# Variability of Infectious Aerosols Produced during Coughing by Patients with Pulmonary Tuberculosis

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***Rationale*: *Mycobacterium tuberculosis* is transmitted by infectious aerosols, but assessing infectiousness currently relies on sputum microscopy that does not accurately predict the variability in transmission.**

***Objectives*: To evaluate the feasibility of collecting cough aerosols and the risk factors for infectious aerosol production from patients with pulmonary tuberculosis (TB) in a resource-limited setting.**

***Methods*: We enrolled subjects with suspected TB in Kampala, Uganda and collected clinical, radiographic, and microbiological data in addition to cough aerosol cultures. A subset of 38 subjects was studied on 2 or 3 consecutive days to assess reproducibility.**

***Measurements and Main Results*: *M. tuberculosis* was cultured from cough aerosols of 28 of 101 (27.7%; 95% confidence interval [CI], 19.9–37.1%) subjects with culture-confirmed TB, with a median 16 aerosol cfu (range, 1–701) in 10 minutes of coughing. Nearly all (96.4%) cultivable particles were 0.65 to 4.7 μm in size. Positive aerosol cultures were associated with higher Karnofsky performance scores ($P = 0.016$), higher sputum acid-fast bacilli smear microscopy grades ($P = 0.007$), lower days to positive in liquid culture ($P = 0.004$), stronger cough ($P = 0.016$), and fewer days on TB treatment ($P = 0.047$). In multivariable analyses, cough aerosol cultures were associated with a salivary/mucosalivary (compared with purulent/mucopurulent) appearance of sputum (odds ratio, 4.42; 95% CI, 1.23–21.43) and low days to positive (per 1-d decrease; odds ratio, 1.17; 95% CI, 1.07–1.33). The within-test (kappa, 0.81; 95% CI, 0.68–0.94) and interday test (kappa, 0.62; 95% CI, 0.43–0.82) reproducibility were high.**

***Conclusions*: A minority of patients with TB (28%) produced culturable cough aerosols. Collection of cough aerosol cultures is feasible and reproducible in a resource-limited setting.**

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

There is no definitive method to determine which patients with tuberculosis (TB) are most infectious, and there is considerable variability of infectiousness among patients based on both epidemiological and experimental studies.

#### What This Study Adds to the Field

We report on the measurement of infectious aerosols from a large group of patients with TB during voluntary coughing. This study demonstrates the feasibility of collecting cough aerosols from patients as a way of detecting infectiousness.

**Keywords:** tuberculosis; cough; air microbiology; infectious disease transmission; infection control

Tuberculosis (TB) continues to be a major cause of global morbidity and mortality, especially among those infected with HIV (1). The spread of multidrug-resistant (MDR) and extensively drug-resistant TB has highlighted the importance of preventing transmission of TB, both in the community and within healthcare facilities (2, 3). *Mycobacterium tuberculosis* is transmitted by fine aerosols (i.e., via the airborne route in infectious droplet nuclei $< 5$ μm in diameter), yet assessment of infectiousness has been based on microscopic examination of sputum for more than a century. Both experimental and epidemiological data suggest that sputum examination for acid-fast bacilli (AFB) is neither a sensitive nor a specific indicator of infectiousness (4–7). Moreover, although the size of aerosol particles is a critical determinant of aerosol deposition in the lungs and a factor that impacts infection control measures, the magnitude and size distribution of aerosols generated by patients with TB is unknown.

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As part of a study of nosocomial transmission of TB in Uganda, we modified the previously described Cough Aerosol Sampling System (CASS) (8) to collect, quantify, and size aerosol particles containing culturable *M. tuberculosis* produced by voluntary coughing in patients with active pulmonary TB. We also evaluated factors associated with cough aerosol production.

Some of the results from this study have been previously reported in the form of abstracts (9, 10).

## METHODS

### Study Population and Measurements

From November 5, 2002 to December 14, 2004, we recruited subjects with suspected TB attending the National Tuberculosis and Leprosy Program Chemotherapy Centre at Mulago Hospital in Kampala, Uganda. Patients were recruited if they had a recent diagnosis (past 7 d) of sputum AFB-positive pulmonary TB from any laboratory. Subjects were included in the final analysis if their sputum was confirmed to be culture-positive for *M. tuberculosis*. Patients with hemoptysis, pneumothorax, or other serious comorbid conditions, and patients who were unable to walk to the procedure room were ineligible. We used a standardized questionnaire to collect demographic and clinical information. The extent of disease on a chest radiograph at baseline was graded on an ordinal scale by an experienced clinician. Subjects were offered HIV testing and a $CD4^+$ lymphocyte cell count was measured in HIV-infected patients; patients with a $CD4^+$ cell count less than 200 cells/μl were referred for antiretroviral treatment according to existing national guidelines (11). All patients were offered TB treatment according to Ugandan National Tuberculosis and Leprosy Program treatment guidelines. Patients found to have MDR-TB were treated when medications became available, as explained elsewhere (12).

### Laboratory Methods

Sputa specimens were processed with the standard digestion and decontamination method using *N*-acetylcysteine-sodium hydroxide (13). Centrifugates were used to prepare smears and cultures on 7H10 agar and in the BACTEC 460 liquid culture system (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer's recommendations (14). Sputum smear microscopy was performed using auramine O fluorescent stain and reported according to the CDC microscopy grading scheme (15). Confirmation of *M. tuberculosis* complex was determined by the BACTEC NAP test (Becton Dickinson). Drug susceptibility testing for first-line TB drugs was done on isolates from sputum using BACTEC 460.

### CASS Method

We used the CASS method previously described (8) with minor modifications. Briefly, the CASS consists of a custom-built stainless steel cylindrical chamber with noncompressible tubing connecting the inlet to a disposable mouthpiece (Figure 1). The chamber holds two Andersen six-stage cascade impactors for viable bioaerosol sampling (Thermo Scientific, Inc., Rockford, IL), each with six plastic Petri plates (Fisher Scientific, Inc., Hanover Park, IL) holding selective 7H11 agar that were loaded in a class II biological safety cabinet. A vacuum pump (GAST, Inc., Benton Harbor, MI) connects the air samplers by tubing (Tygon, Cole Parmer, Inc., Vernon Hills, IL) to fittings that pass through the wall of the chamber. In-line 47-mm filter holders (Cole Parmer, Inc.) loaded with high-efficiency particle air filters (EPM 2000; Whatman, Inc., Piscataway Township, NJ) are placed between the chamber and the vacuum pump for biosafety.

A single-stage impactor (SKC, Inc., Eighty-Four, PA) loaded with the same 7H11 agar was used to sample ambient room air. One settle plate of the same agar was placed inside the chamber and one in the study room to sample large aerosol particles. A timer (GraLab, Inc., Centerville, OH) connected to the vacuum pump was set for 5 minutes. The vacuum pump was calibrated using a primary flow meter (DryCal DC Lite; BIOS, Inc., Butler, NJ), and calibrations were rechecked every 6 months.

### CASS Study Protocol

All studies were performed before the morning meal by one of two trained technicians. During each study, the windows in the study room were open and a fan used to direct airflow from behind the technician, past the subject, and out through the windows. All study personnel wore fit-tested N95 respirators. Before the subject entered the study room, we recorded ambient temperature and relative humidity, and the room air was sampled for 5 minutes to determine if airborne *M. tuberculosis* was present. Subjects were instructed to cough into the CASS mouthpiece as much and as frequently as was comfortable for two 5-minute sessions separated by a rest of approximately 5 minutes. No sputum induction was used. The technician subjectively assessed the cough strength as weak, moderate, or strong. Flow rates through the samplers were recorded. Sputum specimens were collected if produced. The CASS chamber was autoclaved and other components were disinfected after each study.

After the study, the aerosol samplers were removed and transported to the laboratory, where they were unloaded in the biological safety cabinet. Plates were incubated at 37°C. They were read at 1 week to detect any rapidly growing contaminants and then at 3, 6, and 9 weeks to record cfu of *M. tuberculosis*; as there were rarely new cfu at 9 weeks, we used the 6-week count as the outcome measure. Confirmation of *M. tuberculosis* complex was determined by BACTEC NAP. The appearance of sputa specimens expectorated during studies was classified as purulent, mucopurulent, mucosalivary, salivary, or bloody by the microbiology technicians according to laboratory guidelines (16). These data were dichotomized into two groups for analysis: purulent/mucopurulent or salivary/mucosalivary, with two bloody specimens excluded. To assess reproducibility of the CASS method, the last 40 subjects were asked to return (without interrupting TB treatment) for two additional studies on consecutive days, with a goal of three cough aerosol studies per subject.

### Ethical Approvals

Participating patients provided written informed consent in their native language. The study was approved by the AIDS Research subcommittee of the Uganda National Council of Science and Technology, the Institutional Review Boards at the University of Medicine and Dentistry of New Jersey, and the London School of Hygiene and Tropical Medicine.

### Analytic Strategy

Differences in the production of positive cough aerosol cultures were assessed in unadjusted analyses using Fisher exact (categorical data), Wilcoxon rank sum (continuous measures), and Cochran-Armitage trend (ordinal measures) tests. We used Spearman rank correlation to evaluate combinations of continuous or ordinal measures. Variables that were positively associated with cough aerosol cultures in univariate analyses at $P$ less than or equal to 0.2 were included in a stepwise logistic regression model to identify independent predictors of cough aerosol production. Reproducibility between cough aerosol cultures collected during the first and second sessions was assessed using intraclass correlation coefficient (ICC) on log-transformed cfu+1 values and when dichotomized (any versus no cfu) using McNemar test. Concordance of aerosol production between studies on the same patient was assessed using Cohen kappa when there were two measurements per patient and Fleiss kappa when more than two measurements, and the ICC on log-transformed cfu+1 values. Statistical analyses were conducted using SAS 9.1 (Cary, NC). All tests were two-tailed and conducted at the 5% significance level.

## RESULTS

### Characteristics of Patients with TB

We evaluated 112 patients with suspected TB; 101 (90%) had confirmed culture-positive sputum and were further analyzed. Most subjects were men (70%), had advanced radiographic disease (63%), and had high bacillary load as assessed by sputum smear (74% with $\geq$ 3+ AFB) (Table 1). Nearly all (99%) subjects presented with chronic cough. MDR-TB was isolated from eight (8%) participants. Of the 84 (83%) subjects with HIV results, 49 (58%) were HIV infected and had a median CD4 cell count of 112 cells/ml (interquartile range, 33–274).

*Figure 1.* Cough Aerosol Sampling System. View inside of chamber with two Andersen cascade impactors and settle plate (*left*) and set up in procedure room ready for use (*right*).

### Cough Aerosol Cultures

Of the 101 subjects with positive sputum cultures, 28 (27.7%; 95% confidence interval [CI], 19.9–37.1%) produced culture-positive cough aerosols from the first CASS study (first 5-min cough period). Among the positive aerosols, the median was 16 CFU (interquartile range, 5–30) with a range of 1 to 710 cfu (Table 2); 16 (57%) of these subjects produced 10 or more cfu in aerosols. The proportion of patients who generated culture-positive aerosols increased as sputum smear microscopy grade increased (Spearman correlation, 0.40; $P = 0.033$; Figure 2) and as sputum BACTEC days to positive (DTP) decreased (Spearman correlation, −0.31; $P = 0.001$). Although all CASS-positive patients were sputum AFB smear positive, the majority of sputum AFB smear–positive subjects (62 of 90, 69%) did not produce culturable cough aerosols. Conversely, none of the 11 sputum AFB-negative/culture-positive subjects produced cough aerosols.

### Tuberculous Aerosol Particle Size Distribution

The mode of the particle size distribution of culturable aerosols was on stage 5 (1.1–2.0 μm), and nearly all (96.4%) particles collected measured between 0.65 and 4.7 μm in aerodynamic diameter (i.e., deposited in stages 3 to 6 of the Andersen cascade impactors) (Figure 3). Of the 74 settle plates inside the chamber, only 8 (11%) had positive growth. No *M. tuberculosis* was cultured from ambient air, but 45% of these plates were contaminated with mold.

### Factors Associated with Cough Aerosol Cultures

In unadjusted analyses (Table 1), the production of culturable aerosols during the first 5-minute cough period was associated with a higher Karnofsky performance score ($P = 0.016$), higher sputum AFB smear microscopy grade ($P = 0.007$), lower BACTEC DTP ($P = 0.004$), strong cough ($P = 0.016$), and fewer days on TB treatment before enrollment ($P = 0.047$). Other variables marginally associated with aerosol production were a higher CD4 cell count ($P = 0.11$), a salivary or mucosalivary appearance of sputum ($P = 0.077$), and a higher ambient relative humidity at the time of testing ($P = 0.068$). Of 14 subjects with resistance to isoniazid and/or rifampicin, 7 (50%) had culturable aerosols ($P = 0.090$). Factors not associated with cough-generated aerosols were HIV status, number of days of cough before enrollment, extent of disease on chest radiograph, or cavitary disease.

In multivariable analyses (Table 3), only a salivary or mucosalivary appearance of the sputum (odds ratio, 4.42; 95% CI, 1.23–21.4) and lower sputum BACTEC DTP (per 1-d decrease; odds ratio, 1.17; 95% CI, 1.05–1.33) were independently associated with cough aerosols of *M. tuberculosis*. The exclusion of HIV-infected subjects did not significantly change these results (Table 3).

### Reproducibility

There was excellent agreement in the log-cfu+1 between the two 5-minute sessions of coughing in the same session (ICC, 0.83; 95% CI, 0.56–0.88). However, participants were more

**TABLE 1. CHARACTERISTICS OF PARTICIPANTS AT ENROLLMENT ACCORDING TO COUGH AEROSOL SAMPLING SYSTEM RESULTS**

| Characteristic* | All† (*N* = *101*) | Aerosol Negative‡ (*N* = *73*) | Aerosol Positive‡ (*N* = *28*) | *P* Value§ |
|---|---|---|---|---|
| Age, yr | 32 (27–38) | 31 (28–38) | 32 (27–41) | 0.74W |
| Sex | | | | |
| Male | 71 (70) | 50 (70) | 21 (30) | 0.63E |
| Female | 30 (30) | 23 (77) | 7 (23) | |
| Body mass index, kg/m$^2$ | 17.6 (16.2–19.5) | 17.6 (16.2–19.5) | 17.6 (16.4–19.6) | 0.89W |
| Karnofsky score | 90 (80–90) | 80 (80–90) | 90 (80–100) | 0.016W |
| HIV status | | | | |
| Uninfected | 35 (42) | 25 (71) | 10 (29) | 0.61E |
| Infected | 49 (58) | 38 (78) | 11 (22) | 0.11W |
| CD4 count (HIV+ only), cells/μl | 112 (33–274) | 69 (31–253) | 159 (100–594) | |
| TB treatment category | | | | |
| New | 26 (27) | 20 (77) | 6 (23) | 0.61E |
| Retreatment | 71 (73) | 49 (69) | 22 (31) | |
| Any smoking history | | | | |
| Yes | 36 (44) | 30 (83) | 6 (17) | 0.20E |
| No | 46 (56) | 32 (70) | 14 (30) | |
| Days of cough before enrollment | 80 (45–120) | 90 (60–90) | 60 (38–120) | 0.86W |
| Albumin, g/L | 33.7 (29.8–36.9) | 33.7 (29.6–36.6) | 32.2 (30.8–39.1) | 0.56W |
| Chest X-ray findings | | | | |
| Extent of disease | | | | |
| Normal | 5 (6) | 5 (100) | 0 | 0.71E |
| Minimal | 9 (11) | 7 (78) | 2 (22) | |
| Moderate | 16 (20) | 11 (69) | 5 (31) | |
| Advanced | 51 (63) | 38 (75) | 13 (25) | |
| Cavitations | | | | |
| Absent | 36 (37) | 28 (78) | 8 (22) | 0.35E |
| Present | 60 (63) | 40 (67) | 20 (33) | |
| Sputum characteristics | | | | |
| Volume, ml | 7.5 (4–12) | 6.5 (4–12) | 9 (5–12.5) | 0.32W |
| Appearance | | | | |
| Salivary/mucosalivary | 59 (70) | 21 (36) | 38 (64) | 0.077E |
| Purulent/mucopurulent | 23 (28) | 3 (13) | 20 (87) | |
| Bloody | 2 (2) | 1 (50) | 1 (50) | |
| Acid-fast bacilli smear | | | | |
| Negative | 11 (11) | 11 (100) | 0 | 0.007T |
| 1+ | 5 (5) | 4 (80) | 1 (20) | |
| 2+ | 11 (11) | 8 (73) | 3 (27) | |
| 3+ | 24 (24) | 20 (83) | 4 (17) | |
| 4+ | 50 (50) | 30 (60) | 20 (40) | |
| Middlebrook 7H10 agar culture, cfu | | | | |
| 0 | 1 (1) | 1 (100) | 0 | 0.13T |
| 20–100 | 3 (3) | 3 (100) | 0 | |
| 101–200 | 2 (2) | 2 (100) | 0 | |
| >200 | 92 (94) | 64 (70) | 28 (30) | |
| Days to positive BACTEC 460 culture | 8 (4–12) | 8 (5–14) | 5 (3–9) | 0.004W |
| Drug susceptibility testing | | | | |
| Sensitive to I and R | 84 (86) | 63 (75) | 21 (25) | 0.16E |
| Resistant to I or R | 6 (6) | 3 (50) | 3 (50) | |
| Resistant to I and R (MDR) | 8 (8) | 4 (50) | 4 (50) | |
| CASS characteristics | | | | |
| Ambient temperature, °C | 23 (22.1–24.2) | 23 (22.1–24.3) | 22.6 (21.6–23.2) | 0.13W |
| Relative humidity, % | 70.9 (66–74.4) | 70.4 (65.4–73.7) | 73 (68.2–77.4) | 0.068W |
| Cough assessment (subjective) | | | | |
| Weak | 43 (43) | 35 (81) | 8 (19) | 0.016T |
| Moderate | 44 (44) | 31 (70) | 13 (30) | |
| Strong | 13 (13) | 6 (46) | 7 (54) | |
| Days on TB treatment before enrollment | | | | |
| Non-MDR only (n = 75) | 4 (3–6) | 4 (3–6) | 3 (2.5–4) | 0.047W |
| MDR only (n = 6) | 4 (1–69) | 5 (0–132) | 3 (1–69) | >0.99W |
| Aerosol cfu | 0 (0–2) | 0 (0–0) | 16 (5–30) | – |

*Definition of abbreviations*: CASS = Cough Aerosol Sampling System; cfu = colony forming units of *Mycobacterium tuberculosis*; I = isoniazid; IQR = interquartile range; MDR = multidrug resistant; R = rifampicin; TB = tuberculosis.

* Missing data as follows: age (n = 4), BMI (19), Karnofsky score (14), HIV status (17), CD4 (2), TB status (4), smoking history (19), days of cough before enrollment (19), days on TB treatment before enrollment (20), albumin (24), chest X-ray extent of disease (20), cavitations (5), drug susceptibility testing (3), cough assessment (1). Middlebrook culture results were missing (1) or contaminated (2).

† Values are n (column %) or median (IQR).

‡ Values are n (row %) or median (IQR).

§ Categorical variables compared using exact (E) or Cochran Armitage trend (T) tests and continuous variables using a Wilcoxon (W) test.

**TABLE 2. COUGH-GENERATED AEROSOL PRODUCTION ACCORDING TO SPUTUM ACID-FAST BACILLI AND CULTURE RESULTS**

| Characteristic | Level | Statistic | All | Sputum AFB Negative | Sputum AFB 1+ | Sputum AFB 2+ | Sputum AFB 3+ | Sputum AFB 4+ | *P* Value* |
|---|---|---|---|---|---|---|---|---|---|
| Total | | n (row %) | 101 (100) | 11 (11) | 5 (5) | 11 (11) | 24 (24) | 50 (49) | |
| Sputum Middlebrook | 0 | n (col %) | 1 (1) | 1 (11) | 0 | 0 | 0 | 0 | 0.014S |
| 7H10 agar culture, cfu | 20–100 | | 3 (3) | 1 (11) | 0 | 1 (9) | 0 | 1 (2) | |
| | 101–200 | | 2 (2) | 1 (11) | 0 | 0 | 1 (4) | 0 | |
| | >200 | | 92 (94) | 6 (67) | 5 (100) | 10 (91) | 22 (96) | 49 (98) | |
| Sputum BACTEC 460 | | N | 101 | 11 | 5 | 11 | 24 | 50 | <0.001S |
| culture, DTP | | Median | 8 | 16 | 17 | 10 | 9 | 5 | |
| | | (Min, max) | (1, 39) | (3, 32) | (4, 20) | (3, 19) | (3, 17) | (1, 39) | |
| | | (25th, 75th) | (4, 12) | (7, 30) | (14, 18) | (7, 18) | (8, 11) | (3, 8) | |
| Aerosol cfu, (all) | No | n (col %) | 73 (72) | 11 (100) | 4 (80) | 8 (73) | 20 (83) | 30 (60) | 0.007T |
| | Yes | | 28 (28) | 0 | 1 (20) | 3 (27) | 4 (17) | 20 (40) | |
| | | Median | 0 | 0 | 0 | 0 | 0 | 0 | 0.033S |
| | | (Min, max) | (0, 710) | (0, 0) | (0, 4) | (0, 27) | (0, 27) | (0, 710) | |
| | | (25th, 75th) | (0, 2) | (0, 0) | (0, 0) | (0, 2) | (0, 0) | (0, 15) | |
| Aerosol cfu (CASS | | N | 28 | 0 | 1 | 3 | 4 | 20 | |
| positives only) | | Median | 16 | — | 4 | 8 | 6 | 19 | |
| | | (Min, max) | (1, 710) | — | (4, 4) | (2, 27) | (1, 27) | (1, 710) | |
| | | (25th, 75th) | (5, 30) | — | (4, 4) | (2, 27) | (3, 18) | (8, 34) | |

*Definition of abbreviations*: AFB = acid-fast bacilli; CASS = Cough Aerosol Sampling System; cfu = colony forming units of *Mycobacterium tuberculosis*; DTP = days to positive.

Missing results were excluded.

*Cochran Armitage trend test (T), testing whether Spearman Correlation is zero (S), or Wilcoxon rank sum test (W).

likely to produce culturable aerosol in the first versus the second 5-minute period of coughing (McNemar $P = 0.008$). None of the subjects without cfu in the first period produced aerosols in the second.

Of the 40 subjects recruited for the assessment of day-to-day reproducibility, 38 were sputum culture positive; of these, 34 (89%) completed all three studies, and 4 (11%) completed two studies. Of the 38 subjects, 14 (36%) generated cultivable aerosols in at least one of the three sampling periods: 8 (57%) were positive on the first test, an additional 4 (29%) were positive on the second, and another 2 (14%) in the third study. Of those participating in all three studies, 26 of 34 (76%) were concordant on all three studies (Fleiss kappa, 0.62; 95% CI, 0.43–0.82). There were no significant differences in the aerosol cfu between the three cough aerosol studies ($P = 0.67$), and the ICC was 0.62 (95% CI, 0.46–0.76). The pattern of discordance appeared to be random, and discordance mostly involved subjects with less than 10 aerosol cfu, as observed with subjects with same-day discordant results.

### Contamination

Of the 1,344 solid culture plates used for cough aerosol cultivation in the 112 patients screened, 161 (12%) were contaminated with mold. Cough aerosol plate mold contamination was strongly associated with mold isolation from ambient air ($P < 0.001$). Mold collection was positively associated with relative humidity ($P = 0.004$) and negatively associated with temperature ($P < 0.001$).

### Adverse Events

One subject vomited during the procedure; at the time, study personnel were not aware the subject had eaten breakfast before the cough study. Otherwise, the procedure was well tolerated.

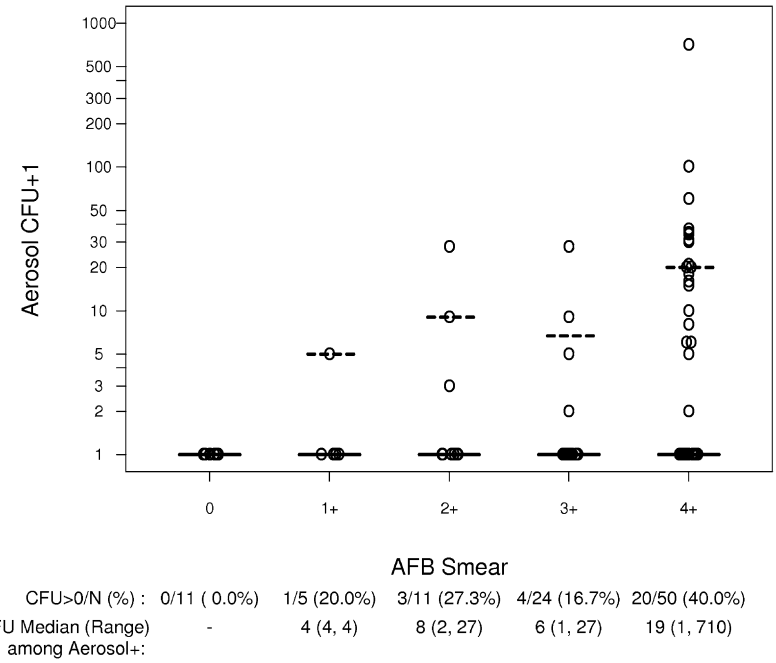


***Figure 2.*** Aerosol cfu by sputum acid-fast bacilli (AFB) smear result.

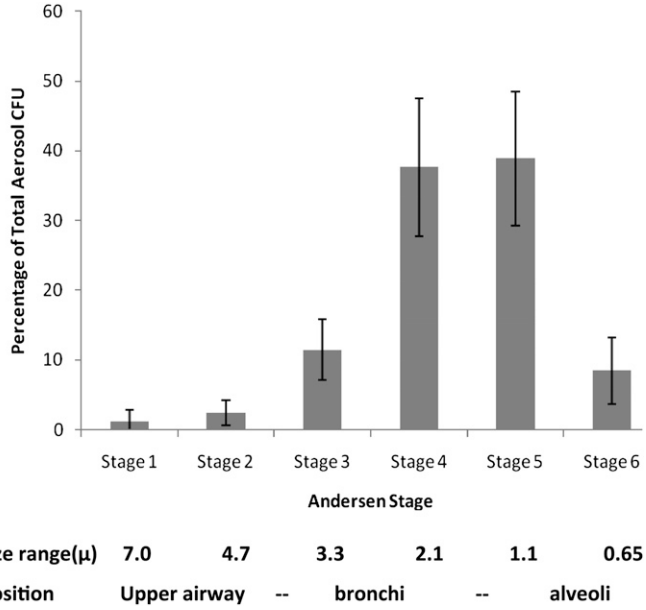


***Figure 3.*** Mean percentage of aerosol cfu on each Andersen stage in subjects producing at least one aerosol cfu.

## DISCUSSION

This is the first study to describe the magnitude, variability, and particle size distributions of aerosols with culturable *M. tuberculosis* from patients with pulmonary TB during voluntary coughing. Most of the subjects in the initial report were studied during sputum induction procedures and had MDR-TB (8). In this larger study, we confirmed the original observation that fewer than one-third of patients with sputum culture–positive TB generate viable tuberculous aerosols during voluntary coughing, and we demonstrated that cough aerosol sampling is feasible in a resource-limited, high-burden setting.

Cough aerosol cultures were best predicted by the sputum bacterial load measured by DTP in liquid culture and by the appearance of the sputum. However, these factors explained only 15% of the variability in culturable cough aerosol production. Thus, it is clear that cough aerosol cultures are not simply a reflection of the sputum bacillary load, even though DTP was the strongest predictor of cough aerosol cultures. DTP may not only reflect bacillary concentration but likely also measures the metabolic capacity or "vitality" of the bacilli. Sputum with a salivary appearance was more highly associated with cough aerosols than purulent sputum. Sputum appearance is most likely a surrogate for viscosity and other rheological, or flow, properties of respiratory secretions. These properties have long been suspected as determinants of infectiousness (17), and experimental data using mucus stimulants suggest that aerosolization is inversely associated with "cohesivity" (18).

Most of the culturable cough aerosols were less than 5 μm, an aerodynamic size range that can be inhaled and deposited in the lower respiratory tract or can remain suspended in room air indefinitely. These data, obtained by direct measurement, are consistent with the early theoretical estimates by Wells that airborne *M. tuberculosis* is transmitted in infectious droplet nuclei of this size range (19). The size distribution of these tuberculous aerosols is similar to that observed in cough aerosols of gram-negative bacteria from patients with cystic fibrosis (20).

The positive association of cough aerosol cultures with cough strength, Karnofsky performance status, and CD4 counts among HIV-infected patients in univariate analyses suggests that healthier ambulatory patients may be more infectious than very ill bedridden patients. Other studies will be needed to confirm this, especially as these associations were not significant in the adjusted model. Similarly, the association of cough aerosol cultures with fewer days of TB treatment and with INH resistance in univariate analyses may have implications for prevention of TB transmission in both healthcare facilities and the community. Isoniazid has the best early bactericidal activity among TB drugs, and further research will be needed to determine if early bactericidal activity is associated with decreased infectiousness.

Our findings are consistent with the concept that sputum smear status should only be considered a risk factor (not the

**TABLE 3. RESULTS OF MULTIPLE LOGISTIC REGRESSION TO PREDICT AEROSOL PRODUCTION**

| Characteristic | Level* | Odds Ratio (95% CI) | *P* Value† |
|---|---|---|---|
| All subjects (N = 101) | | | |
| Sputum appearance | Purulent/mucopurulent | 1 (referent) | |
| | Salivary/mucosalivary | 4.42 (1.23–21.43) | 0.0702 |
| BACTEC 460 culture | Per 1-d decrease | 1.17 (1.05–1.33) | 0.0014 |
| HIV-uninfected only (N = 35) | | | |
| Sputum appearance | Purulent/mucopurulent | 1 (referent) | |
| | Salivary/mucosalivary | 29.36 (2.43–1,365) | 0.0189 |
| BACTEC 460 culture | Per 1-d decrease | 1.53 (1.14–2.63) | 0.0012 |

*Definition of abbreviation*: CI = confidence interval.

*Participants with missing data for dependent or independent variables and bloody sputum type (2 subjects) were excluded from model.

† Likelihood ratio tests.

*sine qua non*) for infectiousness, as suggested by others (21). There is experimental (22–24), classic epidemiologic (25, 26), and molecular epidemiologic (27–30) evidence of considerable variability of infectiousness among sputum smear–positive patients (6). In addition, cough aerosols may provide a better estimate of inhaled dose than the sputum AFB smear and, thus, may help provide insights into TB pathogenesis. In animal models, the inhaled dose of tuberculous aerosols predicts infectivity, severity of disease, and mortality (31).

### Limitations

Although it seems logical that individuals who have cough aerosols of *M. tuberculosis* in a transmissible size are more likely to be infectious than those who do not, this study was not designed to directly measure transmission. In addition, as patients with smear-negative specimens were excluded in our screening, our study cannot estimate the frequency of culturable cough aerosols among these patients.

Although it may have been scientifically preferable to study all patients with TB off therapy, ethically we could not delay treatment of these patients with a high rate of TB-HIV coinfection and on open wards, so most patients were studied after initiating treatment for TB. Thus, it is possible that our data may have underestimated the infectiousness of patients with untreated TB due to an early effect of treatment on infectiousness. However, these data are probably a reasonable estimate of infectiousness of patients with TB who are newly diagnosed and just started on treatment. We chose an exclusion criterion of 7 days of treatment based on the initial data from patients in the United States, most of whom had MDR-TB (8), but there may be differences in the rate at which infectious aerosols decrease after treatment with first-line versus second-line antituberculous drugs. The rate at which patients become noninfectious is unknown, although in our first study in the United States, the cough aerosol cultures of four patients with MDR-TB treated with effective drugs decreased exponentially over a 3-week period (8). Although a review in 1976 suggested that most patients probably become noninfectious within 2 weeks (32), subsequent authors argued against that conclusion (33, 34). The earlier review cited data from household contact studies that did not find additional infections among household contacts after the index TB case was placed on effective treatment. However, such analyses are limited by considerable selection bias, as the contacts who were susceptible or exposed had been infected before the case was treated, removing them from the pool of subjects under subsequent study. In addition, there is experimental evidence that tubercle bacilli remain viable and potentially infectious during early treatment, as guinea pigs were infected by injection with bacilli from the washed sputum from patients treated for 3 to 7 weeks (35, 36). The uncertainty about when patients on treatment become noninfectious is reflected in current guidelines that recommend a conservative approach to removal from respiratory isolation (37). We anticipate that future developments of cough aerosol measurement could provide data to help reduce this uncertainty about when patients become noninfectious.

Another potential limitation of our study is that there may be bacilli in cough-generated aerosols that are viable but not culturable, such as the recently identified lipid-laden bacilli that may be associated with nonreplicating persistence (38). Mold contamination of culture plates was more common in this tropical setting than it was in the high desert of Denver, Colorado (8). In Kampala, 12% of the cough aerosol plates were contaminated with mold compared with only 0.06% in Denver. Although mold contamination did not appear to impair our ability to identify *M. tuberculosis* aerosol production, it might have decreased our total cfu counts in some subjects. As in many other tests of pulmonary function (39, 40), cough-generated aerosol production is effort dependent and probably varies with motivation, strength, sense of well-being, and other factors. However, collection of sputum specimens is also limited by similar issues (41, 42).

### Potential Benefits

The nearly 3-log range of cough aerosol cultures suggests that a minority of patients are more highly infectious than others, consistent with both older (24) and more recent (22) findings of disseminators of TB. In the near future, it may be possible to identify the minority of patients with TB who are most likely infectious using cough aerosol collections with point-of-care devices. Identification of the most highly infectious patients could allow for more cost-effective use of resources, both for infection control in hospitals (e.g., isolation rooms) and for public health control of TB (e.g., active case finding with targeted treatment of contacts exposed to the most highly infectious cases). Such targeted treatment around "superspreaders" of disease is theoretically more efficient in controlling epidemics (43) and might improve TB control. In addition, improved identification of infectious cases may decrease exposure misclassification and improve the precision of future drug and vaccines studies that depend on accurate ascertainment of exposed household contacts. As a major goal of drug therapy is to render patients noninfectious to halt transmission, an improved and validated method of measuring infectiousness could also offer a novel outcome measure used in the evaluation of new treatment regimens. Knowing when patients become noninfectious could also allay concerns about hospital discharges and community-based treatment, especially for patients with MDR-TB or extensively drug-resistant TB.

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### References

1. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003;163:1009–1021.
2. Basu S, Andrews JR, Poolman EM, Gandhi NR, Shah NS, Moll A, Moodley P, Galvani AP, Friedland GH. Prevention of nosocomial transmission of extensively drug-resistant tuberculosis in rural South African district hospitals: an epidemiological modelling study. *Lancet* 2007;370:1500–1507.
3. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006;368:1575–1580.
4. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, Small PM. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet* 1999;353:444–449.
5. Elwood RK, Cook VJ, Hernandez-Garduno E. Risk of tuberculosis in children from smear-negative source cases. *Int J Tuberc Lung Dis* 2005;9:49–55.

6. Fennelly KP. Variability of airborne transmission of mycobacterium tuberculosis: implications for control of tuberculosis in the HIV era. *Clin Infect Dis* 2007;44:1358–1360.
7. Hernandez-Garduno E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM. Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. *Thorax* 2004;59:286–290.
8. Fennelly KP, Martyny JW, Fulton KE, Orme IM, Cave DM, Heifets LB. Cough-generated aerosols of *Mycobacterium tuberculosis*: a new method to study infectiousness. *Am J Respir Crit Care Med* 2004;169: 604–609.
9. Fennelly KP, Jones EC, Menyha H, Peterson SD, Eisenach KD, Okwera A, Mugerwa RD, Ellner JJ. Variability of cough-generated aerosols of *Mycobacterium tuberculosis* in Kampala, Uganda [abstract]. *Am J Resp Crit Care Med* 2004;169:A532.
10. Fennelly KP, Jones-Lopez EC, Menyha H, Ayakaka I, Muchwa C, Joloba M, Okwera A, Mugerwa RD, Eisenach K, Ellner JJ. Reproducibility of sampling cough-generated aerosols of *Mycobacterium tuberculosis* [abstract]. *Proc Am Thorac Soc* 2005;2:A552.
11. van Oosterhout JJ, Laufer MK, Graham SM, Thumba F, Perez MA, Chimbiya N, Wilson L, Chagomerana M, Molyneux ME, Zijlstra EE, *et al*. A community-based study of the incidence of trimethoprim-sulfamethoxazole-preventable infections in Malawian adults living with HIV. *J Acquir Immune Defic Syndr* 2005;39:626–631.
12. Jones-Lopez EC, Ayakaka I, Levin J, Reilly N, Mumbowa F, Dryden-Peterson S, Nyakoojo G, Fennelly K, Temple B, Nakubulwa S, *et al*. Effectiveness of the standard who recommended retreatment regimen (category ii) for tuberculosis in Kampala, Uganda: a prospective cohort study. *PLoS Med* 2011;8:e1000427.
13. Kent PT, Kubica GP. Public health mycobacteriology—a guide for the level III laboratory. Atlanta, GA: US Department of Health and Human Services, Public Health Service, Centers for Disease Control; 1985.
14. Bactec 460 system: product and procedure manual. Sparks, MD: Becton, Dickinson and Company; 1996.
15. ATS/CDC/IDSA. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000;161:1376–1395.
16. Rieder HL, Chonde TM, Myking H, Urbanczik R, Laszlo A, Kim SJ, Van Deun A, Trebucq A. The public health service national tuberculosis reference laboratory and the national laboratory network: minimum requirements, role and operation in a low-income country. Paris, France: International Union Against Tuberculosis and Lung Disease; 1998.
17. Bates JH, Stead WW. Effect of chemotherapy on infectiousness of tuberculosis. *N Engl J Med* 1974;290:459–460.
18. Zayas G, Dimitry J, Zayas A, O'Brien D, King M. A new paradigm in respiratory hygiene: increasing the cohesivity of airway secretions to improve cough interaction and reduce aerosol dispersion. *BMC Pulm Med* 2005;5:11.
19. Wells WF. Airborne contagion and hygiene. Cambridge, MA: Harvard University Press; 1955.
20. Wainwright CE, France MW, O'Rourke P, Anuj S, Kidd TJ, Nissen MD, Sloots TP, Coulter C, Ristovski Z, Hargreaves M, *et al*. Cough-generated aerosols of pseudomonas aeruginosa and other gram-negative bacteria from patients with cystic fibrosis. *Thorax* 2009;64: 926–931.
21. Snider DE Jr, Kelly GD, Cauthen GM, Thompson NJ, Kilburn JO. Infection and disease among contacts of tuberculosis cases with drug-resistant and drug-susceptible bacilli. *Am Rev Respir Dis* 1985;132: 125–132.
22. Escombe AR, Moore DA, Gilman RH, Pan W, Navincopa M, Ticona E, Martinez C, Caviedes L, Sheen P, Gonzalez A, *et al*. The infectiousness of tuberculosis patients coinfected with HIV. *PLoS Med* 2008;5:e188.
23. Riley RL, Mills CC, O'Grady F, Sultan LU, Wittestadt F, Shivipuri DN. Infectiousness of air from a tuberculosis ward-ultraviolet irradiation of infected air: comparative infectiousness of different patients. *Am Rev Respir Dis* 1962;85:511–525.
24. Sultan L, Nyka W, Mills C, O'Grady F, Wells W, Riley RL. Tuberculosis disseminators: a study of the variability of aerial infectivity of tuberculous patients. *Am Rev Respir Dis* 1960;82:358–369.
25. Brooks SM, Lassiter NL, Young EC. A pilot study concerning the infection risk of sputum positive tuberculosis patients on chemotherapy. *Am Rev Respir Dis* 1973;108:799–804.
26. van Geuns HA, Meijer J, Styblo K. Results of contact examination in Rotterdam, 1967–1969. *Bull Int Union Tuberc* 1975;50:107–121.
27. Alland D, Kalkut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W, Drucker E, Bloom BR. Transmission of tuberculosis in New York City: an analysis by DNA fingerprinting and conventional epidemiologic methods. *N Engl J Med* 1994;16:1710–1716.
28. Borgdorff MW, Nagelkerke NJ, de Haas PE, van Soolingen D. Transmission of *Mycobacterium tuberculosis* depending on the age and sex of source cases. *Am J Epidemiol* 2001;154:934–943.
29. Hamburg MA, Frieden TR. Tuberculosis transmission in the 1990s. *N Engl J Med* 1994;330:1750–1751.
30. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, Schecter GF, Daley CL, Schoolnik GK. The epidemiology of tuberculosis in San Francisco: a population-based study using conventional and molecular methods. *N Engl J Med* 1994;16:1703–1709.
31. Glover RE. Infection of mice with *Mycobact. tuberculosis* (bovis) by the respiratory route. *Br J Exp Pathol* 1944;25:141–149.
32. Rouillion A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: the effects of chemotherapy. *Tubercle* 1976;57:275–299.
33. Menzies D. Effect of treatment on contagiousness of patients with active pulmonary tuberculosis. *Infect Control Hosp Epidemiol* 1997;18:582–586.
34. Noble RC. Infectiousness of pulmonary tuberculosis after starting chemotherapy: review of the available data on an unresolved question. *Am J Infect Control* 1981;9:6–10.
35. Cassidy JT. Tubercle bacilli retain pathogenicity after seven weeks chemotherapy. *Med J Aust* 1981;1:588–589.
36. Clancy LJ, Kelly P, O'Reilly L, Byrne C, Costello E. The pathogenicity of *Mycobacterium tuberculosis* during chemotherapy. *Eur Respir J* 1990;3: 399–402.
37. Jensen PA, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings, 2005. *MMWR Recomm Rep* 2005;54:1–141.
38. Garton NJ, Christensen H, Minnikin DE, Adegbola RA, Barer MR. Intracellular lipophilic inclusions of mycobacteria in vitro and in sputum. *Microbiology* 2002;148:2951–2958.
39. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, *et al*. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338.
40. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, *et al*. Interpretative strategies for lung function tests. *Eur Respir J* 2005;26: 948–968.
41. Sakundarno M, Nurjazuli N, Jati SP, Sariningdyah R, Purwadi S, Alisjahbana B, van der Werf MJ. Insufficient quality of sputum submitted for tuberculosis diagnosis and associated factors, in Klaten district, Indonesia. *BMC Pulm Med* 2009;9:16.
42. Tenover FC. Developing molecular amplification methods for rapid diagnosis of respiratory tract infections caused by bacterial pathogens. *Clin Infect Dis* 2011;52:S338–S345.
43. Woolhouse ME, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JL, Ndhlovu PD, Quinnell RJ, *et al*. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proc Natl Acad Sci USA* 1997;94:338–342.