A pilot study to detect airborne *Mycobacterium tuberculosis* exposure in a South African public healthcare facility outpatient clinic

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Summary

Background: Airborne transmission of *Mycobacterium tuberculosis* (TB) remains an occupational health hazard particularly in crowded and resource limited healthcare settings. Aim: The study aimed to quantify airborne TB in a busy outpatient clinic in Gauteng, South Africa.

Methods: Personal (HCWs) and stationary air samples were collected in the Polyclinic and Administrative block. Quantitative real-time PCR was used to detect airborne TB. Walkthrough observations and work practices of HCWs were also recorded.

Findings: TB was detected in 11/49 (22.4%) of the 9/25 (36%) personal and 2/24 (8.3%) stationary samples. Samples from 5 of 10 doctors (50%) and 3 of 13 nurses (23%) were positive. Repeat measurements on different days showed variable results. Most of the HCWs (87.5%) with positive results had been in contact with coughing patients and had not worr respiratory masks despite been training.

Conclusion: The use of air sampling coupled with real-time qPCR is a simple and effective tool to demonstrate the risk of TB exposure. The findings provide an impetus for hospital management to strengthen TB infection prevention and control measures.

INTRODUCTION

Occupational exposure to *Mycobacterium tuberculosis* (TB) is a major health threat for all healthcare workers (HCWs), particularly in South Africa (SA) which is currently ranked third among high TB burden countries.[1,2,3] A study of South African public hospital staff records conducted between January 1999 and June 2004 illustrates this high incidence (1 133 per 100 000) in HCWs, particularly in, young adults (25-29 years).[4,5] Another study showed that South African HCWs were up to three times more likely to acquire TB than the general population (2% and 0.9% respectively) and are at increased risk for multi-drug resistant (MDR) or extensively drug resistant TB.[9,6,13] Inadvertent exposure to TB most often occurs in facilities with undiagnosed or insufficiently treated disease combined with inadequate infection prevention measures.

Studies to detect airborne TB exist, however there are few reports quantifying airborne TB. One study conducted in a hospital setting reported various TB concentrations ranging from 1.43 to 12.06×10^5 DNA copies/m^3.[7] Another study detected airborne TB in the rooms of six of seven patients whose sputum culture tested positive for TB.[8] These studies largely focused on the level of exposure in settings with known active cases of tuberculosis.[9,10] The current study investigated the feasibility and efficacy of using both personal and stationary air sampling devices in an outpatient clinic to assess the extent of airborne TB exposure.

METHODS

This study was conducted in the Polyclinic of a regional public health facility in Gauteng province, SA. The facility provides a weekday service from 7am to 4pm to approximately 170 patients with unknown TB status referred from the emergency department within the hospital, local clinics and other regional hospitals. The estimated waiting period before vital screening and consultation with medical officers is 1to 3 hours. HCWs who participated in the study included medical officers, nurses and phlebotomists.

Walkthrough: Prior to the sampling, a walkthrough was conducted by an occupational hygienist from an approved inspection authority (AIA) to obtain information regarding the Polyclinic's administrative control measures (policies for identifying, segregating coughing patients and training), engineering controls (natural and artificial ventilation, isolation

rooms, disinfection devices) and personal protective equipment (particulate respirator or mask).

Air sampling: A total of 49 air samples, plus eleven field controls were collected from 28th to 31st January 2014 (four days repeat measurements). The purpose of the field controls was to trace sources of contamination in the field by exposing the filters to the same field conditions as the samples. HCWs wore sterile 37-mm filter cassettes containing polytetrafluoroethylene (PTFE) membranes on the collar lapel in their breathing zone (Figure I). Personal samples were collected at a flow rate of 4 L/min, and stationary samples at flow rates of 4 L/min (n = 12) and 12 L/min (n = 12) respectively using low and high volume Gillian sampling pumps (SKC Inc., PA, USA). Stationary samples were collected at heights of 1.5m and 1m to represent the respirable zone of standing and/or seated patients. Sampling pumps were calibrated before and after sampling using a Gillibrator bubble flow

Figure 1. Worker wearing the personal air sampling kit used in the study (courtesy of Ms Buhle Binta).

meter (SKC Inc.PA, USA) and a less than 5% flow rate difference was accepted.[11] An IAQ – Calc TSI model (TSI Instruments Ltd, UK) was used to measure temperature (^{o}C) and relative humidity (% RH). The TSI VelociCalc model: 9555-p (TSI Instruments Ltd, UK) was used to measure the air flow per area. The samples were transported at ambient temperature to the Bioaerosol laboratory at the National Institute for Occupational Health (NIOH) and were stored at -70°C prior to analysis.

TB quantification using real-time PCR: PTFE membranes were extracted in a solution containing 1% Triton X-100 in 10 mM Tris-HCl, pH 8.0. TB was detected from the eluent (triplicate) using the AMPLICOR MTB quantitative real-time polymerase chain reaction (qPCR) assay and the LightCycler 1.5 instrument (Roche, Germany) according to manufacturer's instructions. Primers (KY 18 and KY 75) targeting the 200 bp region of IS6110 gene was used. The attenuated *Mycobacterium tuberculosis* H37Ra strain was used at concentrations 1×10^{8}, 1×10^{6}, 1×10^{4} and 1×10^{2} and 1×10^{1} cells/ml for the standard curve. The unknown TB concentrations were extrapolated from the curve using linear regression. Tests were only accepted if the controls passed and the efficiency of the standard curve was within the accepted limits according to the kit manufacturer. The 95% limit of detection (LOD) determined by probit analysis provided by the kit is 28 target copies/µl. Results were obtained within 3 hours from extraction of sample. For quality control laboratory positive and negative controls as well as a kit positive and negative control were included in the analysis. The final airborne concentration (TB DNA copies/m^{3}) was calculated using the number of TB DNA copies/ml, sampling time and flow rate.

Data analysis: Environmental data were captured in Excel, Microsoft 7 (Microsoft Office, USA) and information on work practices of HCWs were captured and analysed using Epi Info software, version 3.5.1. Descriptive analysis on demographic characteristics of participants and some risk factors was performed and compared with positive air samples.

Ethics approval: The study was approved by the regional hospital management and the research ethics committee, Faculty of Health Sciences, University of Pretoria in SA and the behavioural research ethics board at the University of British Columbia in Vancouver, Canada.

Table I. Characteristics of positive **Mycobacterium tuberculosis** results for stationary and personal air samples of healthcare workers

Sample ID	Positive sample location/occupation	Sample concentration (DNA copies/m^3)	Total time spent on direct patient care (h)	Contact with a coughing patient (yes/no)	Use a mask or respirator (yes/no)	Educated in respirator use (yes/no)
K7	Auxiliary nurse	1.73E+02	7	No	No	Yes
K10	Auxiliary nurse	4.33E+02	4	Yes	No	No
K20	Auxiliary nurse	1.97E+03	6	Yes	No	No
K22	Auxiliary nurse	3.00E+03	7	Yes	No	No
K8	Medical officer	3.22E+02	7.5	Yes	No	Yes
K9	Medical officer	8.00E+03	7.5	Yes	No	Yes
K11	Intern medical officer	2.77E+03	6	Yes	No	No
K12[a]	Medical officer	1.67E+04	7	Yes	No	Yes
K21[a]	Medical officer	3.63E+03	7	Yes	No	Yes
K6	Medical ward[b]	2.05E+02	na	na	na	na
K17	IT room[b]	6.57E+02	na	na	na	na

Seventy-five percent (12/16) of those with negative polymerase chain reaction results indicated that they encountered a coughing patient during the work shift: 44% (7/16) never wore respiratory protection, 31% (5/16) occasionally wore respiratory protection and 25% (4/16) did not disclose such information. Only 38% (6/16) were educated in respirator use and 13% (2/16) were uncertain of the training received. Of the 38% that were trained, one-third never wore respiratory protection, one-third occasionally wore respiratory protection and one-third did not respond.

IT, information technology; na, not applicable.

[a] Same person repeated sampling.

[b] Stationary sample.

RESULTS

Personal air samplers (n = 25) and stationary samplers (n = 24) were collected for approximately 396 and 449 minutes respectively. Eleven of the 49 (22.4%) air samples

tested positive for TB DNA of which nine (36%) were personal and two (8.3%) were stationary air samples (Table I). The TB status of these HCWs was not known as clinical testing of workers was not part of the study. Half of the physician samples (5/10; 50%) tested positive for TB compared with 23% (3/13) of the nurses' samples. One medical officer wore a sampler for three consecutive days and two of these repeats were positive (k12: 1.67×10^4, k21: 3.63×10^3 DNA copies/m^3). A nurse was also evaluated twice with a positive result on day 1 (k20: 1.97×10^3 DNA copies/m^3) and a negative result on day 2 (k46).

Eight stationary samples were collected from the medical wards with active TB patients to evaluate the sensitivity of TB detection and one tested positive. Eight samples were also taken from non-medical areas; one of these also tested positive for TB (the information technology room). All eight stationary samples from the polyclinic tested negative.

The ventilation system was not working during the walkthrough and survey. The facility relied on natural ventilation through open doors and windows, however, at the time of the walkthrough the windows were closed in the Polyclinic. There were also no HEPA filters or ultraviolet germicidal irradiation (UVGI) lights in use. The microclimatic parameters over the four day period of sampling did not vary significantly. The average temperature (^{o}C) in various sampling areas ranged between 25.7 and 28.9. The average relative humidity was between 47.4 – 55.6% and average airflow was 0.01-0.26m/s with the medical floors having the higher airflows.

DISCUSSION

While there is no definitive infectious dose for TB - as infection is linked to susceptibility of the individual and virulence of the microorganism - an estimated infectious dose has been reported as <10 bacilli in humans.[12-14] This is lower that airborne levels detected in this study. Thus this study objectively demonstrated that individuals working in confined spaces at close proximity to undiagnosed TB patients are indeed at risk. The study also found that while both nurses and medical officers (doctors) were exposed to TB, medical officers incurred greater exposure, possibly due to their restricted mobility compared with nurses as they performed their duties.

The results also demonstrated variability in airborne exposure depending upon parameters such as the number of patients on the clinic day and proportion who are infectious, compliance with respiratory etiquette (including masking a suspect patient), and primary job location of the individual being sampled. This variability emphasises the need for repeat measurements to provide a relative exposure profile over time. Although personal samples demonstrated exposure to TB bacilli, all the stationary samples in the Polyclinic were negative probably due to the dilution effect from patient movement and positioning of the sampler in a large space.

One of the samples had been collected in the ward of a HIV positive patient with progressive TB and suspected MDR-TB was positive despite the patient having been on treatment for seven days. The positively result could represent infectious particles in a patient not responding well to treatment, or alternatively this could be attributed to dead bacilli coughed up from necrotic lung cavities.[15-17] The fact that a sample in the area of another re-infected patient with similar medical conditions as the above patient but had been on an even shorter treatment was negative highlights the complex relationship between infectiousness of an individual and airborne TB concentrations. Apart from severity of disease and airborne TB levels, TB transmission is also affected by ventilation rates, humidity, temperature, and respirator usage, application of airborne disinfection (e.g. HEPA filtration, UVGI lights) and duration of exposure.

This study also showed that a supposedly low TB risk area (information technology (IT) room) yielded a positive result. This room is in a quiet area of the facility, approximately 50 m from the Polyclinic and on the floor level above, and is occupied only for short intervals. It is possible that a patient, visitor or unidentified staff member with active TB entered the room during the sampling period. This result highlighted those non-medical areas in a hospital including administration offices can pose a potential risk for TB transmission and should be considered in mitigation strategies.

Although UVGI fixtures had been installed, none were functioning at the time of sampling and no maintenance records were available for the past eight years. In the absence of UVGI, HEPA filtration or mechanical ventilation, the use of natural ventilation can also be effective

in reducing the risk of TB exposure (WHO, 2013). The use of natural ventilation in the Polyclinic was erratic but could be overcome by implementation of strict administrative measures in accordance with national or international guidelines. In SA, TB control strategies have appropriately focused on case management of patients presenting at clinics (diagnosis and treatment) however, there is scope for improvement on airborne infection control.[17] Engineering controls are a cornerstone of disease prevention in occupational health and a SA guideline on utilization of ultraviolet germicidal irradiation (UVGI) technology to control TB transmission recommends its use to minimize risk.[18] In a recent study conducted in Witbank, South Africa, the upper room UVGI showed approximately 86% efficacy in reducing airborne TB levels.[19]

Administrative controls strengthen engineering controls and provide policy and procedural support for disease prevention in HCWs.   Although 55% of the workers had been trained to use a respirator, none actually used them despite being exposed to coughing patients. In addition, surgical masks instead of N95 respirators were available in some work areas, which would provide some protection for the HCW and can also reduce TB transmission if supplied to coughing patients. A policy for regularly screening HCWs for TB was unavailable and a TB screening questionnaire was not used regularly.

The findings from the current study demonstrate that it is feasible to use personal and stationary air sampling coupled with real time quantitative PCR (qPCR) both to assess exposure risk to airborne tuberculosis and as a tool to effect change. Such investigations are most useful when combined with a site assessment that includes both workplace practices and physical parameters including CO_2 measurements as an indicator of inadequate ventilation.[20]   Future research should focus on the probability of TB infection/disease in workers exposed to airborne *Mycobacterium tuberculosis* (MTB) bacteria in congregate settings, as well as on barriers and facilitators implementing infection control measures.

Limitations of the study

The variability of repeat sampling could not be statistically determined due to the small sample size. It should be noted that the concentrations detected included both viable and non-viable organisms and therefore represents the estimated risk and not the actual risk.

Conclusion

Air sampling and qPCR analysis were useful in demonstrating airborne TB exposure in a busy outpatient facility and identifying risk areas not previously recognised. This can be a useful tool coupled with walkthrough assessments in monitoring and evaluating the effectiveness of control measures in work environments. In addition to simple engineering and administrative controls, more emphasis should be placed on compliance with respiratory protection when caring for undiagnosed patients.

Conflict of interest

The authors declare no conflict of interest.

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