	41 (58.6)	29 (41.4)	1		1	
30-39	56	30 (53.6)	26 (46.4)	1.2 (0.6 - 2.3)	0.614	1.3 (0.7 - 2.6)	0.458
40-49	38	13 (34.2)	25 (65.8)	3.4 (1.6 - 7.3)	0.002	6.0 (2.5 - 14.2)	<0.001
≥50	21	12 (57.1)	9 (42.9)	2.1 (0.8 - 5.1)	0.121	2.2 (0.9 - 5.8)	0.096
Ethnicity							
White	94	62 (66.0)	32 (34.0)	1		1	
Non-white	89	33 (37.1)	56 (62.9)	2.93 (1.68 - 5.12)	<0.001	2.2 (1.0 - 4.7)	0.045

Risk factor	Number of study participants	Low particle-producers n (%)	High particle-producers n (%)	Crude odds ratio (95% CI)	Crude odds ratio p-value	Adjusted odds ratio[a](95% CI)	Adjusted odds ratio p-value
Current smoking status							
Non-smoker	109	53 (48.6)	56 (51.4)	1			
Smoker	79	44 (55.7)	35 (44.3)	1.0 (0.5 - 1.8)	0.913		

DISCUSSION

This is the first time bio-aerosol size distribution and variation during normal tidal breathing in intra-thoracic and extra-thoracic tuberculosis patients has been studied using OPC technology. Our findings demonstrate marked variability in bio-aerosol production by tuberculosis patients and provide evidence that those with intra-thoracic tuberculosis are more likely than healthy controls to produce high levels of bio-aerosols in the 1-5µm range. Substantial variation in bio-aerosol production between patients with intra-thoracic tuberculosis may, plausibly be associated with variations in infectivity, since *M.tuberculosis* is an obligate aerosol transmitter. Given that *M.tb* has been found in cough-generated aerosols of the same size range as measured in this study during normal breathing [16] and so it is conceivable that bio-aerosols produced during continuous tidal breathing could transport *M.tb* within a size range that could be inhaled by a susceptible contact and deposited in the distal portions of the lung eliciting M.tb infection. More research, however, is needed to define how such variations correlate with infectivity and transmission risk. Such research may include, for example, studies of the comparative contribution of normal breathing and explosive respiratory events such as cough or sneezing to bio-aerosol production, concentration of pathogen-laden and/or infectious bio-aerosols in exhaled breath, and larger studies assessing how baseline measures of bio-aerosol production in index cases relate to risk of infection in contacts. There is also a need to conduct larger scale research to assess whether bio-aerosol counts change through the course of treatment.

The mechanics of bio-aerosols generation vary depending upon respiratory manoeuvres, namely coughing, sneezing and normal tidal breathing. Bio-aerosols are formed through a rapid passage of airflow over the respiratory surfaces leading to shear forces along the mucous layer, creating wave-like disturbances hence bio-aerosol formation. In a healthy individual a typical cough has a bi-phasic profile, starting with an initial high velocity phase and followed by diminishing flow rate, lasting approximately 0.5 seconds. Coughing is likely to be infrequent and whilst it results in higher flow velocities, the predictive value of cough-generated aerosols over time and outcomes in susceptible contacts is not fully understood. Furthermore explosive respiratory manoeuvres like coughing predominantly generate larger bio-aerosols, depositing in the upper respiratory tract than in alveolar regions and so the assessment of cough alone may be a poor index of infectiousness. By virtue of low inertia, pathogen-laden bio-aerosols measuring less than 5µm produced by continuous normal tidal

breathing will travel greater distances potentially reaching a wider range of susceptible contacts than that produced by infrequent coughing.

It is widely accepted that the spread of *M.tuberculosis* from an index case with pulmonary or laryngeal tuberculosis to susceptible contacts underpins transmission of tuberculosis. Our findings show that the presence of intra-thoracic disease was associated with high bio-aerosol production during normal breathing. This offers additional insights into the pathophysiology of active tuberculosis and presents implications for the risk assessment of suspected index cases. Persistence of bio-aerosol production during normal tidal breathing over treatment course in tuberculosis patients may serve as an additional determinant of infectivity but more large-scale work to explore the relationship between bio-aerosol production and treatment needs to be done.

There are some study limitations: our study measures variations in bio-aerosol production but did not measure infectious particle concentration, limited numbers of cases with extra-thoracic disease limited conclusions in this group, the study was also not sufficiently powered to explain how additional clinical features affected bio-aerosol production, whether bio-aerosol production changed through treatment or to assess the impact of bio-aerosol production on risk of infection in contacts. We only assessed bio-aerosol production during normal tidal breathing and not during coughing and we do not know if the bio-aerosols we detected contained viable *M. tuberculosis* bacilli. There would also be valuable in repeating the work in high incidence settings and in settings where HIV is highly prevalent to determine if findings are generalizable. Due to the highly skewed nature of the data we chose to categorise bio-aerosol counts as above and below median rather than model it as a continuous variable. There is no prior literature on what level of bio-aerosol count should be considered high, so this cut-off is to some extent arbitrary. However it has the advantage of being a common and well understood measure of central tendency and one which helped maintain reasonable sized groups for comparison. . Nevertheless the study provides the first evidence that intra-thoracic tuberculosis increases bio-aerosol particle production in a particle size range that could plausibly transport *M. tuberculosis* and that there is substantial variation in production within tuberculosis patients that may plausibly relate to the degree of infectivity.

Further research on this phenomenon could inform tuberculosis control policy, for example through better mathematical model parameterisation, improved clinical risk assessment of index cases, targeting of infection control and outbreak investigation strategies and case management decisions for isolation of patients with newly emergent strains, and contact tracing measures.

[WORD COUNT: 3,449 WORDS]

Figure legend

Figure 1: Respiratory bio-aerosol measurment using optical particle counter technology using Exhalair system

Figure 2: Log-transformed distribution of 1 to 5µm bio-aerosol production across all 4 groups: healthy/uninfected individuals, healthy/M.tuberculosis-infected, patients with extra-thoracic tuberculosis and patients with intra-thoracic tuberculosis at baseline

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CONFLICTS OF INTEREST

None of the authors have a commercial or other association that might pose a conflict of interest

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