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MAJOR ARTICLE

Host Determinants of Infectiousness in Smear-Positive Patients With Pulmonary Tuberculosis

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Background Epidemiologic data suggests that only a minority of tuberculosis (TB) patients are infectious. Cough aerosol sampling is a novel quantitative method to measure TB infectiousness.

Methods We analyzed data from three studies conducted in Uganda and Brazil over a 13-year period. We included sputum acid fast bacilli (AFB) and culture positive pulmonary TB patients and used a cough aerosol sampling system (CASS) to measure the number of colony-forming units (CFU) of Mycobacterium tuberculosis in cough-generated aerosols as a measure for infectiousness. Aerosol data was categorized as: aerosol negative (CFU = 0) and aerosol positive (CFU > 0). Logistic regression models were built to identify factors associated with aerosol positivity.

Results M. tuberculosis was isolated by culture from cough aerosols in 100/233 (43%) TB patients. In an unadjusted analysis, aerosol positivity was associated with fewer days of antituberculous therapy before CASS sampling (p = .0001), higher sputum AFB smear grade (p = .01), shorter days to positivity in liquid culture media (p = .02), and larger sputum volume (p = .03). In an adjusted analysis, only fewer days of TB treatment (OR 1.47 per 1 day of therapy, 95% CI 1.16-1.89; p = .001) was associated with aerosol positivity.

Conclusion Cough generated aerosols containing viable M. tuberculosis, the infectious moiety in TB, are detected in a minority of TB patients and rapidly become non-culturable after initiation of antituberculous treatment. Mechanistic studies are needed to further elucidate these findings.

Globally, tuberculosis disease is the leading cause of infectious disease mortality with an estimated 10 million new cases and 1.6 million deaths in 2017 [1]. The End TB strategy by the World Health Organization suggests diminishing the large reservoir of latent tuberculosis infection, because providing therapy only for active tuberculosis would not meet the intended target of decreasing incidence by 15%–20% per year [2]. In settings with a high incidence of tuberculosis, most cases of tuberculosis disease occur after recent transmission, rather than reactivation from old exposures [3]. In this context, interventions aimed at preventing new infections and targeting preventive therapy to those at highest risk of progression to tuberculosis disease are likely to have the most significant effect on lowering incidence [4, 5].

Despite recent advances in the fields of tuberculosis diagnostics and treatment, knowledge about host and bacterial factors leading to successful person-to-person transmission of *Mycobacterium tuberculosis* remain largely unknown. A central observation in this field was established by Riley and colleagues in the 1950s [6] when they conclusively demonstrated that tuberculosis is transmitted by fine aerosols [7, 8]. However, abundant epidemiological data have since shown marked variability in tuberculosis transmission, with most secondary infections and disease cases clustered around a minority of pulmonary tuberculosis cases [9, 10]. One difficulty in studying the mechanisms modulating tuberculosis transmission has been the inability to collect and quantify airborne *M. tuberculosis*. Instead, most of the published literature on tuberculosis transmission has used visualization and density of acid-fast bacilli (AFB) in expectorated sputum, a relatively easily obtained clinical specimen, as a surrogate for source infectiousness.

Since first demonstrating the feasibility of culturing *M. tuberculosis* directly from cough-generated aerosol samples in 2004 [10], our group has completed several studies showing that the number of colony-forming units (CFUs) of *M. tuberculosis* in aerosol samples is a quantitative and more precise marker of infectiousness than sputum AFB smear [11–13],

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and have demonstrated reproducibility [14]. In studies from Uganda and Brazil, household contacts of the minority of tuberculosis cases with high aerosol CFU counts (≥10 CFUs) were found to have both a higher risk of infection—as measured by qualitative and quantitative readouts of tuberculin skin test and interferon gamma release assay—and higher rates of secondary tuberculosis disease during follow-up [13, 14]. Therefore, elucidating which host, environmental and bacterial factors are associated with *M. tuberculosis* aerosolization will provide fundamental insights into the pathogen's aerobiology and may lead to interventions to interrupt transmission.

In our group's initial study in Uganda [11], cough-generated aerosol cultures of *M. tuberculosis* were associated with the sputum appearing more salivary than purulent. Cough-generated aerosol cultures were also associated with bacillary load, most notably when the surrogate of faster growth in liquid culture medium was used. However, that study was limited by a modest sample size, a single study site, and a high rate of tuberculosis treatment before aerosol collection. In the current aggregated data analysis, we evaluated clinical, radiographic, and microbiological information from sputum AFB and culture-positive pulmonary tuberculosis case patients in 3 separate cohorts to characterize host factors associated with culturable *M. tuberculosis* in cough-generated aerosol samples.

METHODS

Study Populations

The present study included data from 3 cohorts of adult patients (aged ≥18 years) with pulmonary tuberculosis, cohorts with near-identical inclusion criteria. The studies were conducted in Uganda and Brazil over a 13-year period [11, 12, 14].

Study 1.

This longitudinal cohort study, conducted at the National Tuberculosis and Leprosy Program (NTLP) Chemotherapy Centre at Mulago Hospital in Kampala, Uganda, enrolled patients from November 2002 to December 2004. Patients with suspicion of pulmonary tuberculosis were enrolled, including 112 with suspected tuberculosis, 101 (90%) with culture-proven tuberculosis, and 90 who were sputum AFB positive.

Study 2.

This prospective household contact study was conducted at the same NTLP clinic in Kampala, Uganda. From May 2009 to January 2011, it enrolled 96 participants with sputum AFB-positive, culture-proven pulmonary tuberculosis.

Study 3.

This prospective household contact study was conducted at the Núcleo de Doencas Infecciosas (NDI) in Vitória, Brazil, enrolling patients from April 2013 to June 2015. It included 48 human immunodeficiency virus (HIV)–uninfected participants with sputum AFB-positive (2+ or 3+), culture-proven pulmonary tuberculosis.

All 3 studies excluded patients whose medical conditions could be exacerbated by coughing, who were too ill to consent, or who could not follow the study protocol. When the studies were conducted, Uganda was a country with a high tuberculosis burden and an estimated annual tuberculosis incidence rate of 350 cases per 100 000 inhabitants, and 39% of tuberculosis case patients were HIV infected. The Mulago Hospital NTLP clinic treated 3500–4000 patients with tuberculosis every year, of whom approximately 65% were AFB positive and 10% were receiving retreatment [15]. Brazil is a country with moderate tuberculosis incidence. The NDI has organized a network of 16 tuberculosis clinics in the metropolitan region of Vitória. In Espírito Santo, the tuberculosis incidence is 38 cases per 100 000 inhabitants; the prevalence of HIV infection in the general population is <1%, and 7% in patients with tuberculosis [16]

Measurements

We recorded demographic, clinical and radiographic data in eligible tuberculosis case patients. HIV testing was offered to participants in the Ugandan studies, and CD4 cell counts were obtained in those with HIV infection. In Brazil, HIV testing was performed by the local health system. At least 3 sputum specimens were obtained for AFB smear and culture in solid media (Lowenstein-Jensen in studies 1 and 2, Ogawa-Kudow in study 3) and liquid media (BACTEC 460 in studies 1 and 2 and mycobacteria growth indicator tube [MGIT] 960 in study 3), as described elsewhere [12–14]. Sputum AFB smear microscopic findings were graded as 1+, 2+, or 3+ according to the International Union Against Tuberculosis and Lung Disease classification [17].

Posteroanterior chest radiographs were obtained to assess the extent of disease (normal/minimal disease, moderate advanced disease, and far advanced disease), and record the presence of cavities (yes or no) [18]. Radiographs were interpreted by an experienced radiologist at each site. In studies 2 and 3, cough strength was measured using a visual analog cough scale, as reported elsewhere [18]. Cough peak flow rates were measured using portable peak flow meters [19]. The highest of 3 measures was included in the analysis. The duration of antituberculous therapy was defined as the number of days the participant had received tuberculosis treatment before the cough aerosol sampling system (CASS) procedure was conducted. All participants were referred to their respective national tuberculosis program to start tuberculosis treatment according to national guidelines.

Aerosol Collection Procedure

The 3 studies collected *M. tuberculosis* from cough-generated aerosol samples using the same version of the CASS apparatus

described elsewhere in detail [11]. The CASS study protocol was identical for the 3 cohorts, and aerosol studies were performed by technicians similarly trained by one of us (K. P. F.), as described elsewhere [11]. Briefly, 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. The technician subjectively assessed the cough strength as weak or strong. The CASS chamber was autoclaved, and other components were disinfected after each study. During each CASS study, the windows in the study room were open, and a fan was used to direct airflow from behind the technician, past the subject, and out through the windows. All study personnel wore fit-tested N95 respirators.

After study completion, the aerosol samplers were removed and transported to the respective laboratory (Makerere University in Uganda and NDI in Brazil), where they were unloaded within a biological safety cabinet. Plates were incubated at 37°C. In Uganda, plates were read at weeks 1, 3, 6, and 9 to record CFU counts of *M. tuberculosis*; in Brazil, plates were read weekly for 6 weeks. We used the 6-week CFU count as the outcome measure. The appearance of sputum specimens expectorated during aerosol studies was classified as purulent, mucopurulent, mucosalivary, salivary, or bloody by the microbiology technicians, according to international laboratory guidelines [20]. These data were dichotomized into 2 groups for this analysis: purulent/mucopurulent or salivary/mucosalivary, with 2 bloody specimens excluded.

Statistical Analysis

The primary study outcome was the cough-generated aerosol status of patients with tuberculosis. We categorized tuberculosis case patients as aerosol negative (0 CFUs) or aerosol positive (≥1 CFU). The exposure variables included baseline clinical, radiographic and microbiological data from tuberculosis case patients. We calculated descriptive statistics to identify clinical and demographic differences between study cohorts. To evaluate the effect of antituberculous therapy on cough-generated aerosol results, we excluded 8 patients with multidrug-resistant tuberculosis, because response to treatment in such patients is expected to be slower than in drug-susceptible tuberculosis. To identify factors associated with *M. tuberculosis* growth in aerosol cultures we used χ^2 tests for categorical and Kruskal-Wallis tests for numerical variables. Variables with a *P* value <.1 in the bivariate analysis and those considered clinically important (eg, HIV status and the presence of cavities on chest radiographs) were included in a multivariable logistic regression model. All analyses were performed with Stata 14 software.

Ethics Statement

The studies were approved by the Makerere University Faculty of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, the Comitê de Ética em Pesquisa do Centro de Ciências da Saúde–Universidade Federal do Espírito Santo, the Comissão Nacional de Ética em Pesquisa, and the institutional review boards of Boston University Medical Center and New Jersey Medical School–Rutgers University (formerly UMDNJ). We obtained written informed consent and assent in the preferred local language in accordance with age-specific ethical guidelines.

RESULTS

Among the 3 study cohorts, 275 patients with pulmonary tuberculosis were initially considered eligible for inclusion into this study. Of these, 42 (15%) were excluded, mostly because they had negative sputum AFB smear (n = 14) or culture (n = 10) results (Figure 1). Thus, the present analysis included 233 sputum AFB-positive, culture-positive tuberculosis cases. All the excluded patients with negative smear and culture sputum results also had negative culture results from cough-generated aerosol samples.

The baseline characteristics of the 3 cohorts by study site are shown in Table 1. Overall, patients were young (median age, 30 years; interquartile range, 25–40 years), with a preponderance of male patients (65%), and most had a high bacillary burden, as indicated by AFB smear grade 3+ in 152 (65%) and far advanced disease on chest radiograph in 133 (63%) of 209 patients. Participants from the 2 Ugandan cohorts were more likely to be HIV infected, to have salivary sputum ($P < .001$), and to have received more days of antituberculous therapy before the CASS procedure ($P < .001$). We show the correlation between aerosol CFU count and sputum AFB smear grade in Figure 2.

Culturable *M. tuberculosis* From Cough-Generated Aerosol Samples

Of the 233 participants, 100 (43%) had culturable *M. tuberculosis* from cough-generated aerosol samples, of whom 43 (18%) had low and 57 (24%) had high aerosol CFU counts (defined as 1–9 vs ≥10 CFUs). In an unadjusted analysis, aerosol positivity was associated with fewer days of antituberculous therapy before CASS sampling ($P < .001$), higher sputum AFB smear grade ($P = .01$), shorter time to positivity in liquid culture medium ($P = .02$), and larger sputum volume ($P = .03$) (Table 2). In an adjusted analysis, only shorter duration of antituberculous therapy before CASS was associated with aerosol positivity (odds ratio [OR], 1.47 per day of therapy; 95% confidence interval [CI] 1.16–1.89; $P = .001$) (Table 3). This association remained statistically significant (OR, 1.38 per day; 95% CI, 1.05–1.82; $P = .02$) when the outcome variable was restricted to patients with high aerosol CFU counts. Cough generated aerosol production according to sputum AFB is shown in Table 4.

Effect of Antituberculous Treatment on Aerosol Results

Of 206 participants (88%) with available treatment information, we cultured *M. tuberculosis* from cough-generated aerosol samples in 9 of 43 (20%) with >2 days of antituberculous

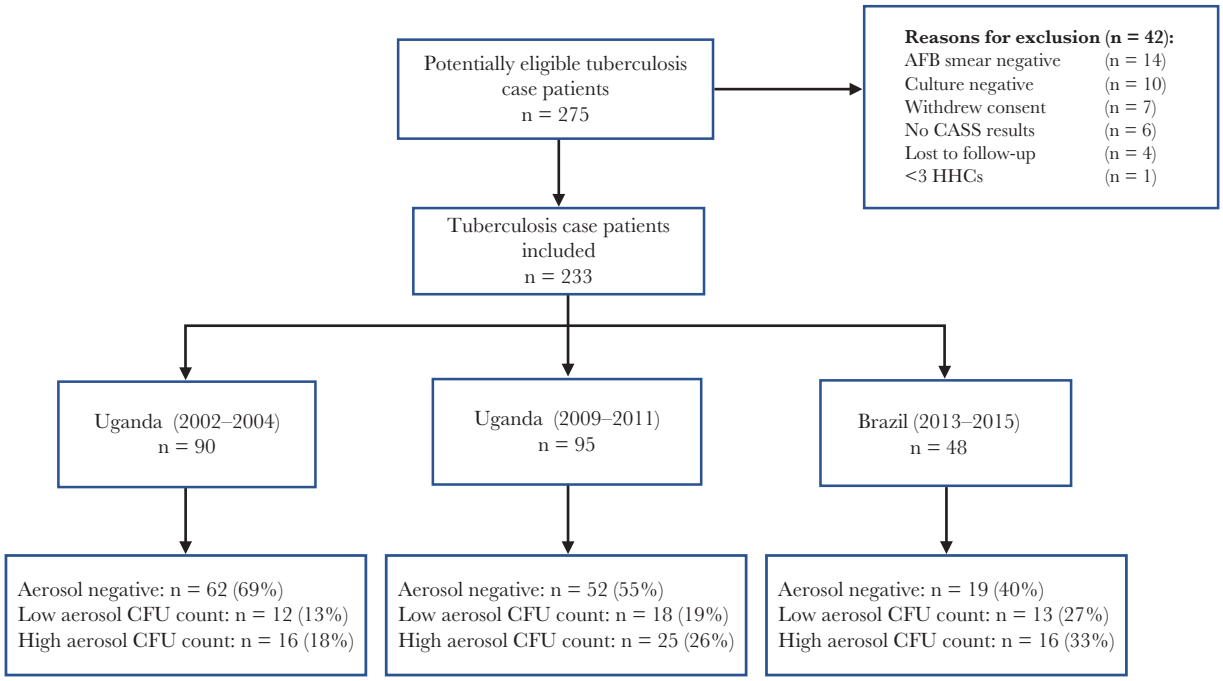

Figure 1. Study population and aerosol results in each participating cohort of pulmonary tuberculosis cases patients. Abbreviations: AFB, acid-fast bacilli; CASS, cough aerosol sampling system; HHCs, household contacts.

therapy, 40 of 99 (40%) with 1–2 days of therapy, and 38 of 64 (59%) with no treatment before collection of aerosol samples. When they were compared with those receiving >2 days of tuberculosis therapy, the OR for aerosol positivity was 2.56 (95% CI, 1.11–5.92; $P = .03$) for those with 1–2 days of therapy, and 5.5 (2.3–13.4; $P < .001$) for those without treatment (Figure 3A). In contrast, the effect of antituberculous therapy on sputum AFB smear grade (Figure 3B; $P = .1$) or sputum culture results (100% remained culture positive) was not apparent.

Effect of HIV Infection on Aerosol Results

Of 217 (93%) participants with available information on HIV status, 64 (29%) were HIV infected. The proportions of HIV-infected patients (24 of 64 [38%]; OR, 0.75; 95% CI, .41–1.36) and HIV-uninfected patients (68 of 153 [44%]) with culturable *M. tuberculosis* in aerosol samples were similar ($P = .35$). Among 53 participants with available CD4 cell count information, 16 (30%) had CD4 cell counts <100 cells/mm^3. Aerosol positivity was higher for those with CD4 cell counts >100 cells/mm^3 (17 of 37 [46%]) than for those with CD4 cell counts <100 cells/mm^3 (2 of 16 [13%]; OR, 5.95; 95% CI, 1.06–33.3; $P = .02$). Among aerosol-positive patients, the median (interquartile range) CFU cell count for those who were HIV negative was 20 (5–52) CFUs, compared with 8 (2–16) CFUs for those with HIV infection.

DISCUSSION

In this aggregated data analysis of 233 sputum AFB-positive/culture-positive pulmonary tuberculosis case patients from 3 cohorts, 43% of participants had culturable *M. tuberculosis* from cough-generated aerosol samples, of whom only 25% had high aerosol CFU counts (>10 CFUs). Culturable *M. tuberculosis* was inversely associated with the duration of antituberculous therapy before aerosol collection, and only partially related to common markers of sputum bacillary burden. Thus, these data are consistent with the findings of epidemiological studies showing an association between bacillary load and transmission; however we suggest that the sputum AFB smear result is best considered a risk factor for infectiousness, not the *sine qua non* for infectiousness, as it is often considered. In addition, aerosol positivity was not related to cough severity, self-reported duration of symptoms, or other standard markers of infectiousness, such as presence of cavities or extent of disease on chest radiographs.

The observation that only a minority of patients with tuberculosis generate culturable aerosol samples is striking and unlikely to be explained by an insensitive method of aerosol collection. Investigators studying 2 different cohorts from South Africa, using a modified CASS and a novel respiratory aerosol collection chamber, reported similar rates (23%–43%) of *M. tuberculosis* in aerosol samples from patients with pulmonary tuberculosis [21, 22]. Based on our data linking culturable aerosol samples and risk of tuberculosis infection and disease [12–14] we posit that aerosol positivity represents a unique subgroup of individuals with enhanced ability to expel culturable aerosolized *M. tuberculosis,* who therefore could be acting as disease superspreaders [23]. A better understanding of host and bacterial factors associated with *M. tuberculosis* aerosolization could provide fundamental insights into lung physiology,

Table 1. Characteristics of Study Participants by Site

Characteristic	Patients, No. (%)[a]			*P* Value
	Uganda (2002–2004) (n = 90)	Uganda (2009–2011) (n = 95)	Brazil (2013–2015) (n = 48)	
Age, median (IQR), y	32 (27–39)	29 (23–40)	32 (23–45)	.10
Sex				
Male	67 (74)	48 (51)	36 (75)	.001
Female	23 (26)	47 (49)	12 (25)	
HIV infection	43 (56)	21 (23)	0	<.001
Duration of symptoms, median (IQR), wk	12 (8–20)	12 (8–16)	12 (7–24)	.74
Duration of antibiotic treatment, median (IQR), d	4 (3–5)	1 (1–2)	0 (0-0)	<.001
Extent of disease on chest radiograph				
Normal/minimal	8 (11)	8 (9)	3 (6)	.41
Moderate	15 (21)	30 (33)	12 (25)	
Far advanced	48 (68)	52 (58)	33 (69)	
Cavitations	49 (69)	60 (67)	41 (85)	.05
Sputum appearance				
Salivary/mucosalivary	49 (68)	39 (41)	2 (4)	<.001
Purulent/mucopurulent	23 (32)	55 (59)	44 (96)	
Sputum AFB smear grade				
1+	16 (18)	15 (16)	3 (6)	.07
2+	24 (27)	14 (15)	9 (19)	
3+	50 (56)	66 (69)	36 (75)	
Time to sputum culture positivity,[b] median (IQR) d	7 (4–11)	6 (4–8)	5 (4–7)	.09
Aerosol CFU count				
Median (IQR)	0 (0–4)	0 (0–11)	2 (0–45)	.003
Mean (SD)	13.9 (75.6)	16.2 (49.5)	33.1 (65.2)	
Range	0–710	0–378	0–333	
Aerosol negative	62 (69)	52 (55)	19 (40)	.02
Aerosol positive				
Low aerosol CFU count (1–9 CFUs)	12 (13)	18 (19)	13 (27)	
High aerosol CFU count (≥10 CFUs)	16 (18)	25 (26)	16 (33)	

Abbreviations: AFB, acid-fast bacilli; CFUs, colony-forming units; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

[a]Data represent no. (%) of patients unless otherwise specified.

[b]Time to positivity in liquid culture medium (mycobacteria growth indicator tube [MGIT] 960).

immunology and bacterial aerobiology. Furthermore, timely identification of superspreaders would allow targeted LTBI strategies and more effective infection control interventions.

Some findings of our study are noteworthy. First, aerosol positivity was only partially related to commonly used measures of sputum bacillary burden, such as AFB smear grade and time

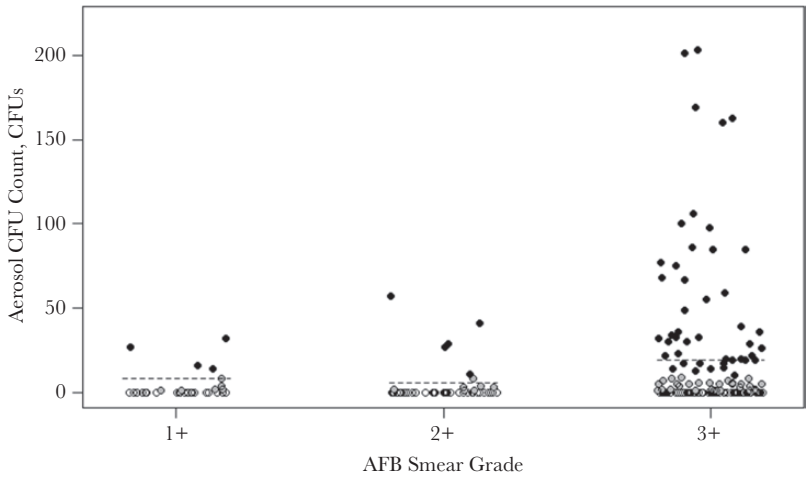

Figure 2. Culturable *Mycobacterium tuberculosis* colony-forming unit (CFU) count from cough-generated aerosol samples and sputum acid-fast bacilli (AFB) smear grade.

Table 2. Unadjusted Analysis of Characteristics Associated With Cough-Generated Aerosol Cultures From Patients With Pulmonary Tuberculosis

Characteristic[a]	Patients, No. (%)[b]				
			Aerosol Positive		
	All Patients	Aerosol Negative	Low CFU Count (1–9 CFUs)	High CFU Count (≥10 CFUs)	*P* Value[c]
Total group	233	133 (57)	43 (18)	57 (25)	…
Age, median (IQR), y	30 (25–40)	31 (25–39)	31 (25–44)	30 (24–41)	.75
Sex					
Male	151 (65)	85 (56)	28 (18)	38 (25)	.94
Female	82 (35)	48 (59)	15 (18)	19 (23)	
BMI,[d] median (IQR)	19 (17–21)	19 (17–21)	19 (17–20)	19 (17–21)	.53
HIV status					
Infected	64 (30)	40 (63)	14 (22)	10 (15)	.20
Uninfected	153 (70)	85 (56)	27 (18)	41 (27)	
Karnofsky performance score					
50–80	61 (29)	39 (64)	8 (13)	14 (23)	.35
90–100	154 (71)	84 (54)	32 (21)	38 (25)	
BCG status					
Present	147 (70)	88 (60)	25 (17)	34 (23)	.36
Absent/uncertain	62 (30)	31 (50)	15 (24)	16 (26)	
Duration of symptoms, median (IQR), wk	12 (8–20)	12 (8–20)	12 (8–16)	12 (8–16)	.33
Duration of antibiotic treatment, median (IQR), d	1 (0–3)	2 (1–4)	1 (0–2)	1 (0–2)	<.001
Radiographic findings					
Cavities					
Yes	150 (72)	87 (58)	24 (16)	39 (26)	.37
No	59 (28)	33 (56)	14 (24)	12 (20)	
Extent of disease					.
Normal/minimal	19 (9)	13 (68)	5 (26)	1 (5)	24
Moderately advanced	57 (27)	29 (51)	12 (21)	16 (28)	
Far advanced	133 (64)	78 (59)	21 (16)	34 (25)	
Cough parameters					
Cough strength					.
Weak	101 (33)	60 (59)	20 (20)	21 (21)	52
Strong	132 (67)	73 (55)	23 (17)	36 (27)	
Visual analog cough scale score, median (IQR)	5 (4–7)	5 (4–7)	5 (4–7)	5 (4–7)	.92
Peak expiratory flow rate, median (IQR), L/min	240 (160–320)	230 (170–270)	230 (170–230)	280 (200–350)	.16
Sputum characteristics					
Sputum volume, median (IQR), mL	5 (4–10)	5 (3–10)	5 (3–8)	7 (5–13)	.03
Sputum appearance					.
Salivary/mucosalivary	90 (42)	52 (58)	15 (17)	23 (25)	59
Purulent/mucopurulent	122 (58)	64 (53)	27 (22)	31 (25)	
AFB smear grade					
1+	34 (15)	23 (67)	6 (18)	5 (15)	.01
2+	47 (20)	35 (74)	7 (15)	5 (11)	
3+	152 (65)	75 (49)	30 (20)	47 (31)	
Time to sputum culture positivity,[e] median (IQR), d	6 (4–9)	7 (4–10)	5 (4–8)	5 (3–6)	.02

Abbreviations: AFB, acid-fast bacilli; BCG, bacille Calmette-Guérin; BMI, body mass index; CFU, colony-forming unit; HIV, human immunodeficiency virus; IQR, interquartile range.

[a]Data were missing for age in 2 patients, for body mass index in 23, for HIV status in 18, Karnofsky performance score in 20, BCG status in 27, duration of symptoms in 16, chest radiography in 26, sputum volume in 31, AFB smear grade in 2, sputum appearance in 69, and time to sputum culture positivity in 22.

[b]Data represent no. (%) of patients unless otherwise specified.

[c]*P* values were estimated using χ^2 tests for categorical variables and Kruskal-Wallis tests for numerical variables.

[d]Calculated as the weight in kilograms divided by height in meters squared.

[e]Time to positivity in liquid culture medium (mycobacteria growth indicator tube [MGIT] 960).

to positivity in liquid culture medium. We suggest that, like AFB smear, time to positivity is another risk factor for transmission, as it has been inversely associated, with enhanced tuberculosis transmission in household contacts [24]. Second, none of the host factors we studied was associated with aerosol positivity in the adjusted analysis, although it is important to emphasize that we did not evaluate other factors that could facilitate aerosolization, such as 24-hour cough-frequency [25] or

Table 3. Logistic Regression Model of Factors Associated With Growth of *Mycobacterium tuberculosis* in Cultures of Cough-Generated Aerosol Samples[a]

Variable	Samples, No.	OR (95% CI)	*P* Value
Antibiotic treatment duration[b]	206	1.47 (1.16–1.89)	.001
Sputum volume[c]	197	1.01 (.96–1.06)	.64
Cavitations on chest radiograph	150	0.59 (.27–1.29)	.19
Time to sputum culture positivity, d[d]	214	0.98 (.92–1.03)	.43
Sputum AFB smear grade			
3+	34	1.55 (.50–4.83)	.45
2+	57	0.43 (.11–1.72)	
1+	152	Reference standard	
HIV infection	64	1.61 (.39–3.76)	.27

Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

[a]Comparing samples from aerosol-positive (high and low aerosol colony-forming unit counts) versus aerosol-negative patients.

[b]Estimated per 1-day decrease in antibiotic use.

[c]Estimated per 1-mL increase in sputum volume.

[d]Estimated per 1-day increase in time to positivity for culture in liquid medium (mycobacteria growth indicator tube [MGIT] 960).

forced expiratory volume [26]. Alternatively, it is possible that bacterial survival in aerosol samples is modulated by yet unknown phenotypic or genotypic factors, such as lipid wall content, as suggested for nontuberculous mycobacteria [27]. Third, our results suggest that patients with pulmonary tuberculosis with advanced HIV are less likely to produce culturable aerosol samples, a finding consistent with transmission studies in this population [28]. Finally, cough-generated aerosol samples become rapidly sterile after antituberculous therapy is initiated, at a faster rate of decline compared with AFB smear and culture [29].

The marked decrease in tuberculosis infectiousness promptly after initiation of proper therapy and well before sputum smear conversion has been suggested elsewhere [30]. While in high-incidence settings significant delays in diagnosis and treatment initiation lead to continuous tuberculosis transmission [31], in low-incidence settings patients with tuberculosis often remain under respiratory isolation until either 3 sputum smears become negative or at least 2 weeks of proper tuberculosis therapy is provided, despite little evidence to support this recommendation [32].

In the seminal studies by Riley and colleagues [6, 7], tuberculosis transmission to guinea pigs ceased almost immediately after tuberculosis therapy. Furthermore, transmission in tuberculosis wards in Peru occurred only from patients with unsuspected drug-resistant tuberculosis or from those with delayed tuberculosis treatment initiation [33]. We posit that effective therapy could decrease tuberculosis infectiousness in 2 ways: (1) by decreasing bacterial burden and (2) by impairing the innate ability of the mycobacteria to survive in aerosol samples. If our results are confirmed by future studies, it could have consequential implications for infection control policies. In fact, the concept that nosocomial tuberculosis transmission is driven mainly by unsuspected or untreated tuberculosis cases

Table 4. Cough-Generated Aerosol Results According to Sputum Smear Microscopy Grade

Result	All Patients	AFB 1+ (n = 34)	AFB 2+ (n = 47)	AFB 3+ (n = 152)	*P* Value
Time to sputum culture positivity, d[a]	(n = 214)	(n = 30)	(n = 42)	(n = 142)	
Median (IQR)	6 (4–9)	10 (7–13)	8 (7–10)	5 (3–6)	.02
Mean (SD)	7.2 (5.7)	10.2 (4.9)	8.9 (4.1)	6.1 (6.0)	
Range	1–42	2–20	1–27	1–42	
Aerosol negative, No. (%)	133 (57)	23 (68)	35 (74)	75 (49)	.004
Aerosol positive, No. (%)	100 (43)	11 (32)	12 (26)	77 (51)	
Aerosol CFU Count, CFUs					
All patients					
Median (IQR)	0 (0–8)	0 (0–2)	0 (0–1)	1 (0–19)	.001
Mean (SD)	18.8 (63.9)	12.9 (57.0)	4.0 (11.4)	24.7 (73.5)	
Range	0–710	0–333	0–57	0–710	
Aerosol-positive patients	(n = 100)	(n = 11)	(n = 12)	(n = 77)	
Median (IQR)	16 (4–36)	8 (2–27)	6 (3–28)	19 (5–49)	.12
Mean (SD)	44.9 (92.2)	40 (97.8)	15.6 (18.6)	48.7 (97.8)	
Range	1–710	1–333	1–57	1–710	

Abbreviations: AFB, acid-fast bacilli; IQR, interquartile range; SD, standard deviation.

[a]Time to positivity in liquid culture medium (mycobacteria growth indicator tube [MGIT] 960).

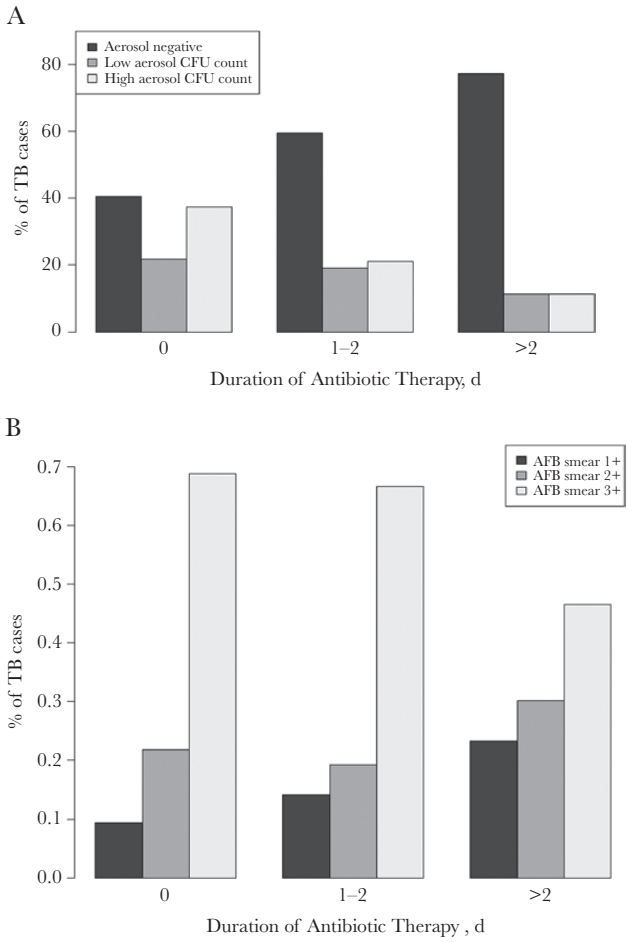

Figure 3. *A,* Culturable aerosol samples and duration of antibiotic therapy before cough aerosol sample collection. *B,* Sputum acid-fast bacilli (AFB) smear grade and duration of antibiotic therapy before sample collection.

is the basis of the find cases actively, separate safely and treat effectively strategy that aims to control tuberculosis spread in congregate settings by actively identifying unsuspected tuberculosis cases and prompt initiating tuberculosis therapy in high-incidence settings [34].

In the current study, 57% of patients with pulmonary tuberculosis did not produce culturable *M. tuberculosis* in aerosol samples, despite evidence of culture-positive disease in sputum. Nevertheless, studies using polymerase chain reaction (PCR) have identified *M. tuberculosis* in aerosol samples from close to 90% of patients with tuberculosis [35, 36]. The mechanisms behind the apparent improved yield of PCR compared with culture in aerosol samples is unclear; a possible explanation is that, when exposed to the stresses of aerosolization and airborne desiccation, some tuberculous bacilli may survive in a nonculturable state, as observed in other bacteria [37]. It is also likely that PCR is detecting nonviable bacilli that may be aerosolized from the respiratory tract but unable to replicate and cause disease in the exposed host after inhalation.

In our previous studies linking aerosol results to infection outcomes, household contacts of aerosol-negative patients had higher rates of tuberculin skin test positivity than community controls [12, 14], suggesting that aerosol-negative patients with tuberculosis may still transmit *M. tuberculosis.* However, when compared with infected contacts of aerosol-positive tuberculosis cases, aerosol negative contacts had significantly lower interferon gamma release assay responses and mostly have culture-negative tuberculosis disease during follow-up [12]. Taken together, these data suggest that measurements from cough-generated aerosol samples identify a subgroup of high-risk patients with pulmonary tuberculosis who transmit qualitatively different tuberculosis infection to their close contacts, with clustering of secondary tuberculosis cases around the aerosol-positive phenotype.

Our study has limitations. First, because we included sputum AFB-positive patients with mostly advanced radiographic tuberculosis disease, our results might not be generalizable to other important groups, such as those with smear-negative or subclinical tuberculosis. Second, other factors, such as time of contact and proximity to the index tuberculosis case patient, are also important to assess infectiousness [38]. Third, the data on CASS as a marker of infectiousness are based on only 2 household contact studies. Fourth, we performed aerosol sampling at only 1 point, which could lead to exposure misclassification. Fifth, most of our cohort were already receiving tuberculosis treatment at the time of CASS collection. Finally, the CASS device in its present form is designed as a research tool rather than a point-of-care device, which could limit its applicability in resourced-constrained settings. Although it would presumably be advantageous to have a more rapid signal (such as PCR) indicating the presence of *M. tuberculosis* in biological aerosols [36], such a test may not be able to discriminate the viability of the detected DNA.

In conclusion, we found that only a minority of sputum smear–positive patients with tuberculosis produce culturable *M. tuberculosis* in cough-generated aerosol samples. Aerosol production is only partially related to measures of bacillary burden, such as AFB smear grade or time to positivity in liquid medium. Antituberculous therapy seems to rapidly decrease bacterial viability in aerosol samples. Future mechanistic studies should further evaluate the role of the aerosol-positive phenotype in tuberculosis transmission, and the efficacy and cost-effectiveness of interventions aimed at targeting preventive therapy based on aerosol CFU counts rather than sputum AFB smear results as currently recommended.

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