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Factors associated with sputum culture non-completion and negativity in pulmonary tuberculosis cases in the U.S., 2011–2019

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Abstract

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By Lydia Rautman

Background
Sputum culturing is the gold standard for tuberculosis (TB) disease diagnosis because of its high sensitivity and utility in phenotypic drug sensitivity testing. Around one-quarter of verified TB cases in the United States 2011–2019 had either no sputum culture completed or a negative result, representing opportunities for missed cases and further transmission.

Methods
We used verified TB cases in the National Tuberculosis Surveillance System in bivariate and multivariable models to evaluate the association between predictors and outcomes of 1) sputum culture non-completion and 2) negative sputum culture result.

Results
Odds of sputum culture non-completion were higher among individuals with a non-sputum culture completed, long-term care facility residents, pediatric and elderly patients, and individuals whose care provider was not from a health department. Odds of negative sputum culture were higher among pediatric patients, individuals who had previously had TB, and patients with no cavitation on chest x-rays.

Discussion
In children, high odds of sputum culture non-completion were due to patient inability to expectorate sputum and high odds of negative sputum culture were due to increased rates of paucibacillary disease. In elderly patients and long-term care facility residents, odds of sputum culture non-completion were high due to difficulty obtaining a sputum specimen and incidental diagnosis. Higher suspicion for TB could be the driver behind lower odds of sputum culture non-completion in patients with a non-health department care provider and higher odds of negative sputum culture among patients who had previously had TB. Sputum culture-negative patients seem more likely to present with less cavitation and fewer TB symptoms.

Conclusion
TB sputum culture non-completion is due to patient inability to produce a sample and lack of consideration of TB on the differential diagnosis; a negative result occurs primarily in the presence of paucibacillary TB and in patients with poor immune function. Sputum culture should be performed when possible when considering TB, but non-sputum culture specimens may be available as an alternative. A negative result for any laboratory test should not always rule out TB and clinical evidence should be considered in supporting a diagnosis.

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CHAPTER 1: INTRODUCTION

Context

Tuberculosis (TB) remains a central concern in global health: according to World Health Organization estimates, in 2019 there were 10 million incident TB disease cases and 1.4 million TB deaths globally [1]. Compared with the global incident TB disease case rate of 130 cases per 100,000 persons, the United States has one of the lowest TB case rates in the world at 2.7 per 100,000 persons [1, 2]. The U.S. has sought to eliminate TB from the country, and incidence has steadily decreased since TB reporting began in 1953. The current incidence rate of TB in the U.S. is 5% of what it was seven decades previously, representing enormous efforts of public health programs to treat, manage, and prevent TB disease [2]. Nonetheless, despite major steps toward its goal, the U.S. struggles with achieving total elimination. One barrier to TB elimination is difficulty recognizing cases early, especially among individuals without apparent epidemiologic risk factors. As sputum culturing is the gold standard for diagnosing TB, identifying cases can be especially challenging when sputum culture results are unavailable for a patient or when the sputum culture result is negative, which is insufficient for a diagnosis. A delayed diagnosis can result in insufficient treatment, avoidable morbidity and mortality, and further transmission of TB.

Key issues

Sputum culturing provide critical information for the diagnosis and treatment of a TB patient when considered in conjunction with other diagnostic tools. Although the gold standard diagnostic method, around one-quarter of verified TB cases in the United States 2011–2019 had either no sputum culture completed or a negative result. Sputum culture non-completion could be due to the physician not ordering a sputum culture, inability of the patient to produce a sample, non-viability of the sample, or inability of the lab to culture the sample. There is a lack of even preliminary research on factors associated with sputum culture non-completion, but deeper investigation of this issue could illuminate intervention points

for TB programs where the process could be improved and culture completion rates increased, where possible. Increasing culture completion rates may result in higher treatment success and more complete molecular surveillance data, contributing to TB prevention and intervention efforts.

A negative sputum culture, while less common than a positive sputum culture in patients with pulmonary TB, is insufficient for making a diagnosis and could be accompanied by atypical presentation of disease. A negative result could suggest low bacillary load in expectorated sputum due to poor immune function [3-5], or an earlier stage of disease [6, 7], which is less transmissible and can be treated with a shorter drug regimen [8, 9]. In these cases, if not followed-up with other diagnostic methods, a negative culture can lead to a delayed diagnosis and progression to a more advanced or infectious stage with higher morbidity and mortality of the TB patient [10, 11], especially among those with risk factors [12], and can result in further transmission. Understanding in which populations a sputum culture is more likely to be negative will help clinicians utilize TB diagnostic methods to enable an accurate diagnosis. This will improve case and outbreak recognition, and enable programs to better address and prevent TB transmission.

Aims, purpose, and significance

This study utilizes National Tuberculosis Surveillance System (NTSS) data from 2011–2019 to investigate factors associated with sputum culture non-completion and negativity in TB cases with pulmonary involvement. Specifically, we aim to identify the following:

1. Which demographic, clinical, or laboratory characteristics are associated with sputum culture non-completion among pulmonary TB cases?
2. Which demographic, clinical, or laboratory characteristics are associated with sputum culture negativity among pulmonary TB cases?

While TB elimination efforts in the U.S. have been largely successful over the last nearly 60 years, a sputum culture completion rate of approximately 90% leaves much room for improvement, especially considering the importance of culture completion in diagnosing cases, recognizing outbreaks, determining treatment, and preventing further transmission. Determining factors associated with a sputum culture not being completed will facilitate and target efforts to increase this completion rate. A sputum culture result can be critically informative, but may need to be carefully considered in the context of other diagnostic methods. A deeper understanding of characteristics associated with culture negativity may improve recognition of culture-negative TB. Ultimately, both of these analyses seek to improve our TB elimination efforts through earlier and more accurate diagnosis, treatment, and prevention.

Definition of terms and concepts

AIDS	Acquired immune deficiency syndrome
AFB	Acid-fast bacilli
CDC	Centers for Disease Control
CT scan	Computed tomography scan
Culture completion	A sputum specimen was cultured with a result of either “positive” or “negative.”
Culture negativity	A sputum specimen from a TB case was cultured with a result of “negative.”
Culture non-completion	No sputum culture result was available for a diagnosed TB case
HIV	Human immunodeficiency virus
IGRA	Interferon-gamma release assay
LTBI	Latent tuberculosis infection
MDR(-TB)	Multidrug-resistant (TB)
MTB(C)	*Mycobacterium tuberculosis* (complex)
NAA(T)	Nucleic acid amplification (test)
NH	Non-Hispanic
(non-)IDU	(Non-) injection drug use
NTSS	National Tuberculosis Surveillance System
PTB	Pulmonary TB
SNP	Single nucleotide polymorphism
TB	Tuberculosis
TST	Mantoux tuberculin skin test
U.S.	United States
WGS	Whole genome sequencing
XDR(-TB)	Extensively drug-resistant (TB)

CHAPTER 2: LITERATURE REVIEW

World and United States epidemiology

Tuberculosis is the leading cause of death by a single infectious agent globally, making the disease one of the top health problems public health entities face today [1]. Approximately one-quarter of the world population is infected with *Mycobacterium tuberculosis* in either a latent or active state [2]. TB incidence in the U.S. has been steadily decreasing since the 1953, when TB reporting began, aside from a small resurgence in the 1990s with the AIDS epidemic [3]. In 2019 the U.S. saw its lowest incidence of TB at a rate of 2.7 cases per 100,000 persons. Drug resistant TB case rates have remained stable for 20 years, with resistance to isoniazid being the most common and occurring in 9% of isolates that were tested for drug susceptibility [4]. In 2019, 71.4% of cases reported were among non-U.S.–born persons, and diabetes, alcohol use, HIV coinfection, non-injection drug use (non-IDU), injection drug use (IDU), homelessness, and incarceration remained important risk factors among cases. The majority of TB cases occurred in California, Texas, New York, and Florida: in 2019, 51% of TB cases were reported from those states [4].

Incidence of active disease has been declining in the U.S., with 8,916 reported cases in the U.S. in 2019 [5]. This risk is not evenly distributed and is higher in several vulnerable populations. A Morbidity and Mortality Weekly Report (MMWR) from the Centers for Disease Control (CDC) reported that in 2017, rates of TB among non-U.S.–born persons living in the U.S. was nearly 15 times greater than among U.S.-born persons, with the highest rates among non-U.S.–born persons being among non-Hispanic (NH) Asians and NH Blacks [6]. Among U.S.-born persons, TB rates were highest among NH Native Hawaiians/Pacific Islanders, followed by NH American Indians and Alaska Natives, NH Blacks, NH Asians, Hispanics, and NH Whites.

Tuberculosis disease can result from either reactivation of a previously acquired infection or recent transmission. A study using source-case methods to analyze TB surveillance data found that from 2011–2014, approximately 60% of TB cases in the U.S. were among non-U.S.–born individuals, although only

8% of recent transmission was attributed to non-U.S.–born cases [7]. It appeared that most cases among non-U.S.–born individuals resulted from reactivation of or progression to active disease from a previous infection acquired abroad where TB rates are higher than those in the U.S. This highlights the importance of focusing on early diagnosis of TB cases in immigrants, so as to prevent further spread within these communities.

Pathogenesis

Respiratory droplets carrying *Mycobacterium tuberculosis* bacteria are expectorated by a patient with active pulmonary TB, typically through coughing, but can also be produced by sneezing, singing, talking, or simply breathing. Depending on the size of the droplets, they can stay in the air for several minutes up to hours after expectoration. These droplet nuclei can be inhaled by a susceptible individual and the bacteria make their way to the individual's lungs.

If the immune system is intact, a cell-mediated immune response is developed within 2–8 weeks after infection and further replication of the bacteria is halted for the vast majority of infected individuals. The immune system continues to mount a response, clearing the infection completely in 10% of cases and halting growth in the other 90%, where the bacilli become dormant, resulting in latent tuberculosis infection (LTBI) [8]. In 5–10% of LTBI cases, dormant bacilli will at some later point cause reactivation TB, a form of active TB disease, which in pulmonary form can be infectious to others [8, 9]. Half of these reactivation cases occur in the first two years after exposure and the other half at some point later in time [10]. Medical conditions that increase the risk for individuals with a latent infection to develop active disease include coinfection with HIV, diabetes, renal failure, and general immunosuppression [11, 12]. If the immune response is weak, the tubercule bacilli will replicate uncontrolled, causing primary TB, most commonly in the form of pulmonary TB. [8, 13]. Primary TB is most commonly associated with children under the age of 5 years and advanced immunosuppression like AIDS [12].

Clinical features and treatment

Pulmonary TB (PTB) affects the lungs alone and makes up 80% of cases and presents with symptoms including a cough that lasts for several weeks, chest pain, coughing up blood or sputum, fatigue, weight loss, loss of appetite, chills, fever, and night sweats [14]. PTB usually causes abnormalities on the chest x-ray, including evidence of cavities. As cavities increase access of TB organisms to the respiratory tract for expectoration, cases with cavitation are considered more infectious and tests conducted on sputum are more likely to be positive [15, 16].

Patients with naïve or weak immune systems may have a large growth of bacteria in the lungs due to inability to control the infection. Paradoxically, as a weak immune system is often unable to create cavities [12, 17], there may be few organisms present in the sputum and these individuals may have a negative result for a diagnostic test. This is most notably demonstrated with more frequent smear-negative disease in patients with HIV [18, 19]. Although not yet demonstrated, weak immune response could have similar implications for sputum culture results. In pediatric patients, lymphatic involvement is common and miliary disease, indicative of disseminated TB in the setting of immunosuppressed individuals, may also be evident on chest x-ray [20].

Extrapulmonary TB (EPTB) may result when the immune response fails to contain *M. tuberculosis* and the bacteria reach the bloodstream to be carried to a different site or sites in the body, where they replicate and cause further infection [13]. Comprising approximately one-fifth of TB disease cases, EPTB is much less common than PTB and can be caused by a smaller number of bacilli, making it difficult to diagnose due to lower likelihood of a positive result on a laboratory test [20, 21]. Risk of extrapulmonary involvement in TB infection increases with immunosuppression and is most notably associated with AIDS [22]. Patients with concurrent pulmonary and extrapulmonary TB may present with atypical and nonspecific symptoms that vary depending on the site of infection [20, 23]. Because of this, presentation

of EPTB may be more difficult to recognize as TB, delaying the diagnosis and in serious cases resulting in hospitalization or death, particularly for immunocompromised patients [24].

If untreated, both pulmonary and extrapulmonary TB can be fatal. In 2018, there were 542 deaths attributed to all forms of TB in the U.S. [21]. Drug-susceptible TB disease is usually treated with a regimen of first-line anti-TB drugs including isoniazid, rifampin, ethambutol, and pyrazinamide for a total of 6 to 9 months [25]. Drug-resistant, multidrug-resistant (MDR), and extensively drug-resistant (XDR) TB require different treatment regimens. Special consideration is also taken in determining a treatment regimen for individuals with HIV because of drug interactions [25].

Diagnosis

A TB case is verified by fulfillment of specified laboratory and clinical criteria; when laboratory data are not available and clinical criteria are not met, a case may be verified via provider diagnosis. Laboratory diagnostic options for case verification are a positive sputum or non-sputum culture, a positive nucleic acid amplification test (NAAT), or a positive acid-fast bacilli (AFB) smear if *M. tuberculosis* complex (MTBC) cannot be isolated from the specimen. Where culture, NAAT, and AFB smear are not completed or are negative, a clinical diagnosis can be made if the chest x-ray or CT scan is abnormal, tuberculin skin test (TST) or interferon-gamma release assay (IGRA) is positive, and there is documented improvement of the patient on an anti-TB treatment regimen [26]. In verifying a case, laboratory criteria supersede fulfillment of clinical criteria; of the possible laboratory diagnostic tools, isolation of MTBC via sputum culture is the gold standard. It is the most sensitive laboratory method to diagnose TB disease and is able to detect MTBC starting at counts as low as 10 organisms [27]. Due to its slow growth rate—generally 4 weeks in liquid broth media and up to 6–8 weeks on solid media—it is often completed in conjunction with other faster laboratory tests. However, culturing from a sputum specimen remains the gold standard for diagnostic testing and is necessary for phenotypic drug susceptibility testing, which

informs clinical decisions about treatment regimens [28]. Culture is also necessary for genotyping, which informs epidemiologic investigation of recent transmission [29].

Culture and smears may be negative in patients with sub-clinical infection, which could be indicative of early-stage disease [30]. Diagnostic tests may also have lower sensitivity in children and immunocompromised patients. Research on this phenomenon in patients with HIV suggests it could be due to the inability of the impaired immune system to produce cavities, through which mechanism TB organisms become present in the sputum [16, 31].

When the patient is unable to expectorate a sputum specimen for culture, obtainment of an alternative specimen is acceptable. A gastric aspirate specimen is usually the non-sputum specimen collected in pediatric patients [20]. Another alternative for pediatric or adult patients is the bronchial fluid specimen, obtained by bronchoscopy; however, as an aerosol-generating procedure, it is not recommended unless absolutely necessary and only when infection control measures can be ensured [20, 32, 33]. The AFB smear is a diagnostic tool that can quantify patient infectiousness based on grading the number of bacilli present in sputum samples visualized in microscopic fields [34]. While smear microscopy is inexpensive, it has a higher likelihood for false negatives, as it will only register an infection above a concentration of 10,000 bacilli per mL sputum [35]. The NAAT is a molecular method for detection of MTBC and rifampin resistance [36]. NAA testing is sensitive, timely, and is the standard of care; however, NAA testing does not replace the need for AFB smear and culture [37].

Genotyping methods

Quicker recognition of outbreaks can be facilitated through the use of whole genome sequencing (WGS), a genotyping method that enables epidemiologists to compare TB isolates and discern recent transmission, recognizable by identical or closely related genetic sequences of the isolates [28, 38]. WGS identifies and quantifies single nucleotide polymorphisms (SNPs) to determine the genetic relationship of

2+ isolates. The SNPs identified are mapped onto a phylogenetic tree to illustrate relationships and focus and inform epidemiologic investigations. This helps epidemiologists and researchers determine key trends and high-risk groups, as well as detect and address outbreaks. In order to perform WGS, positive culture of a specimen is necessary.

Factors associated with TB infection

Several social risk factors have been identified as increasing an individual's risk of TB infection. All categories of substance use can increase an individual's risk for TB, possibly through a combination of biologic processes, such as a weakening of the immune system resulting in higher susceptibility [39], and social mechanisms, such as how the environment for partaking in substance use—typically an indoor, crowded space—is also ideal for TB transmission via respiratory droplets due to the nature of inhalation drugs [40, 41]. Substance use also presents a barrier to TB control because of the stigma, as well as the reluctance to name contacts associated with illegal activities, which can increase the likelihood of missing contacts, increasing the potential for further transmission [40].

Homelessness places individuals at higher risk for exposure to M. tuberculosis because of communal living spaces and shared air space [42, 43]. This compounds with a number of factors that disproportionately affect persons experiencing homelessness and compromise the immune system, including HIV, substance use, malnutrition, and other chronic health conditions, increasing susceptibility to infection with TB [39, 44-46]. This is further exacerbated by the lower rates at which homeless individuals receive treatment for early-stage TB, leading to more infectious TB when untreated [44, 45, 47].

Occupation, correctional facilities, and residence in a long-term care facility also represent important factors associated with TB infection. Occupation as a healthcare worker is frequently considered a risk factor for TB infection and disease [48]. TB incidence and risk factors for TB are much higher and rates

of treatment completion are lower among inmates in state and federal prisons compared with the general non inmate population [49-51]. Similar to homeless shelters and correctional facilities, long-term care facilities impose a greater risk of TB infection on their elderly residents due to the nature of nursing homes as communal living spaces, the poorer health of the residents, and the opportunity for nosocomial infection through the provision of healthcare [52, 53].

Pediatric TB

In 2019, children under 15 comprised 4% of TB disease cases in the U.S. [54]. Children represent an especially important population in TB due to the unique epidemiology and challenges presented in diagnosing young patients. Infection in children, especially very young children, is most commonly primary tuberculosis, due to the inability of the immune system to control the infection [12, 55]. Young children usually do not form cavities in the lungs [17], are less infectious and have lower rates of relapse [20]; this also means they may be more likely to have negative results for sputum smears and cultures. While cavitation is rare, extrapulmonary involvement and disseminated TB are more common among very young children, but adolescents are more likely to have TB disease presentation similar to that in adults, which may include radiographic evidence of cavitation [17, 20].

Non-specific symptoms and radiographic findings make pediatric TB difficult to recognize, compounded by difficulty isolating the MTBC organism. Sputum specimens are frequently unable to be obtained from pediatric patients due to their inability to expectorate; gastric aspirates are most often collected as an alternative specimen for culturing [20, 55]. However, microbiologic tests on both sputum and gastric aspirate specimens are less sensitive in children due to the typically paucibacillary appearance of TB disease in children [56, 57], although culturing both sputum and gastric aspirate specimens can increase sensitivity significantly [58]. Owing to the difficulty of obtainment and low sensitivity of specimens from children for laboratory tests, children are more often diagnosed on the basis of clinical

criteria, including TST and chest x-ray results, and factoring in epidemiologic evidence, such as linking to an adult source case [17, 55]. When a culture cannot be completed or is negative for the child, specimens from the source adult case are then critical for drug susceptibility testing and determination of a treatment regimen for the child.

Human immunodeficiency virus (HIV)

HIV coinfection presents a unique challenge for TB prevention and control. HIV coinfection with TB results in different clinical presentation, more severe disease, and a higher risk of death [24, 59, 60]. During TB infection, the production of interferon gamma (INF-γ), among other cytokines, plays a role in inhibiting intracellular growth of the bacteria. HIV infection decreases the production of IFN-γ along with CD4+ T-lymphocytes, significantly increasing a patient's risk of reinfection or reactivation [59]. Even when matching on CD4+ T-cell count, patients with HIV who are coinfected with TB have twice the risk of death compared with patients with HIV who do not have TB coinfection [60].

Due to immunodeficiency accompanying more advanced stages of HIV/AIDS, patients with HIV are more likely to progress from LTBI to active TB disease compared with patients without HIV [61], and are more likely to present with extrapulmonary involvement [22]. Because decreased immune response results in less cavitation, patients with HIV-TB coinfection are more likely to have negative smear results, even though they often have a higher mycobacterial load [16, 18, 19, 31]. While not yet demonstrated, this process could have similar implications for sputum cultures in patients with HIV. Patients with more advanced HIV may present with milder symptoms or symptoms atypical of TB, and less frequently with radiographic evidence of cavitation [20]. These factors can make it difficult to diagnose and treat an HIV-TB coinfection in time, particularly in vulnerable populations like persons experiencing homelessness, among whom rates of HIV are higher. Prompt diagnosis of the coinfection is also imperative for proper treatment: a normal TB treatment regimen with rifampicin has the potential to interfere with antiretroviral

therapy and patients with HIV may require alternative TB therapy [24, 62]. Drug resistant TB, particularly strains resistant to isoniazid and rifampicin, is more prevalent among patients with HIV compared with HIV-seronegative patients and may be acquired due to poor adherence to TB treatment and malabsorption of anti-TB drugs, as well as interactions with antiretroviral drugs that result in resistance [24, 63].

Barriers to diagnosis and treatment

As rates of TB continue to decline in the U.S., the challenge has been in identifying remaining barriers to diagnosis and treatment. Some main contributors to delays in diagnosis are low physician awareness of TB epidemiology and failure to screen for TB. [64]. As TB incidence decreases, fewer physicians have experience diagnosing and managing TB cases and are less likely to consider TB as a possible diagnosis for patients presenting with non-specific TB symptoms [65]. Failing to identify TB can result in prolonged infection and further transmission. A commentary published in the *International Journal of Infectious Diseases* pointed out how despite relatively higher rates of TB among migrants, prisoners, persons experiencing homelessness, and individuals who report drug or alcohol use, TB detection is delayed in these vulnerable populations [66]. The report described further barriers to diagnosis, including misconceptions about TB, stigma, poor TB awareness among healthcare workers, lack of specialist services, and language/cultural barriers. Further reviews have made similar suggestions for improvement for diagnosis among immigrants, including universal access to care along migration pathway, TB-specific screening adapted to migrant groups, and linking of screening to treatment [67].

Sputum culture non-completion

Culturing of an MTBC isolate is the gold standard for diagnostic testing for active TB disease and is critical for resistance testing. For the purposes of this study, the term “sputum culture completion” is used to describe when a sputum culture was collected and cultured, regardless of whether the culturing

result was positive or negative; "sputum culture non-completion" refers to a verified TB case with no sputum culture result available and it is assumed that no sputum culture was done. Sputum culture results were not available for approximately 10% of TB cases with pulmonary involvement from 2011–2019. A lack of culturing could result from the physician not ordering a sputum culture, inability of the patient to produce a sample, non-viability of the sample, or inability of the lab to culture the sample or report results.

Previous research on characteristics associated with culture non-completion in the United States is limited. One widely acknowledged challenge to sputum culture completion is pediatric age; this is because children under 12 years generally do not produce sputum that is adequate for culture [20]. Traditional TB diagnostic methods used for adults, such as a sputum culture, may be difficult to obtain and less sensitive in children, and are consequently bypassed; TB diagnosis among children is often based on a positive TST, clinical and radiographic indication, as well as epidemiologic risk factors [17, 20, 68]. Not completing a sputum culture for a suspected TB patient could result in missed cases, inappropriate treatment if phenotypic drug susceptibility testing is not performed, and gaps in molecular surveillance data.

Sputum culture negativity

A TB case is verified ideally via positive sputum culture. This may not be possible when a sputum specimen cannot be obtained from the patient or when the sputum culture result is negative. A negative sputum culture can be due to low bacilli populations in the lungs (as seen in paucibacillary disease), poor access of cavitary lesions to the respiratory tract, poor specimen quality, or errors during specimen handling [69, 70]. Approximately 15–20% of adults with pulmonary TB disease have negative sputum cultures and are diagnosed on the basis of other laboratory, clinical, and radiologic findings [20, 71]. This percentage is estimated to be even higher among children, where the culture may be less sensitive due to lower mycobacterial load in the sputum [12, 17, 20, 68, 72]. A patient can be diagnosed with culture-

negative TB via positive NAAT or clinical and radiographic presentation consistent with TB, as well as improvement of symptoms or condition with antituberculosis treatment [69, 71].

Limited preliminary research has investigated factors associated with a negative culture result. A study in Hong Kong in 1981 among patients with sputum smear-negative disease found sputum culture negative patients to have fewer radiographic abnormalities and report fewer TB symptoms, including blood-streaked sputum and frank hemoptysis [73]. In a more recent sub-project of a larger cross-sectional study, Nguyen et al. looked at clinical and radiographic differences between sputum culture-negative and positive pulmonary TB patients (N = 99) in New York City [74]. They found sputum culture-negative patients to less frequently report classic TB symptoms like cough, sputum production, and weight loss, and to less frequently have cavitation on radiographic imaging compared with sputum culture-positive patients. A larger follow-up study (N = 796) reported similar findings and called for further research on the presentation of culture-negative TB disease, so as to improve diagnosis of this likely underrecognized disease state [71]. A prospective cohort study conducted by Nakiyingi et al. analyzed predictors for positive MTB cultures among 418 HIV-positive smear-negative TB patients in Uganda. They found culture negativity to be associated with previous TB treatment [75].

Culture-negative TB presents an important challenge for diagnosis and may be one cause of underdiagnosis, given that the proportion of culture-negative TB patients is estimated to be as high as 20% [12, 71]. Culture-negative TB can result from lack of cavitation in immunodeficient patients, but it can alternatively represent an early stage on the TB disease continuum because of the likelihood of progression from culture-negative to culture-positive TB if treatment is not initiated [74, 76, 77]. A better understanding of factors associated with culture negativity may help to enhance diagnostic methods to more quickly and accurately detect and treat TB. Early diagnosis and treatment of TB are associated with lower morbidity and mortality of the patient [73, 78], particularly among individuals with HIV [79]. Early treatment can additionally prevent further transmission, as culture-negative TB is typically less

transmissible than culture-positive TB due to its relatively lower mycobacterial burden [69, 74]. Under some circumstances, culture-negative TB can be treated with a shorter drug regimen of four months of chemotherapy compared with the six to nine months of treatment typical for culture-positive cases [69, 80]. Therefore, in addition to reducing patient morbidity and mortality and preventing further transmission, accurate diagnosis of early-stage culture-negative TB disease can minimize the treatment burden with a shorter regimen time.

Summary

The positive sputum culture is the gold standard for diagnosis in a patient with active TB disease, superior to other laboratory tests for its high sensitivity, utility in species identification and phenotypic drug susceptibility testing, and its role in the broader context of TB elimination via molecular surveillance. Because of slow growth rates, a sputum culture is often performed in conjunction with other diagnostic tests but is nevertheless the gold standard in high-resource areas like the U.S.

There are two reasons why a TB patient may not be diagnosed via positive sputum culture: the sputum culture was not completed or the sputum culture result was negative. In this two-part study, we explore demographic characteristics, risk factors, clinical features, and laboratory results associated with both scenarios. Investigating why sputum cultures are not able to be completed could help us increase rates of sputum culturing or understand in which cases alternative diagnostic methods should be considered. Understanding factors associated with sputum culture negativity could help raise clinician awareness of underrecognized atypical TB presentations, thereby reducing morbidity and further transmission resulting from delayed diagnosis. While we have made great strides in lowering the incidence of TB in the United States over the last several decades, improving our understanding of factors associated with culture non-completion and culture negativity could make these tools more impactful in our efforts to eliminate TB.

References

1. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization, **2020**.
2. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS medicine **2016**; 13(10): e1002152.
3. Centers for Disease Control. Tuberculosis (TB): TB Incidence in the United States. TB Incidence in the United States, 1953-2019 TB Cases and Case Rates per 100,000 Population. Available at: https://www.cdc.gov/tb/statistics/tbcases.htm.
4. Centers for Disease Control. Tuberculosis (TB): Trends in Tuberculosis 2019. Available at: https://www.cdc.gov/tb/publications/factsheets/statistics/tbtrends.htm.
5. Centers for Disease Control. Tuberculosis (TB): Data and Statistics. Available at: https://www.cdc.gov/tb/statistics/default.htm. Accessed October 20.
6. Stewart RJ, Tsang CA, Pratt RH, Price SF, Langer AJ. Tuberculosis—United States, 2017. Morbidity and Mortality Weekly Report **2018**; 67(11): 317.
7. Yuen CM, Kammerer JS, Marks K, Navin TR, France AM. Recent transmission of tuberculosis—United States, 2011–2014. PloS one **2016**; 11(4): e0153728.
8. Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clinical and Developmental Immunology **2011**; 2011.
9. Cruz-Knight W, Blake-Gumbs L. Tuberculosis: An Overview. Primary Care: Clinics in Office Practice **2013**; 40(3): 743-56.
10. Centers for Disease Control. Tuberculosis (TB): Fact Sheets. **2014**.
11. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS med **2008**; 5(7): e152.
12. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. The Lancet **2003**; 362(9387): 887-99.
13. Kaufmann SH. Tuberculosis: Deadly combination. Nature **2008**; 453(7193): 295-6.
14. Centers for Disease Control. Tuberculosis (TB): Signs & Symptoms. Available at: https://www.cdc.gov/tb/topic/basics/signsandsymptoms.htm.
15. Shaw JB, Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. American review of tuberculosis **1954**; 69(5): 724-32.
16. Ong CW, Elkington PT, Friedland JS. Tuberculosis, pulmonary cavitation, and matrix metalloproteinases. American journal of respiratory and critical care medicine **2014**; 190(1): 9-18.
17. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. Emerging infectious diseases **1995**; 1(4): 115.
18. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. The Lancet **2007**; 369(9578): 2042-9.
19. Gathua S, Waiyaki P. Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis. Am Rev Respir Dis **1993**; 147: 958-61.
20. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med **2000**; 161: 1376-95.
21. Centers for Disease Control. Tuberculosis Cases and Percentages by Case Verification Criterion and Site of Disease: United States, 1993–2019. **2020**.
22. Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. American family physician **2005**; 72(9): 1761-8.
23. Kim J-H, Kim ES, Jun K-I, et al. Delayed diagnosis of extrapulmonary tuberculosis presenting as fever of unknown origin in an intermediate-burden country. BMC infectious diseases **2018**; 18(1): 426.
24. Aaron L, Saadoun D, Calatroni I, et al. Tuberculosis in HIV-infected patients: a comprehensive review. Clinical microbiology and infection **2004**; 10(5): 388-98.
25. Centers for Disease Control. Tuberculosis (TB): Treatment for TB Disease. **2016**.
26. Centers for Disease Control. National Tuberculosis Surveillance System (NTSS), data set reference guide. **2015**.

27. van Zyl-Smit RN, Binder A, Meldau R, et al. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. PloS one **2011**; 6(12): e28815.
28. Hobby GL, Holman AP, Iseman MD, Jones JM. Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. Antimicrobial agents and chemotherapy **1973**; 4(2): 94-104.
29. Forbes BA, Hall GS, Miller MB, et al. Practice guidelines for clinical microbiology laboratories: mycobacteria. Clinical microbiology reviews **2018**; 31(2).
30. Furin J, Cox H, Pai M. Tuberculosis. The Lancet **2019**; 393(10181): 1642-56.
31. Walker NF, Clark SO, Oni T, et al. Doxycycline and HIV infection suppress tuberculosis-induced matrix metalloproteinases. American journal of respiratory and critical care medicine **2012**; 185(9): 989-97.
32. Stem DR. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care facilities, 1994. **1994**.
33. Larson JL, Ridzon R, Hannan MM. Sputum induction versus fiberoptic bronchoscopy in the diagnosis of tuberculosis. American journal of respiratory and critical care medicine **2001**; 163(5): 1279-80.
34. Centers for Disease Control. Tuberculosis (TB). **2019**.
35. Mase S, Ramsay A, Ng V, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. The International Journal of Tuberculosis and Lung Disease **2007**; 11(5): 485-95.
36. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. New England Journal of Medicine **2010**; 363(11): 1005-15.
37. Centers for Disease Control. Tuberculosis (TB): Report of an Expert Consultation on the Uses of Nucleic Acid Amplification Tests for the Diagnosis of Tuberculosis, **2012**.
38. Centers for Disease Control. Tuberculosis (TB): TB Genotyping. Available at: https://www.cdc.gov/tb/programs/genotyping/.
39. Rehm J, Samokhvalov AV, Neuman MG, et al. The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. BMC public health **2009**; 9(1): 450.
40. Mitruka K, Oeltmann JE, Ijaz K, Haddad MB. Tuberculosis outbreak investigations in the United States, 2002–2008. Emerging infectious diseases **2011**; 17(3): 425.
41. Oeltmann JE, Oren E, Haddad MB, et al. Tuberculosis outbreak in marijuana users, Seattle, Washington, 2004. Emerging infectious diseases **2006**; 12(7): 1156.
42. Barnes PF, El-Hajj H, Preston-Martin S, et al. Transmission of tuberculosis among the urban homeless. Jama **1996**; 275(4): 305-7.
43. Haddad MB, Wilson TW, Ijaz K, Marks SM, Moore M. Tuberculosis and homelessness in the United States, 1994-2003. Jama **2005**; 293(22): 2762-6.
44. Centers for Disease Control Prevention. Tuberculosis outbreak associated with a homeless shelter-Kane County, Illinois, 2007-2011. MMWR Morbidity and mortality weekly report **2012**; 61(11): 186.
45. Bamrah S, Yelk Woodruff R, Powell K, Ghosh S, Kammerer J, Haddad M. Tuberculosis among the homeless, United States, 1994–2010. The international journal of tuberculosis and lung disease **2013**; 17(11): 1414-9.
46. Wolitski RJ, Kidder DP, Fenton KA. HIV, homelessness, and public health: critical issues and a call for increased action. AIDS and Behavior **2007**; 11(2): 167.
47. Centers for Disease Control. Tuberculosis transmission in a homeless shelter population--New York, 2000-2003. MMWR Morbidity and mortality weekly report **2005**; 54(6): 149.
48. Menzies D, Joshi R, Pai M. Risk of tuberculosis infection and disease associated with work in health care settings [State of the Art Series. Occupational lung disease in high-and low-income countries, Edited by M. Chan-Yeung. Number 5 in the series]. The International Journal of Tuberculosis and Lung Disease **2007**; 11(6): 593-605.
49. Centers for Disease Control. Tuberculosis (TB): TB in Correctional Facilities in the United States. **2020**.
50. MacNeil JR, Lobato MN, Moore M. An unanswered health disparity: tuberculosis among correctional inmates, 1993 through 2003. American Journal of Public Health **2005**; 95(10): 1800-5.
51. Braun MM, Truman BI, Maguire B, et al. Increasing incidence of tuberculosis in a prison inmate population: association with HIV infection. Jama **1989**; 261(3): 393-7.
52. Stead WW, Lofgren J, Warren E, Thomas C. Tuberculosis as an endemic and nosocomial infection among the elderly in nursing homes. New England Journal of Medicine **1985**; 312(23): 1483-7.

53. Rajagopalan S, Yoshikawa TT. Tuberculosis in long-term-care facilities. Infection control and hospital epidemiology **2000**; 21(9): 611-5.
54. Centers for Disease Control. Tuberculosis Cases, Percentages, and Case Rates per 100,000 Population by Age Group: United States, 1993–2019. **2020**.
55. Piccini P, Chiappini E, Tortoli E, de Martino M, Galli L. Clinical peculiarities of tuberculosis. BMC infectious diseases **2014**; 14(1): 1-12.
56. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. The Lancet **2005**; 365(9454): 130-4.
57. Marais BJ. Advances in the clinical diagnosis of TB in children. Pediatric research **2008**; 63(2): 116.
58. Singh S, Singh A, Prajapati S, et al. Xpert MTB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. BMC microbiology **2015**; 15(1): 1-10.
59. Zhang M, Gong J, Iyer DV, Jones BE, Modlin RL, Barnes PF. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. The Journal of clinical investigation **1994**; 94(6): 2435-42.
60. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. American journal of respiratory and critical care medicine **1995**; 151(1): 129-35.
61. Daley CL, Small PM, Schecter GF, et al. An outbreak of tuberculosis with accelerated progression among persons infected with the Human Immunodeficiency Virus: an analysis using restriction-fragment—length polymorphisms. New England journal of medicine **1992**; 326(4): 231-5.
62. Centers for Disease Control Arizona. Prevention and treatment of tuberculosis among pa-tients infected with human immunodeficiency virus: prin-ciples of therapy and revised recommendations. Mmwr **1998**; 47(RR–20).
63. Munsiff SS, Joseph S, Ebrahimzadeh A, Frieden TR. Rifampin-monoresistant tuberculosis in New York city, 1993–1994. Clinical infectious diseases **1997**; 25(6): 1465-7.
64. Golub JE, Bur S, Cronin W, et al. Patient and health care system delays in pulmonary tuberculosis diagnosis in a low-incidence state. The International journal of tuberculosis and lung disease **2005**; 9(9): 992-8.
65. Lönnroth K, Migliori GB, Abubakar I, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. European Respiratory Journal **2015**; 45(4): 928-52.
66. Heuvelings C, de Vries S, Grobusch M. Tackling TB in low-incidence countries: improving diagnosis and management in vulnerable populations. International Journal of Infectious Diseases **2017**; 56: 77-80.
67. Lönnroth K, Mor Z, Erkens C, et al. Tuberculosis in migrants in low-incidence countries: epidemiology and intervention entry points. The International Journal of Tuberculosis and Lung Disease **2017**; 21(6): 624-36.
68. Cruz AT, Starke JR. Pediatric tuberculosis. Pediatrics in review **2010**; 31(1): 13.
69. Blumberg HM, Burman WJ, Chaisson RE, Daley CL. American thoracic society/centers for disease control and prevention/infectious diseases society of America: treatment of tuberculosis. American journal of respiratory and critical care medicine **2003**; 167(4): 603.
70. Mccarter YS, Robinson A. Quality evaluation of sputum specimens for mycobacterial culture. American journal of clinical pathology **1996**; 105(6): 769-73.
71. Nguyen M-VH, Levy NS, Ahuja SD, Trieu L, Proops DC, Achkar JM. Factors associated with sputum culture-negative vs culture-positive diagnosis of pulmonary tuberculosis. JAMA network open **2019**; 2(2): e187617-e.
72. Zar HJ, Connell TG, Nicol M. Diagnosis of pulmonary tuberculosis in children: new advances. Expert review of anti-infective therapy **2010**; 8(3): 277-88.
73. Hong Kong Chest Service, Tuberculosis Research Centre M, Council BMR. A study of the characteristics and course of sputum smear-negative pulmonary tuberculosis. Tubercle **1981**; 62(3): 155-67.
74. Nguyen M-VH, Jenny-Avital ER, Burger S, Leibert EM, Achkar JM. Clinical and radiographic manifestations of sputum culture-negative pulmonary tuberculosis. PLoS One **2015**; 10(10): e0140003.

75. Nakiyingi L, Nonyane BA, Ssengooba W, et al. Predictors for MTB culture-positivity among HIV-infected smear-negative presumptive tuberculosis patients in Uganda: application of new tuberculosis diagnostic technology. PLoS One **2015**; 10(7): e0133756.
76. Achkar JM, Jenny-Avital ER. Incipient and subclinical tuberculosis: defining early disease states in the context of host immune response. Journal of Infectious Diseases **2011**; 204(suppl_4): S1179-S86.
77. Drain PK, Bajema KL, Dowdy D, et al. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clinical microbiology reviews **2018**; 31(4).
78. Cowie R, Langton M, Escreet B. Diagnosis of sputum smear-and sputum culture-negative pulmonary tuberculosis. South African medical journal= Suid-Afrikaanse tydskrif vir geneeskunde **1985**; 68(12): 878.
79. Mtei L, Matee M, Herfort O, et al. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. Clinical infectious diseases **2005**; 40(10): 1500-7.
80. Dutt AK, Moers D, Stead WW. Smear-and culture-negative pulmonary tuberculosis: four-month short-course chemotherapy. Am Rev Respir Dis **1989**; 139(4): 867-70.

CHAPTER 3: MANUSCRIPTS

Manuscript: Sputum culture non-completion

Title: Factors associated with sputum culture non-completion in verified tuberculosis cases in the U.S., 2011–2019

Running title: Non-completion of TB sputum cultures

Journal for first submission: Clinical Infectious Diseases

Contribution of student: Surveillance data from the National Tuberculosis Surveillance System was used for this study, which included data collection and reporting by clinicians and state and local health departments. The database is managed by the Centers for Disease Control and Prevention Division of Tuberculosis Elimination. Julie Self designed the research project; Lydia Rautman conducted data analysis, developed figures and tables, and wrote the following manuscript. Steve Kammerer, Vincent Marconi, Julie Self, Ben Silk, Jonathan Wortham, and Monica Youngblood provided insights on the topic; Johnathan A. Edwards, Steve Kammerer, Vincent Marconi, Julie Self, and Jonathan Wortham edited manuscript drafts.

Key points

- Sputum culture may not be possible for tuberculosis patients unable to expectorate sputum
- Non-sputum specimens and subsequent test results should be chosen and interpreted carefully
- Clinicians should prioritize TB on differential diagnosis in patients with non-specific symptoms

Abstract

Background

Sputum culturing is the gold standard for tuberculosis (TB) disease diagnosis because of its high sensitivity and utility in phenotypic drug sensitivity testing, species identification and genotyping. Around 10% of verified TB cases in the United States 2011–2019 had no sputum culture result available, representing opportunities for missed cases, improper treatment and gaps in surveillance data.

Methods

We used National Tuberculosis Surveillance System data in bivariate and multivariable models to evaluate the association between sputum culture non-completion and demographic characteristics, risk factors, and clinical and laboratory characteristics.

Results

We found higher odds of sputum culture non-completion among individuals with a non-sputum culture completed, long-term care facility residents, pediatric and elderly patients, and individuals whose care provider was not from a health department.

Conclusions

High odds of sputum culture non-completion in pediatric patients were likely due to patient inability to expectorate sputum. In elderly and long-term care facility residents, high odds of sputum culture non-completion may have been due to difficulty obtaining a sputum specimen and incidental diagnosis. Higher odds of sputum culture non-completion in patients with a non-health department care provider may be due to higher suspicion of health department clinicians for TB. Overall, TB sputum culture non-completion is due to patient inability to produce a sample and lack of consideration of TB on the differential diagnosis. We recommend minimally invasive and hazardous specimen alternatives when patients are unable to produce sputum and encourage clinicians to familiarize themselves with TB epidemiology and consider testing for TB in patients with risk factors.

Introduction

The United States has one of the lowest active tuberculosis (TB) disease case rates in the world at 2.7 per 100,000 persons [1]. As rates of TB continue to decline in the U.S., the challenge has been identifying remaining barriers to diagnosis and treatment. Active TB disease develops in 5–10% of persons infected with *Mycobacterium tuberculosis* [2]. TB disease can be diagnosed based on a *Mycobacterium tuberculosis* complex (MTBC) positive culture, MTBC nucleic acid amplification test (NAAT), acid-fast bacilli (AFB) smear ("sputum smear"), or clinical diagnosis. Culturing MTBC from an expectorated sputum specimen can take several weeks but its high sensitivity, utility in species identification and drug susceptibility testing and role in molecular surveillance have established sputum culturing as the gold standard diagnostic method [2].

While TB can affect many sites in the body, pulmonary TB comprises 80% of all TB cases [3]. Sputum specimen culturing was performed for 90% of TB cases with pulmonary involvement during 2011–2019, leaving 10% of cases for which there was no sputum culture result available. Not culturing a sputum specimen can result in missed cases if a less sensitive method is used, insufficient treatment if phenotypic drug susceptibility testing is not conducted, and gaps in molecular surveillance data, which create barriers to detecting and preventing outbreaks.

Surveillance characteristics associated with sputum culture non-completion in the United States have yet to be thoroughly explored. We used National Tuberculosis Surveillance System (NTSS) data to investigate factors associated with sputum culture non-completion in TB cases with pulmonary involvement. Specifically, we identified which demographic, clinical, or laboratory characteristics were associated with sputum culture non-completion. Results from this study could inform recommendations for clinical or laboratory practices to increase sputum culture completion among reported TB cases.

Methods

Our analyses included TB cases in the NTSS, which stores data on all verified TB cases from the 50 states and the District of Columbia along with demographic, clinical, and risk factor data [4]. Inclusion criteria were pulmonary involvement and a date between January 2011–December 2019. We assumed that no sputum culture had been completed for individuals for whom no sputum culture result was available.

We performed analyses to assess the association between predictor variables and the outcome of sputum culture non-completion. Predictor variables included demographic characteristics, risk factors, and clinical and laboratory characteristics. The race/ethnicity variable was a combination of self-reported patient race and ethnicity. Self-reported Hispanic or Latino individuals were categorized as of Hispanic ethnicity regardless of race; self-reported non-Hispanic (NH) persons were categorized by self-reported race.

We combined missing and unknown values for the frequency analysis. We stratified variables on sputum culture completion status and compared proportions with Chi-squared tests or Monte Carlo simulations for expected cell counts <5. We excluded cases with missing data for bivariate and multivariable analyses, except where the proportion of these values exceeded 3%. We conducted bivariate analyses between each predictor and the outcome to calculate crude odds ratios and 99% confidence intervals. For the multivariable analysis, we performed backward selection procedures with Schwarz Bayesian information criterion for model selection and calculated adjusted ORs and 99% confidence intervals. We cleaned and analyzed the data in SAS 9.3 [5] and produced visualizations of ORs in R Studio 1.3.1093 [6].

Emory University Institutional Review Board waived HIPAA authorization and informed consent under expedited review. CDC exempted this study from IRB review on the basis of being a public health surveillance activity as defined in 45 CFR 46.102.

Results

From January 2011 through December 2019, 89,419 TB cases were reported in NTSS. Of these, 63,382 (71%) had pulmonary involvement. The median age (interquartile range [IQR]) of these cases was 49 (31–64) years old and 64% were male (Table 1). No sputum culture result was available for 6,343 cases (10%); approximately one-third of these were pediatric. Of this 10%, 75% had a non-sputum culture result available and 34% had a NAAT completed. Approximately 19% of cases with no sputum culture had no other micrologic or molecular testing (sputum or non-sputum culture, NAAT, sputum smear) done. The median age (IQR) among cases with a sputum culture completed was 49 (32–64); among cases with no sputum culture completed it was 43 (4–68). Of all patients in the analysis, 65% (N = 40,896) were non-U.S.–born; this proportion was 43% (N = 2,741) among cases without a sputum culture and 67% (N = 38,046) among cases with a sputum culture. We excluded cases with missing data (11%) for bivariate and multivariable analyses; 56,337 cases remained. All variables had a significant bivariate association with sputum culture non-completion (Table 2). The final model selected for the multivariable analysis had 14 main effects.

Of the 10% of patients with no sputum culture, 61% were microbiologically confirmed via positive non-sputum culture or positive NAAT. Of the 2,128 individuals with no microbiologic confirmation (not including those for whom sputum culture was negative), 525 (25%) were not pediatric, elderly, or a resident of an LTCF.

Compared with adult patients, odds of not having a sputum culture result were higher among both pediatric (Table 2, adjusted odds ratio (aOR) = 30.48, 99% Confidence Interval (CI): 25.55, 36.35) and elderly patients (aOR = 1.26, 99% CI: 1.10, 1.44). The median age (IQR) for pediatric cases was 3 (1–8) years old and 67% of all pediatric cases did not have a sputum culture result available. The sputum culture non-completion proportion among non-U.S.–born patients was 6%; among U.S.-born patients it was 16%.

There was no significant relationship between HIV positivity and sputum culture completion (Table 2). Compared with those with a negative HIV test result, there were higher odds of not having a sputum culture result among those with an HIV test result of "Not offered" (OR = 2.96, 99% CI: 2.60, 3.38), "Refused" (OR = 2.34, 99% CI: 1.23, 4.46), or "Result unknown/Indeterminate" (OR = 1.69, 99% CI: 1.35, 2.11). Patients with TB risk factors (RF) had lower odds of not having a sputum culture done compared with those with no TB risk factors (aOR = 0.84, 99% CI: 0.75, 0.95). However, the opposite was observed for patients residing in long-term care facilities (LTCFs), who had higher odds of not having a sputum culture available compared with patients not residing in LTCFs (aOR = 1.46, 99% CI: 1.14, 1.87). A higher proportion of patients in LTCFs (42%) compared with patients not in LTCFs (30%) had any of the following underlying medical conditions: immunosuppression, recipient of an organ transplant, end-stage renal disease, TNF-alpha antagonist therapy, HIV co-infection, or diabetes mellitus compared with patients not in LTCFs.

Patients whose healthcare provider was not a health department had higher odds of not having a sputum culture result available compared with those who sought care at a health department (aOR = 2.11, 99% CI: 1.90, 2.34). Patients for whom no NAAT was completed also had higher odds of not having a sputum culture done compared with patients for whom a NAAT was completed (aOR = 2.51, 99% CI: 2.29, 2.76).

Completion of a culture with a specimen other than sputum was positively associated with sputum culture non-completion (aOR = 6.94, 99% CI: 6.24, 7.73). Of all patients with no sputum culture result available, 71% had a culture of a non-sputum specimen available (Table 3; 52% of pediatric patients, 75% of adult patients, and 87% of elderly patients). In pediatric patients without sputum culture, gastric aspirates comprised the largest proportion (79%) of non-sputum specimens used for culture. Bronchial fluid was the most common non-sputum specimen in adults (49%) and the elderly (59%), followed by lung biopsy (19% in both adults and elderly). Among individuals with no sputum culture but a culture of

a non-sputum specimen done, “incidental” was the reason for evaluation in 43% of adults and 52% of the elderly, compared with 5% in children. The median age (IQR) for patients with no sputum culture done but a gastric aspirate culture done was 1 (1–4); for bronchial fluid it was 64 (43–78) and for lung biopsy cultures it was 62 (46–74).

Among individuals with no sputum culture, a non-sputum culture was done for 92% of the patients in LTCFs, compared with 73% not in LTCFs. For these patients in LTCFs without sputum cultures, bronchial fluid was the non-sputum specimen most frequently cultured (64%). Of patients with no microbiologic or molecular confirmation (sputum culture was not done and both non-sputum culture and NAAT were not done or negative; N = 2,128), 66% were children, 25% were adults, and 9% were elderly. Of these patients, 525 individuals were not pediatric or elderly and did not reside in a long-term care facility.

Discussion

We found significantly higher odds of sputum culture non-completion for 1) individuals who had a non-sputum culture done, 2) long-term care facility residents, 3) pediatric and elderly patients, and 4) individuals whose care provider was not from a health department. We also found that a lack of sputum culture was associated with lack of a NAAT, highlighting greater implications of sputum culturing and more challenges to timely and accurate TB diagnoses.

Completion of a culture with a specimen other than sputum was associated with sputum culture non-completion. There appeared to be two scenarios in which a non-sputum culture was done: in young children and some adult and elderly cases as an alternative to obtaining an expectorated sputum specimen; and adults and the elderly as a workup for the diagnosis of another condition that resulted in an incidental TB diagnosis.

Children had higher rates of non-culturing of both sputum and non-sputum cultures, likely because of the challenges in obtaining adequate specimens from young children [2]. A gastric aspiration was the non-sputum specimen collected from the vast majority of children without sputum culture, concurrent with guidelines for diagnosis in pediatric patients [2]. Gastric aspirate specimens can be used for both AFB smears and cultures, but the sensitivity of both is quite low, estimated to be around 30-60% in children [7-10]. Challenges in diagnosing childhood TB are widely recognized; as most laboratory tests have decreased sensitivity in children, we may be missing cases if clinicians rely on these for TB diagnosis in children [11]. If positive, a sputum culture provides valuable information about drug susceptibility, so this test should be completed wherever possible. However, as delayed diagnosis can quickly lead to serious complications in children [12], a negative result should not rule out TB and clinicians should also consider clinical, radiographic, and epidemiologic findings to support the diagnosis.

Among adults and the elderly with no sputum culture but a non-sputum culture performed, a lung biopsy and bronchial fluid were the specimens used in the majority of cases. A lung biopsy is an invasive procedure most commonly used to diagnose lung cancer and interstitial lung disease [10], so it is likely that most of these TB diagnoses were incidental. Bronchial fluid, obtained via bronchial lavage, can be used to diagnose a number of conditions including tuberculosis, interstitial lung disease, and pulmonary sarcoidosis [13]. The procedure can be used for a TB diagnosis when a patient is unable to expectorate, for instance when the patient is intubated, but is aerosol-generating and can pose infection risks for the clinician or others in a shared space if proper infection control measures are not taken [2, 14, 15]. In incidental cases where TB was not suspected at the time of the sampling, it seems plausible that adequate infection control measures were not taken. Aerosol-generating procedures should not be performed on patients who may have infectious TB except where absolutely necessary and where infection control measures can be ensured [14, 16]. If a patient is able, an expectorated sputum sample may be a safer testing option in terms of invasiveness and infection control. When expectoration is not possible, a sputum

induction is a cost-effective, less invasive and potentially more sensitive alternative to bronchial fluid [17, 18]. Clinicians, especially primary care providers, should consider TB more often on the differential diagnosis and utilize standard TB diagnostic methods, such as sputum culture, wherever possible.

Long-term care facilities include rehabilitation facilities, hospitals, nursing and residential homes, and mental care facilities; because of the nature of these institutions, individuals in LTCFs are more likely to be frail or disabled in a manner that would make sputum expectoration challenging [19, 20]. We found that individuals residing in LTCFs had lower odds of having a sputum culture done, but a larger proportion had a non-sputum culture completed, most frequently with bronchial fluid and lung biopsies, compared with those not residing in LTCFs. The increased use of bronchial fluid for culture is not ideal due to concerns over infection control [14-16]; instead, sputum induction should be considered as an alternative [18, 21]. Some of these cases were incidental TB diagnoses; as a large proportion of LTCF residents were foreign-born, this indicates a lack of consideration of TB despite TB risk factors. Our findings emphasize the need for special consideration of diagnostic tools in patients less frequently able to produce a traditional sputum sample, as well as increased awareness of TB risk factors. These will be critical in catching and treating cases and preventing further transmission in this vulnerable population.

We expected and observed a low proportion of children with TB having a sputum culture result, driven, as demonstrated in previous studies, by the challenges in collecting an expectorated sputum sample from very young children [2, 22]. Elderly patients had lower odds of sputum culture completion compared with adult patients. Low culturing rates in this population are concerning because of the higher rates of TB reactivation and generally poorer health [20, 23]; however, we found that culturing rates of non-sputum specimens, especially of lung biopsies and bronchial fluid, are high in this age group. Lung biopsies and some bronchial fluid specimens are often used for diagnosis of other conditions like lung cancer and likely represent incidental TB diagnosis. Lack of consideration of TB on the differential diagnosis could be due to low physician suspicion of TB infection, recognized as a challenge in low-

incidence countries where clinical expertise diminishes as incidence decreases [24]. Some bronchial fluid cultures may have been completed for as a TB diagnostic where the patient was unable to expectorate sputum, but this method is less specific and more hazardous than other alternatives. When a patient presents with non-specific symptoms, especially if the patient has TB risk factors, TB should be more thoroughly considered and ruled out with an established TB diagnostic tool, such as sputum culture. If the patient is unable to expectorate, sputum induction should once again be considered before bronchoscopy as an alternative [17].

Patients whose healthcare provider was not a public health department had significantly lower odds of having a sputum culture completed than health department patients. Healthcare providers other than those from health departments work in various settings including in-patient hospital care, nursing homes, assisted living facilities, correctional institutions, the Indian Health Service (IHS), primary care offices, and other clinical outpatient clinics. Our findings may suggest that health department clinicians may have higher suspicion for TB compared with a primary care physician who very rarely sees TB, which may reflect decreasing proficiency in clinical recognition of TB with decreasing TB incidence [24]. Additionally, health department physicians may have better access to TB diagnostic tools than other care providers. Another study examining factors associated with delayed pulmonary TB diagnosis found that delays were longer for patients seeking care from primary care physicians, compared with emergency room or public health clinic patients [25]. From our findings it is difficult to identify any one type of healthcare provider as a driver of low sputum culture completion rates, but this warrants further research on the relationship between provider type and utilization of diagnostic tools. This research could reduce delays in TB diagnoses and treatment initiation, which could ultimately minimize morbidity and mortality through improvement of TB infection outcomes and prevention of further transmission.

The NAAT is often used for its quick turnaround when culture results are not immediately available; however, stratifications revealed that for many patients without a sputum culture result, no

NAAT was performed. This may indicate that TB was not adequately considered during the initial stage of diagnosis but was diagnosed incidentally via a non-sputum culture or via clinical assessment, which may reflect low awareness or misconceptions surrounding TB epidemiology. This finding emphasizes the need for continued vigilance of physicians for established TB risk factors and consideration of TB for a patient presenting with non-specific symptoms.

Conclusion

Sputum culturing is the gold-standard method for diagnosing pulmonary TB and is unparalleled in its sensitivity and vital in determining treatment. This paper assessed factors associated with sputum culture non-completion and found that this resulted primarily from patient inability to expectorate sputum and lack of consideration of TB on the differential diagnosis. Of patients without a sputum culture, the majority was microbiologically confirmed via positive non-sputum culture or NAAT. Non-sputum cultures can also be used for determining drug susceptibility but can involve invasive or hazardous procedures; NAAT is useful for diagnosis but lacks drug susceptibility information.

Of those without any microbiologic confirmation, the majority were pediatric, elderly, or a resident of an LTCF, representing populations typically less able to produce a sputum sample. For these populations, alternative culture specimens such as gastric aspirates in children and sputum induction in others are recommended, but should also be interpreted with caution due to lower sensitivity in patients with paucibacillary disease and lower mycobacterial load in the sputum, common in children and immunocompromised individuals.

Finally, the remaining patients represent a population for whom it is not clear in the surveillance data why they were not molecularly or microbiologically confirmed with standard TB diagnosis methods. Although making up only a small proportion of cases, each patient represents a potentially delayed diagnosis, incorrect treatment, other missed cases, or opportunity for further transmission. We made

preliminary recommendations for appropriate alternative diagnostic approaches dependent on target group and encourage clinicians to prioritize TB on the differential diagnosis. Enhancing clinician awareness of TB risk factors and understanding of TB diagnostic methods may improve TB diagnosis strategies, leading to reduced delays in diagnoses and improvements in outbreak detection and transmission prevention.

References

1. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization, **2020**.
2. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med **2000**; 161: 1376-95.
3. Kaufmann SH. Tuberculosis: Deadly combination. Nature **2008**; 453(7193): 295-6.
4. Centers for Disease Control. National Tuberculosis Surveillance System (NTSS), data set reference guide. **2015**.
5. SAS Institute I. Cary, NC, **2013**.
6. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC. Boston, MA, **2020**.
7. AM LB. Effectiveness of smears and cultures in gastric aspirate samples in the diagnosis of tuberculosis. Anales espanoles de pediatria **2000**; 53(5): 405-11.
8. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. The Lancet **2005**; 365(9454): 130-4.
9. Lobato MN, Loeffler AM, Furst K, Cole B, Hopewell PC. Detection of Mycobacterium tuberculosis in gastric aspirates collected from children: hospitalization is not necessary. Pediatrics **1998**; 102(4): e40-e.
10. Bonnave P-E, Raoult D, Drancourt M. Gastric aspiration is not necessary for the diagnosis of pulmonary tuberculosis. European journal of clinical microbiology & infectious diseases **2013**; 32(4): 569-71.
11. Subgroup STPCT. Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children. Chapter 1: Introduction and Diagnosis of Tuberculosis in Children. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease **2006**; 10(10): 1091-7.
12. Roya-Pabon CL, Perez-Velez CM. Tuberculosis exposure, infection and disease in children: a systematic diagnostic approach. Pneumonia **2016**; 8(1): 1-18.
13. Davidson KR, Ha DM, Schwarz MI, Chan ED. Bronchoalveolar lavage as a diagnostic procedure: a review of known cellular and molecular findings in various lung diseases. Journal of Thoracic Disease **2020**; 12(9): 4991.
14. Stem DR. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care facilities, 1994. **1994**.
15. Agerton T, Valway S, Gore B, et al. Transmission of a highly drug-resistant strain (strain W1) of Mycobacterium tuberculosis: community outbreak and nosocomial transmission via a contaminated bronchoscope. Jama **1997**; 278(13): 1073-7.
16. Larson JL, Ridzon R, Hannan MM. Sputum induction versus fiberoptic bronchoscopy in the diagnosis of tuberculosis. American journal of respiratory and critical care medicine **2001**; 163(5): 1279-80.
17. Brown M, Varia H, Bassett P, Davidson RN, Wall R, Pasvol G. Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate. Clinical infectious diseases **2007**; 44(11): 1415-20.
18. Anderson C, Inhaber N, Menzies D. Comparison of sputum induction with fiber-optic bronchoscopy in the diagnosis of tuberculosis. American journal of respiratory and critical care medicine **1995**; 152(5): 1570-4.
19. Wunderlich GS, Kohler PO. Profile of Long-Term Care. Improving the quality of long-term care **2001**.
20. Lane NE, Wodchis WP, Boyd CM, Stukel TA. Disability in long-term care residents explained by prevalent geriatric syndromes, not long-term care home characteristics: a cross-sectional study. BMC geriatrics **2017**; 17(1): 1-14.
21. Biswas S, Das A, Sinha A, Das SK, Bairagya TD. The role of induced sputum in the diagnosis of pulmonary tuberculosis. Lung India: Official Organ of Indian Chest Society **2013**; 30(3): 199.
22. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. Emerging infectious diseases **1995**; 1(4): 115.
23. Shea KM, Kammerer JS, Winston CA, Navin TR, Horsburgh Jr CR. Estimated rate of reactivation of latent tuberculosis infection in the United States, overall and by population subgroup. American journal of epidemiology **2014**; 179(2): 216-25.

24. Lönnroth K, Migliori GB, Abubakar I, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. European Respiratory Journal **2015**; 45(4): 928-52.
25. Golub JE, Bur S, Cronin W, et al. Patient and health care system delays in pulmonary tuberculosis diagnosis in a low-incidence state. The International journal of tuberculosis and lung disease **2005**; 9(9): 992-8.
26. United States Census Bureau. Geography & ACS. Available at: https://www.census.gov/programs-surveys/acs/geography-acs.html.

Tables and figures

Table 1: Demographic characteristics, risk factors, and clinical/laboratory characteristics of pulmonary TB cases based on sputum culture completion, 2011–2019

	Total (N = 63,382)			
	Sputum culture not completed	Sputum culture completed	Unknown/ Missing	
	No. (%)	No. (%)	No. (%)	p-value
Total cases	6,343 (10.01%)	56,854 (89.70%)	185 (0.29%)	
Demographic characteristics				
Sex				<.001
Female	2,737 (43.15%)	20,210 (35.55%)	81 (43.78%)	
Male	3,606 (56.85%)	36,641 (64.45%)	104 (56.22%)	
Unknown	0 (0.00%)	3 (0.01%)	0 (0.00%)	
Age group				<.001
0-14	2,000 (31.53%)	989 (1.74%)	6 (3.24%)	
15-64	2,461 (38.80%)	41,972 (73.82%)	106 (57.30%)	
65+	1,881 (29.65%)	13,889 (24.43%)	73 (39.46%)	
Unknown	1 (0.02%)	4 (0.01%)	0 (0.00%)	
Origin				<.001
Non-U.S.–born	2,741 (43.21%)	38,046 (66.92%)	109 (58.92%)	
U.S.-born	3,593 (56.65%)	18,779 (33.03%)	75 (40.54%)	
Unknown	9 (0.14%)	29 (0.05%)	1 (0.54%)	
Race/ethnicity				<.001
American Indian/Alaska Native	76 (1.20%)	816 (1.44%)	2 (1.08%)	
Asian	1,627 (25.65%)	18,433 (32.42%)	63 (34.05%)	
NH Black	1,399 (22.06%)	11,660 (20.51%)	29 (15.68%)	
Hispanic or Latino	1,932 (30.46%)	16,908 (29.74%)	44 (23.78%)	
Native Hawaiian/Other Pacific Islander	109 (1.72%)	517 (0.91%)	3 (1.62%)	
NH White	1,133 (17.86%)	8,130 (14.30%)	41 (22.16%)	
Multiple races or unknown	67 (1.06%)	390 (0.69%)	3 (1.62%)	
Region				<.001
Midwest	1,040 (16.40%)	6,604 (11.62%)	30 (16.22%)	
Northeast	887 (13.98%)	6,048 (10.64%)	12 (6.49%)	
South	2,832 (44.65%)	24,026 (42.26%)	45 (24.32%)	
West	1,584 (24.97%)	20,176 (35.49%)	98 (52.97%)	
Primary occupation				<.001
Occupation with TB risk[a]	164 (2.59%)	2,809 (4.94%)	9 (4.86%)	
Not seeking	2,514 (39.63%)	9,722 (17.10%)	35 (18.92%)	
Retired	1,211 (19.09%)	9,114 (16.03%)	46 (24.86%)	
Unemployed	1,209 (19.06%)	14,043 (24.70%)	41 (22.16%)	
Other	1,056 (16.65%)	19,850 (34.91%)	36 (19.46%)	
Missing or Unknown	189 (2.98%)	1,316 (2.31%)	18 (9.73%)	
Risk factors				

[a] Includes correctional facility staff, healthcare workers, and migrant workers

Long-term care facility				<.001
No	6,065 (95.62%)	55,837 (98.21%)	172 (92.97%)	
Yes	254 (4.00%)	941 (1.66%)	5 (2.70%)	
Missing or Unknown	24 (0.38%)	76 (0.13%)	8 (4.32%)	
Corrections				<.001
No	6,158 (97.08%)	54,047 (95.06%)	174 (94.05%)	
Yes	139 (2.19%)	2,644 (4.65%)	4 (2.16%)	
Missing or Unknown	46 (0.73%)	163 (0.29%)	7 (3.78%)	
Substance use[b]				<.001
Any substance use	501 (7.90%)	9,970 (17.54%)	24 (12.97%)	
No substance use	5,687 (89.66%)	46,146 (81.17%)	145 (78.38%)	
Missing or Unknown	155 (2.44%)	738 (1.30%)	16 (8.65%)	
Homeless				<.001
No	6,095 (96.09%)	52,805 (92.88%)	171 (92.43%)	
Yes	181 (2.85%)	3,639 (6.40%)	7 (3.78%)	
Missing or Unknown	67 (1.06%)	410 (0.72%)	7 (3.78%)	
HIV Status				<.001
Negative	3,951 (62.29%)	48,748 (85.74%)	130 (70.27%)	
Positive	244 (3.85%)	3,180 (5.59%)	11 (5.95%)	
Not offered	1,576 (24.85%)	2,641 (4.65%)	12 (6.49%)	
Refused	287 (4.52%)	1,203 (2.12%)	6 (3.24%)	
Result unknown/Indeterminate	32 (0.50%)	149 (0.26%)	0 (0.00%)	
Unknown or Missing	253 (3.99%)	933 (1.64%)	26 (14.05%)	
Previous TB				<.001
No	6,076 (95.79%)	53,535 (94.16%)	168 (90.81%)	
Yes	179 (2.82%)	3,014 (5.30%)	13 (7.03%)	
Missing or Unknown	88 (1.39%)	305 (0.54%)	4 (2.16%)	
Risk factors for infection[c]				<.001
No RF	2,171 (34.23%)	14,057 (24.72%)	66 (35.68%)	
Some RF	4,172 (65.77%)	42,797 (75.28%)	119 (64.32%)	
Clinical and laboratory characteristics				
Disease site				<.001
Pulmonary only	5,166 (81.44%)	49,943 (87.84%)	152 (82.16%)	
Both pulmonary and extrapulmonary disease	1,177 (18.56%)	6,911 (12.16%)	33 (17.84%)	
Provider type				<.001

[b] Any reported alcohol use, injection drug use or non-injection drug use in the 12 months prior to diagnostic evaluation for TB.

[c] Risk factors include non-U.S.–born status, homelessness or substance use in the 12 months prior to diagnostic evaluation for TB, being a contact of a TB patient but had been missed in a contact investigation, or having contact with an infectious TB patient within 2 years of diagnosis. “Some risk factors” is indicated for patients who meet at least one criterion.

Health Department	3,119 (49.17%)	36,599 (64.37%)	76 (41.08%)	
All other	2,640 (41.62%)	14,662 (25.79%)	95 (51.35%)	
Both	578 (9.11%)	5,516 (9.70%)	13 (7.03%)	
Unknown or Missing	6 (0.09%)	77 (0.14%)	1 (0.54%)	
Chest radiograph				<.001
Abnormal with no cavitation	4,792 (75.55%)	36,019 (63.35%)	125 (67.57%)	
Abnormal with cavitation	652 (10.28%)	15,311 (26.93%)	29 (15.68%)	
Normal	434 (6.84%)	2,933 (5.16%)	8 (4.32%)	
Not done	383 (6.04%)	2,138 (3.76%)	10 (5.41%)	
Missing	82 (1.29%)	453 (0.80%)	13 (7.03%)	
Non-sputum culture completion				<.001
Not done	1,600 (25.22%)	37,757 (66.41%)	43 (23.24%)	
Done	4,738 (74.70%)	18,855 (33.16%)	116 (62.70%)	
Missing	5 (0.08%)	242 (0.43%)	26 (14.05%)	
TST completion				<.001
Not done	3,536 (55.75%)	35,949 (63.23%)	119 (64.32%)	
Done	2,731 (43.06%)	20,201 (35.53%)	46 (24.86%)	
Missing or Unknown	76 (1.20%)	704 (1.24%)	20 (10.81%)	
IGRA completion				<.001
Not done	3,610 (56.91%)	25,412 (44.70%)	69 (37.30%)	
Done	2,482 (39.13%)	29,506 (51.90%)	94 (50.81%)	
Missing or Other	251 (3.96%)	1,936 (3.41%)	22 (11.89%)	
NAAT completion				<.001
Not done	4,138 (65.24%)	19,520 (34.33%)	70 (37.84%)	
Done	2,167 (34.16%)	37,097 (65.25%)	102 (55.14%)	
Missing or Other	38 (0.60%)	237 (0.42%)	13 (7.03%)	

Table 2: Crude and adjusted ORs for sputum culture non-completion by patient characteristic, 2011–2019

	Total (N = 56,337)				
	Sputum culture not completed	Bivariate analysis		Multivariable analysis	
	N (%)	cOR (99% CI)	p-value	aOR (99% CI)	p-value
Total cases	5,438 (9.65%)				
Demographic characteristics					
Sex					
Female	2,382 (43.80%)	1.40 (1.30, 1.51)	<.001	1.20 (1.09, 1.31)	<.001
Male	3,056 (56.20%)	Reference	-	Reference	-
Age					
0-14	1,845 (33.93%)	36.81 (32. 68, 41.47)	<.001	30.48 (25.55, 36.35)	<.001
15-64	2,038 (37.48%)	Reference	-	-	-
65+	1,555 (28.60%)	2.36 (2.15, 2.58)	<.001	1.26 (1.10, 1.44)	<.001
Race/Ethnicity					
NH American Indian/ Alaska Native	59 (1.08%)	0.59 (0.41, 0.84)	<.001	0.45 (0.28, 0.72)	<.001
NH Asian	1,380 (25.38%)	0.62 (0.55, 0.69)	<.001	0.85 (0.73, 0.99)	.007
NH Black	1,205 (22.16%)	0.85 (0.76, 0.96)	<.001	0.85 (0.74, 0.99)	.006
Hispanic or Latino	1,683 (30.95%)	0.83 (0.74, 0.92)	<.001	0.99 (0.86, 1.15)	.923
NH Native Hawaiian/ Other Pacific Islander	99 (1.82%)	1.57 (1.17, 2.12)	<.001	1.39 (0.90, 2.15)	.051
NH White	969 (17.82%)	Reference	-	Reference	-
Multiple races or unknown	43 (0.79%)	1.06 (0.69, 1.63)	.730	0.93 (0.54, 1.62)	.749
Region					
Midwest	894 (16.44%)	2.05 (1.82, 2.31)	<.001	2.07 (1.78, 2.40)	<.001
Northeast	727 (13.37%)	1.87 (1.65, 2.11)	<.001	1.66 (1.42, 1.94)	<.001
South	2,474 (45.49%)	1.51 (1.38, 1.65)	<.001	1.78 (1.58, 2.01)	<.001
West	1,343 (24.70%)	Reference	-	Reference	-
Primary occupation					
Occupation with TB risk	140 (2.57%)	0.21 (0.17, 0.27)	<.001	0.77 (0.59, 1.01)	.012
Not seeking	2,304 (42.37%)	Reference	-	Reference	-
Retired	1,046 (19.24%)	0.50 (0.45, 0.55)	<.001	0.87 (0.74, 1.03)	.032
Unemployed	1,018 (18.72%)	0.32 (0.29, 0.35)	<.001	0.92 (0.79, 1.06)	.114
Other	930 (17.10%)	0.20 (0.18, 0.22)	<.001	0.80 (0.69, 0.93)	<.001
Risk factors					
Long-term care facility					
No	5,233 (96.23%)	Reference	-	Reference	-
Yes	205 (3.77%)	2.40 (1.95, 2.94)	<.001	1.46 (1.14, 1.87)	<.001
Corrections					
No	5,328 (97.98%)	Reference	-		
Yes	110 (2.02%)	0.47 (0.36, 0.60)	<.001		
HIV Status					
Negative	3,575 (65.74%)	Reference	-	Reference	-
Positive	187 (3.44%)	0.84 (0.69, 1.03)	.030	0.84 (0.67, 1.05)	.041
Not offered	1,390 (25.56%)	7.44 (6.74, 8.20)	<.001	2.96 (2.60, 3.38)	<.001

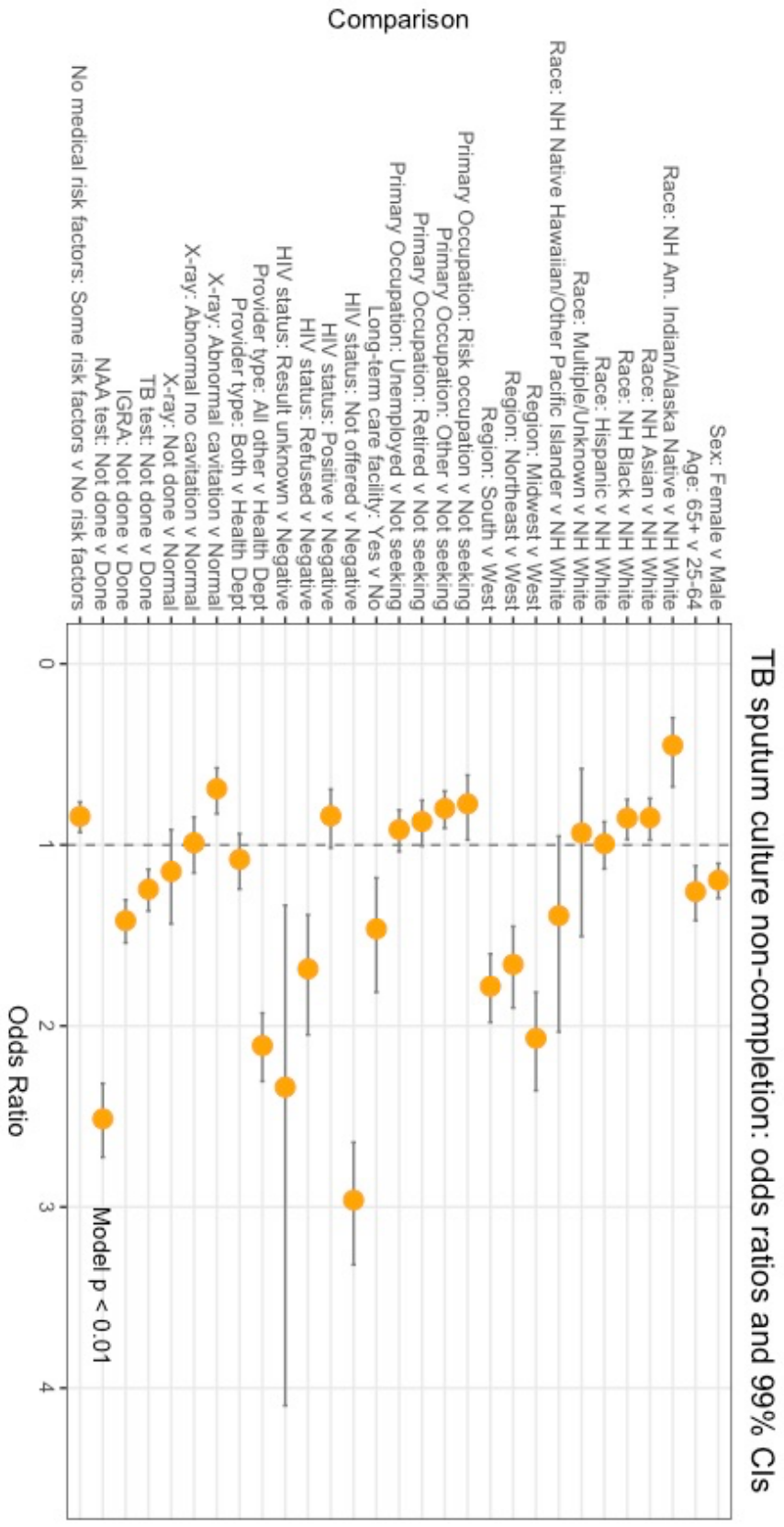
Refused	259 (4.76%)	2.99 (2.49, 3.59)	<.001	1.69 (1.35, 2.11)	<.001
Result unknown/ Indeterminate	27 (0.50%)	2.69 (1.56, 4.66)	<.001	2.34 (1.23, 4.46)	<.001
Risk factors for infection					
No RF	1,838 (33.80%)	Reference	-	Reference	-
Some RF	3,600 (66.20%)	0.64 (0.59, 0.70)	<.001	0.84 (0.75, 0.95)	<.001
Previous TB					
No	5,284 (97.17%)	Reference	-		
Yes	154 (2.83%)	0.52 (0.42, 0.64)	<.001		
Clinical and laboratory characteristics					
Disease site					
Pulmonary only	4,443 (81.70%)	Reference	-		
Both pulmonary and extrapulmonary disease	995 (18.30%)	1.63 (1.48, 1.79)	<.001		
Provider type					
Health Department	2,856 (52.52%)	Reference	-	Reference	-
All other	2,065 (37.97%)	2.01 (1.85, 2.17)	<.001	2.11 (1.90, 2.34)	<.001
Both	517 (9.51%)	1.23 (1.08, 1.40)	<.001	1.08 (0.92, 1.27)	.219
Chest radiograph					
Abnormal with no cavitation	4,200 (77.23%)	0.91 (0.79, 1.06)	.105	0.99 (0.83, 1.18)	.871
Abnormal with cavitation	585 (10.76%)	0.30 (0.25, 0.35)	<.001	0.69 (0.56, 0.85)	<.001
Normal	378 (6.95%)	Reference	-	Reference	-
Not done	275 (5.06%)	1.07 (0.86, 1.33)	.458	1.15 (0.89, 1.49)	.170
Non-sputum culture completion					
Not done	1,421 (26.13%)	Reference	-	Reference	-
Done	4,017 (73.87%)	5.72 (5.27, 6.22)	<.001	6.94 (6.24, 7.73)	<.001
TST completion					
Not done	2,955 (54.34%)	0.68 (0.63, 0.74)	<.001	1.25 (1.12, 1.38)	<.001
Done	2,483 (45.66%)	Reference	-	Reference	-
IGRA completion					
Not done	3,167 (58.24%)	1.66 (1.54, 1.78)	<.001	1.42 (1.29, 1.56)	<.001
Done	2,271 (41.76%)	Reference	-	Reference	-
NAAT completion					
Not done	3,621 (66.59%)	3.76 (3.48, 4.06)	<.001	2.51 (2.29, 2.76)	<.001
Done	1,817 (33.41%)	Reference	-	Reference	-

Table 3: Type of non-sputum culture performed among pulmonary TB patients without a sputum culture result available, 2011–2019

No sputum culture result available (N = 5,438)				
Non-sputum culture type	Age group			
	0-14 (N = 1,845)	15-64 (N = 2,038)	65+ (N = 1,555)	Overall
Bronchial fluid	54 (2.93%)[d]	752 (36.90%)	793 (51.00%)	1,599 (29.40%)
Gastric aspirate	757 (41.03%)	16 (0.79%)	5 (0.32%)	778 (14.31%)
Lung	22 (1.19%)	298 (14.62)	263 (16.91)	583 (10.72%)
Pleural fluid	7 (0.38%)	86 (4.22%)	66 (4.24%)	159 (2.92%)
Lymph node	13 (0.70%)	71 (3.48%)	21 (1.35%)	105 (1.93%)
Cerebral spinal fluid	45 (2.44%)	33 (1.62%)	12 (0.77%)	90 (1.66%)
Other type of non-sputum culture	57 (3.09%)	282 (13.84%)	194 (12.48%)	533 (9.80%)
No non-sputum culture done	890 (48.24%)	500 (24.53%)	201 (12.93%)	1,591 (29.26%)

[d] Denominator: total number of patients in age group with no sputum culture done.

Figure 1: Odds ratios and 99% CIs, factors associated with TB sputum culture non-completion, 2011–2019[e]



[e] Odds ratios for age 0-14 and non-sputum culture completed not displayed due to values outside of plot range.

Manuscript: Sputum culture negativity

Title: Factors associated with negative sputum culture in verified tuberculosis patients in the U.S., 2011–2019

Short title: Factors associated with negative TB sputum culture

Journal for first submission: Chest

Contribution of student: Surveillance data from the National Tuberculosis Surveillance System was used for this study, which included data collection and reporting by clinicians and state and local health departments. The database is managed by the Centers for Disease Control and Prevention Division of Tuberculosis Elimination. Julie Self designed the research project; Lydia Rautman conducted data analysis, developed figures and tables, and wrote the following manuscript. Johnathan A. Edwards, Steve Kammerer, Vincent Marconi, Julie Self, Ben Silk, and Jonathan Wortham provided insights on the topic and edited manuscript drafts.

Abstract

Background
Sputum culture is the gold standard diagnostic tool for TB disease in part due to its high sensitivity. Yet, one-fifth of verified TB cases 2011–2019 with a sputum culture done had a negative sputum result. Negative sputum cultures could result in missed cases and subsequent transmission of TB; identifying factors associated with a negative result could help us understand in which populations sputum culture sensitivity is lower.

Research question
What demographic characteristics, risk factors, and clinical or laboratory characteristics were associated with a negative sputum culture result in pulmonary TB patients 2011–2019?

Methods
We used verified TB cases in the National Tuberculosis Surveillance System to assess the association between predictors and a negative sputum culture result in bivariate and multivariable models.

Results
We found higher odds of a negative sputum culture result among pediatric patients, individuals who had previously had TB, and patients with no cavitation on chest x-rays.

Interpretation
In children, higher odds of negative sputum culture result likely resulted from lower mycobacterial load in the sputum. Higher odds of negative sputum culture among patients who had previously had TB could be due to higher suspicion of clinicians, enabling diagnosis of early-stage disease. Chest x-ray findings are supported by other studies that found culture negativity to be associated with less cavitation and fewer TB symptoms. Sputum culture negativity among TB patients occurs where mycobacterial load in the sputum is low; these patients can be difficult to diagnose via laboratory test because diagnostic tests are not sensitive enough to detect them. Sputum culture should be performed when possible, but a negative result should not always rule out TB, and clinical evidence should be considered in making a diagnosis.

Key words
TB diagnostics, sputum culture, paucibacillary disease, cavitation

Abbreviations
TB, tuberculosis

Introduction

Tuberculosis disease (TB) remains a persistent challenge in global health; in 2019 there were 10 million incident TB disease cases globally according to World Health Organization estimates [1]. The incidence rate of TB in the United States is one of the lowest in the world, representing successful efforts of public health programs to treat, manage, and prevent TB disease [2]. TB is verified microbiologically through isolation of *Mycobacterium tuberculosis* from culture, positive nucleic acid amplification test (NAAT) results reflecting *M. tuberculosis genetic material*, or positive smear results for acid-fast bacilli (AFB); nonetheless, isolation of *Mycobacterium tuberculosis* complex (MTBC) via sputum culture is important for diagnosing pulmonary disease because of its high sensitivity [3], utility in phenotypic drug resistance testing [4], and role in genotyping [5]. From 2011–2019, 82% of TB cases in the U.S. were confirmed via positive culture, the majority being from a sputum specimen. Among the remaining 18% of cases, the sputum culture was either negative (78%) or not done (22%).

Some negative cultures could result from errors in specimen collection, improper laboratory management, poor specimen quality, or low specimen quantity. More frequently, negative culture results in TB patients with paucibacillary disease or lack of cavitation in the lungs. Paucibacillary disease, a form of TB disease caused by a smaller number of bacteria [6], is common in children and may present with non-specific but significant symptoms [7]. In healthy adult patients, paucibacillary disease is associated with early stages on the TB disease continuum [7, 8]; as the disease progresses, bacillary loads typically increase, leading to higher diagnostic test sensitivity as well as a more infectious case. In these cases, delays in treatment could result in further transmission or disease-related morbidity. Identification of characteristics associated with these early stages might help clinicians recognize these cases and start treatment sooner. Good immune function is required to create cavities in the lungs, which are therefore rare in children and immunosuppressed individuals [9, 10]. As cavities provide access for TB bacilli to the respiratory tract for expectoration, patients without cavities may be more likely to have a negative test

result; this is most notably seen in patients with HIV-TB coinfection who often have smear-negative disease [11, 12]. TB disease is especially dangerous in these populations as it can quickly lead to complications [7, 13], so understanding factors associated with a negative sputum culture could help clinicians understand when there is substantial risk of a false negative and other diagnostic methods should be considered.

There is limited literature on factors associated with sputum culture negativity among verified TB cases. Research on TB in children has estimated that positive cultures only occur in 30–40% of pediatric cases [13]. Some older [14] and more recent studies [15, 16] found that sputum culture-negative TB patients have less cavitation on chest radiographs and present less frequently with classic TB symptoms compared with sputum culture-positive patients.

Although patients can be verified as cases via alternative criteria, patients presenting with paucibacillary disease whose diagnosis is missed or delayed because of a negative sputum culture represent missed opportunities to detect and treat TB before it becomes more advanced and infectious. Identifying characteristics associated with negative sputum cultures could help clinicians recognize cases, minimizing delays in diagnosis of sputum culture-negative TB cases and thereby improving outcomes [17]. This study analyzes the National Tuberculosis Surveillance System (NTSS) data to identify demographic characteristics, risk factors, and clinical or laboratory results associated with a negative sputum culture result.

Methods

We used data from the NTSS, containing verified TB cases reported in the 50 United States and DC by state and local health departments. NTSS is managed by the Centers for Disease Control and Prevention (CDC) [18]. We included all cases with pulmonary TB and an available sputum culture result reported 2011–2019.

We analyzed variables related to patient demographics, clinical characteristics, and laboratory tests and assessed each one's association with the outcome of a negative sputum culture result. We classified patient age into three broad categories based upon diagnostic and clinical differences generally observed in patients older than 65 years [19], as well as those ages 0-14 years and 15-64 years old [9]. The race/ethnicity variable is a combination of the self-reported patient race and ethnicity. If a patient's self-reported ethnicity was Hispanic or Latino, then the patient was classified as such regardless of race; non-Hispanic (NH) individuals were classified by race. Region was determined according to the U.S. Census Bureau's American Community Survey (ACS) region classifications [20]. For the variable indicating primary occupation, "correctional facility staff", "healthcare worker" and "migrant worker" were combined and recoded "occupations associated with higher TB risk." Variables for initial chest x-ray results and x-ray evidence for one or more lung cavities were combined. A composite variable combined alcohol use, injection drug use, and non-injection drug use to denote any or no reported substance use in the previous 12 months.

For the frequency analysis, we combined missing and "unknown" values due to low frequencies. We compared proportions of the levels in each variable stratified on sputum culture results with chi-squared tests; where expected cell counts <5 we used 10,000 Monte Carlo simulations to produce the associated p-value. The combined "unknown" and missing values category was excluded after the chi-squared analysis, except where the proportion of cases in this category exceeded 3%.

We ran each variable in a univariable logistic regression model to estimate the association with a negative sputum culture result. Crude odds ratios, 99% confidence intervals, and p-values were recorded. For multivariable analysis, we ran 21 main effects in a model with sputum culture negativity as the outcome, using an alpha of 0.01 and backward selection with Schwarz BIC as the model choice method. The resulting model had 14 main effects and collinearity diagnostics confirmed there was no collinearity. Model fit and testing statistics, Wald chi-square values, and p-values were generated and adjusted ORs

and 99% confidence intervals were calculated. Statistical analyses were run in SAS 9.3 [21]; figures illustrating ORs were produced in RStudio 1.3.1093 [22].

Emory University Institutional Review Board (IRB) granted this study expedited approval and waived HIPAA authorization and informed consent. CDC determined this project to be public health surveillance as defined in 45 CFR 46.102, thereby exempt from IRB review.

Results

During 2011–2019, 89,419 TB cases were reported to NTSS. Among these, 56,854 cases (64%) met the criteria of pulmonary involvement and a sputum culture result available in NTSS. Sixty-four percent were male and 67% were non-U.S.–born (Table 4). Pediatric cases made up 2% of the cases, adults comprised 74% and elderly cases were 24%. After removing observations with missing data, our dataset included 50,982 (90%) cases, 11,349 (22%) of which had a negative sputum culture result. Of sputum culture-negative patients, 61% were male and 67% were non-U.S.–born. Five percent of sputum culture-negative cases were pediatric, 73% were adult, and 22% were elderly. The median age (interquartile range [IQR]) of sputum culture-negative and sputum culture-positive patients were 48 (31–63) and 49 (32–64), respectively.

Children were more likely than adults to have a negative sputum culture result (Table 5; aOR = 2.44, 99% CI: 1.94, 3.06). The median age (IQR) of children with negative sputum cultures and positive sputum cultures were 9 (5–12) and 12 (7–14). The primary reason for evaluation differed between children with negative and positive sputum cultures, specifically for contact of TB patient (34% of sputum culture-negative children v 23% of sputum culture-positive children), abnormal x-ray result (24% v 15%), and TB symptoms (23% v 48%).

Patients who reported previous TB were more likely than those without previous TB to have a negative sputum culture result (aOR = 2.08, 99% CI: 1.80, 2.40). Among patients who previously had TB, negative sputum cultures were more common among non-U.S.–born (33%) compared with U.S.-born

persons (24%); among patients without previous TB diagnoses, negative sputum cultures were equally common among non-U.S.–born and U.S.-born. The median number of years (IQR) since the previous TB episode was 7 (3–18) among sputum culture-negative individuals, compared with 14 (5–32) among sputum culture-positive patients.

Individuals with any kind of substance use reported were less likely to have a negative sputum culture result compared with those who had no reported substance use (aOR = 0.74, 99% CI: 0.67, 0.82). Cases coinfected with HIV were less likely to have a negative sputum culture result compared with HIV-negative individuals (aOR = 0.83, 99% CI: 0.71, 0.97. A greater proportion of HIV-positive patients had normal x-ray results compared with HIV-negative patients (17% and 7%, respectively), and a lower proportion of HIV-positive patients had abnormal results with evidence of cavitation compared with HIV-negative patients (11% v 14%), as well as abnormal results without evidence of cavitation (68% v 77%).

Patients with an initial x-ray result of "Abnormal with no cavitation" were more likely to have a negative sputum culture result compared with those with a "normal" initial x-ray (aOR = 1.2, 99% CI: 1.05, 1.36); this pattern remained when compared with patients with x-ray results that were abnormal with cavitation (aOR = 1.15, 99% CI: 1.04, 1.27). "Abnormal x-ray" was more frequently the reason for evaluation in sputum culture-negative patients compared with sputum culture-positive patients (30% v 21%), while "TB symptoms" was less frequent (45% in sputum culture-negative compared with 63% in sputum culture-positive). Compared with patients with a negative result, there were lower odds of negative sputum culture result among patients with a positive sputum smear (aOR = 0.08, 99% CI: 0.07, 0.09), NAAT (aOR = 0.17, 99% CI: 0.16, 0.19), or IGRA (aOR = 0.57, 99% CI: 0.51, 0.64).

Odds of negative sputum culture were higher among patients with either negative (aOR = 3.73, 99% CI: 3.31, 4.19) or positive (aOR = 1.47, 99% CI:1.36, 1.59) non-sputum culture results. Among patients with a negative sputum culture, a non-sputum culture was completed in 47% of cases. Where sputum culture was negative, TB was able to be diagnosed via positive culture of a non-sputum specimen

in an additional 29% of cases (57% of concurrent pulmonary and extrapulmonary cases, 23% of pulmonary only cases). Verification via positivity of a culture from a non-sputum sample after a negative sputum culture was successful in 6% of pediatric cases, 27% of adults, and 43% of elderly patients.

Discussion

We found higher odds of a negative sputum culture result for 1) children, 2) patients who had previously had TB, and 3) patients with x-ray results that were abnormal but showed no evidence of cavitation. These groups may represent populations who could be at greater risk for a misdiagnosis if other clinical, radiographic, or epidemiologic evidence is not also considered.

Children with TB disease are more likely to have a negative sputum culture result compared with adults and negative sputum culture result was associated with younger age. Children, especially very young children, often have difficulty producing sputum specimens, and specimens that are collected might give a negative culture result due to poor quality [23]. However, in children with negative sputum culture results who also had other non-sputum specimens tested, (often gastric aspirate, the other specimens were often culture negative as well, suggesting higher rates of paucibacillary disease in children. These two considerations make it unsurprising that sputum culture is markedly less sensitive for case verification in children than in adult TB patients [24]. Many of the pediatric cases in the NTSS were diagnosed not via laboratory results but per clinical case definition. It is clear that laboratory tests must be used with caution in diagnosing TB in all patients, but especially in children. While a positive sputum or non-sputum culture can provide valuable phenotypic drug sensitivity information, untreated TB in young children can quickly lead to life-threatening complications including meningitis and disseminated TB [7, 13], so a negative smear or culture should never rule out TB in a child. Physicians should be aware of the often-atypical presentation of TB disease in children and should continue to diagnose pediatric TB patients based on a combination of clinical, epidemiologic, and radiographic evidence.

Negative sputum culture result was associated with previous TB and less time since the previous TB episode. We considered that some of these patients could still have been on a treatment regimen from a previous episode; however, the variable indicated only previous TB episodes that had ended more than a year previous to the reported case [18]. Higher odds of negative sputum culture for these patients could be because TB was caught in an earlier disease stage due to heightened suspicion of the clinician for TB. As TB incidence has declined over time, fewer clinicians have experience with TB and the index of suspicion for TB in a patient with nonspecific symptoms and no TB risk factors may be quite low [25]. When managing a patient who has had previous TB, a clinician may be more likely to test for TB sooner either from their own experience previously treating TB in that patient or the patient's medical records; this may result in diagnosis of earlier-stage (and often paucibacillary) disease. These findings echo those of a study on presumed TB patients with HIV coinfection in Uganda [26], warranting further investigation on this topic. Having previous TB is a significant risk factor for subsequent episodes; while it is encouraging that these cases may be caught early, a next step toward TB elimination might entail focusing on helping TB patients with a history of previous episodes manage co-morbidities and reduce factors that may predispose to a subsequent episode. Further studies on factors associated with multiple TB episodes could inform these efforts.

The proportion of patients with "abnormal x-ray" as the reason for evaluation was higher in sputum culture-negative patients compared with sputum culture-positive patients; the proportion reporting "TB symptoms" as the reason for evaluation was lower. This suggests patients with negative sputum cultures (and likely early-stage or paucibacillary disease) may not as frequently present with classic TB symptoms but may have abnormal chest x-ray results that increase suspicion for TB disease. We found that patients with sputum culture-negative disease were less likely to present with cavitation on the chest x-ray compared with patients with positive sputum cultures. This finding is similar to previous studies, which suggested that sputum culture negativity is associated with less frequent TB symptoms and less

radiographic cavitation [14-16]. These findings may be informative about presentation of paucibacillary-appearing disease, but were limited by the variables available for analysis. Studies with more detailed symptomological data will be critical in developing a better understanding of these less common presentations of TB.

We expected to see higher odds of sputum negativity among individuals with HIV because of the atypical presentation (including paucibacillary disease) that may accompany advanced TB-HIV coinfection [27]. We instead observed an association between HIV positivity and sputum culture positivity. This could be because immunosuppressed patients with HIV are more likely to progress to advanced disease with a higher bacillary load more quickly, or because immune function is adequate to enable cavitation. However, individuals with HIV more frequently had normal x-ray results and less frequently had x-ray results that were abnormal, with or without evidence of cavitation, compared with HIV-negative patients. At CD4+ T-cell counts over 200, indicating adequate immune function, HIV-related TB presents similarly to TB in HIV-negative patients; it is only at advanced stages of HIV that TB presentation is atypical due to profound immunodeficiency [27, 28]. Most evaluations of the presentation of TB-HIV coinfection in the U.S. are based on data from the HIV/AIDS epidemic and TB resurgence in the 1990s. These patients were often highly immunodeficient and perhaps do not an accurately represent patients with HIV diagnosed in later years, who have better prognoses from access to newly developed and better antiretrovirals, improved adherence and management of comorbidities [29], which may result in higher CD4+ T-cell counts. NTSS data does not include CD4+ T-cell count, so we are unable to make any definitive conclusion about the extent to which patients with HIV in the dataset are immunodeficient, but this area warrants further investigation.

Odds of a negative sputum culture were higher in both patients with positive and negative non-sputum culture compared to patients without a non-sputum culture. This indicates that where clinicians are unable to confirm TB via sputum culture, another non-sputum culture is often attempted, with varying

degrees of success. Among sputum culture-negative individuals, confirmation via a non-sputum culture is more successful among concurrent extrapulmonary and pulmonary patients than in pulmonary only patients, likely because of the availability of other sites for culturing. Confirmation by positive non-sputum culture following a negative sputum culture is much less successful in children compared with adults and elderly patients. While culturing from a sputum specimen is the gold standard, culturing from other sites can also be helpful in identifying *M. tuberculosis*, depending on the patient, method, and specimen.

Conclusion

Continued improvements in TB prevention, diagnosis, and management will help us reach our goal of TB elimination in the U.S. Verification of a case via positive sputum culture is the gold standard; however, a large proportion of cases are unable to be verified this way either because of a negative sputum culture result or because a sputum culture was not attempted. During 2011–2019 one-fifth of TB patients with any sputum culture result available had a negative result. We expected to see the distribution of negative sputum culture results to be driven by children and immunocompromised adult patients due to their higher rates of paucibacillary disease and lack of cavitation frequently accompanied by negative cultures or AFB smears. We did observe higher sputum culture negativity among children, although pediatric patients only comprised a small proportion of culture-negative individuals. These sputum culture-negative children were more often diagnosed via clinical criteria, a reminder to clinicians that TB diagnosis is especially challenging in children due to decreased sensitivity of laboratory-based diagnostic tools in pediatric patients. As pediatric TB can quickly lead to serious complications, a negative sputum culture or smear in a child should never rule out TB and clinicians may consider clinical or epidemiologic evidence in supporting a diagnosis.

Sputum culture negativity was associated with previous TB and shorter time since the previous episode of TB. We posit that this connection is driven by higher suspicion of TB for these patients and could suggest the success of catching these cases early in the disease; the next step should involve targeting these patients for preventative measures, such as managing co-morbidities that increase the risk of another episode. In concordance with previous literature, we found that sputum culture-negative patients had less cavitation on chest x-rays. Additionally, a smaller proportion of sputum culture-negative patients were evaluated for TB symptoms compared with sputum culture-positive cases. While our findings do support those of previous studies, it is difficult to make broad generalizations about the frequency of symptoms or radiologic indications without more symptomologic variables. Our findings warrant further investigation on the presentation of sputum culture-negative TB and any differences between early-stage TB or paucibacillary-appearing disease. Because these may be less frequently accompanied by classic TB symptoms and cavitation on a chest x-ray, sputum culture-negative TB may be underrecognized, particularly by clinicians who do not often see TB cases. Further research on and an increased awareness of atypical disease presentation could help clinicians identify cases earlier, initiate treatment sooner, and prevent TB transmission.

References

1. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization, **2020**.
2. Centers for Disease Control. Tuberculosis (TB): Trends in Tuberculosis 2019. Available at: https://www.cdc.gov/tb/publications/factsheets/statistics/tbtrends.htm.
3. Schluger NW, Burzynski J. PNEUMONIA | Mycobacterial. In: Laurent GJ, Shapiro SD. Encyclopedia of Respiratory Medicine. Oxford: Academic Press, **2006**:451-6.
4. Hobby GL, Holman AP, Iseman MD, Jones JM. Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. Antimicrobial agents and chemotherapy **1973**; 4(2): 94-104.
5. Centers for Disease Control. Tuberculosis (TB): TB Genotyping. Available at: https://www.cdc.gov/tb/programs/genotyping/.
6. Centers for Disease Control. Tuberculosis (TB): Children. **2020**.
7. Roya-Pabon CL, Perez-Velez CM. Tuberculosis exposure, infection and disease in children: a systematic diagnostic approach. Pneumonia **2016**; 8(1): 1-18.
8. Blumberg HM, Burman WJ, Chaisson RE, Daley CL. American thoracic society/centers for disease control and prevention/infectious diseases society of America: treatment of tuberculosis. American journal of respiratory and critical care medicine **2003**; 167(4): 603.
9. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med **2000**; 161: 1376-95.
10. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. Emerging infectious diseases **1995**; 1(4): 115.
11. Gathua S, Waiyaki P. Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis. Am Rev Respir Dis **1993**; 147: 958-61.
12. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. The Lancet **2007**; 369(9578): 2042-9.
13. Cruz AT, Starke JR. Pediatric tuberculosis. Pediatrics in review **2010**; 31(1): 13.
14. Hong Kong Chest Service, Tuberculosis Research Centre M, Council BMR. A study of the characteristics and course of sputum smear-negative pulmonary tuberculosis. Tubercle **1981**; 62(3): 155-67.
15. Nguyen M-VH, Jenny-Avital ER, Burger S, Leibert EM, Achkar JM. Clinical and radiographic manifestations of sputum culture-negative pulmonary tuberculosis. PLoS One **2015**; 10(10): e0140003.
16. Nguyen M-VH, Levy NS, Ahuja SD, Trieu L, Proops DC, Achkar JM. Factors associated with sputum culture-negative vs culture-positive diagnosis of pulmonary tuberculosis. JAMA network open **2019**; 2(2): e187617-e.
17. Virenfeldt J, Rudolf F, Camara C, et al. Treatment delay affects clinical severity of tuberculosis: a longitudinal cohort study. BMJ open **2014**; 4(6).
18. Centers for Disease Control. National Tuberculosis Surveillance System (NTSS), data set reference guide. **2015**.
19. Lee JH, Han DH, Song JW, Chung HS. Diagnostic and therapeutic problems of pulmonary tuberculosis in elderly patients. Journal of Korean medical science **2005**; 20(5): 784.
20. United States Census Bureau. Geography & ACS. Available at: https://www.census.gov/programs-surveys/acs/geography-acs.html.
21. SAS Institute I. Cary, NC, **2013**.
22. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC. Boston, MA, **2020**.
23. Mccarter YS, Robinson A. Quality evaluation of sputum specimens for mycobacterial culture. American journal of clinical pathology **1996**; 105(6): 769-73.
24. Marais BJ. Advances in the clinical diagnosis of TB in children. Pediatric research **2008**; 63(2): 116.
25. Lönnroth K, Migliori GB, Abubakar I, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. European Respiratory Journal **2015**; 45(4): 928-52.
26. Nakiyingi L, Nonyane BA, Ssengooba W, et al. Predictors for MTB culture-positivity among HIV-infected smear-negative presumptive tuberculosis patients in Uganda: application of new tuberculosis diagnostic technology. PLoS One **2015**; 10(7): e0133756.

27. Burman WJ, Jones BE. Clinical and radiographic features of HIV-related tuberculosis. In: Seminars in respiratory infections, 2003:263-71.
28. Aaron L, Saadoun D, Calatroni I, et al. Tuberculosis in HIV-infected patients: a comprehensive review. Clinical microbiology and infection **2004**; 10(5): 388-98.
29. Trickey A, May MT, Vehreschild J-J, et al. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. The lancet HIV **2017**; 4(8): e349-e56.

Tables and figures

Table 4: Demographic characteristics, risk factors, and clinical/laboratory characteristics of pulmonary TB cases by sputum culture result, 2011–2019

	Total (N = 56,854)		
	Negative sputum culture	Positive sputum culture	
	N (%)	N (%)	*P*-value
Total cases	12,614 (22.19%)	44,240 (77.81%)	
Demographic characteristics			<.001
Sex			
Female	4,899 (38.84%)	15,311 (34.61%)	
Male	7,714 (61.15%)	28,927 (65.39%)	
Unknown	1 (0.01%)	2 (0.00%)	
Age group			<.001
0-14	584 (4.63%)	405 (0.92%)	
15-64	9,170 (72.70%)	32,802 (74.15%)	
65+	2,860 (22.67%)	11,029 (24.93%)	
Unknown	0 (0.00%)	4 (0.01%)	
Origin			.500
Non-U.S.–born	8,422 (66.77%)	29,624 (66.96%)	
U.S.-born	4,188 (33.20%)	14,591 (32.98%)	
Unknown	4 (0.03%)	25 (0.06%)	
Race/ethnicity			<.001
American Indian/Alaska Native	111 (0.88%)	705 (1.59%)	
Asian	3,945 (31.27%)	14,488 (32.75%)	
NH Black	2,952 (23.40%)	8,708 (19.68%)	
Hispanic or Latino	3,497 (27.72%)	13,411 (30.31%)	
Native Hawaiian/Other Pacific Islander	133 (1.05%)	384 (0.87%)	
NH White	1,915 (15.18%)	6,215 (14.05%)	
Multiple races or unknown	61 (0.48%)	329 (0.74%)	
Region			<.001
Midwest	1,785 (14.15%)	4,819 (10.89%)	
Northeast	1,502 (11.91%)	4,546 (10.28%)	
South	5,626 (44.60%)	18,400 (41.59%)	
West	3,701 (29.34%)	16,475 (37.24%)	
Primary occupation			<.001
Occupation with TB risk[f]	635 (5.03%)	2,174 (4.91%)	
Not seeking	2,664 (21.12%)	7,058 (15.95%)	
Retired	1,805 (14.31%)	7,309 (16.52%)	
Unemployed	3,129 (24.81%)	10,914 (24.67%)	
Other	4,047 (32.08%)	15,803 (35.72%)	
Missing or Unknown	334 (2.65%)	982 (2.22%)	
Risk factors			
Long-term care facility			.872

[f] Includes correctional facility staff, healthcare workers, and migrant workers.

No	12,389 (98.22%)	43,448 (98.21%)	
Yes	210 (1.66%)	731 (1.65%)	
Missing or Unknown	15 (0.12%)	61 (0.14%)	
Corrections			<.001
No	11,859 (94.01%)	42,188 (95.36%)	
Yes	731 (5.80%)	1,913 (4.32%)	
Missing or Unknown	24 (0.19%)	139 (0.31%)	
Substance use[g]			<.001
No substance use	10,888 (86.32%)	35,258 (79.70%)	
Any substance use	1,585 (12.57%)	8,385 (18.95%)	
Missing or Unknown	141 (1.12%)	597 (1.35%)	
Homeless			<.001
No	11,854 (93.97%)	40,951 (92.57%)	
Yes	648 (5.14%)	2,991 (6.76%)	
Missing or Unknown	112 (0.89%)	298 (0.67%)	
HIV Status			<.001
Negative	10,660 (84.51%)	38,088 (86.09%)	
Positive	745 (5.91%)	2,435 (5.50%)	
Not offered	578 (4.58%)	2,063 (4.66%)	
Refused	391 (3.10%)	812 (1.84%)	
Result unknown/Indeterminate	30 (0.24%)	119 (0.27%)	
Unknown or Missing	210 (1.66%)	723 (1.63%)	
Previous TB			<.001
No	11,641 (92.29%)	41,894 (94.70%)	
Yes	914 (7.25%)	2,100 (4.75%)	
Missing or Unknown	59 (0.47%)	246 (0.56%)	
Risk factors for infection[h]			<.001
No risk factors	8,999 (71.34%)	29,316 (66.27%)	
Some risk factors	3,615 (28.66%)	14,924 (33.73%)	
Clinical and laboratory characteristics			
Disease site			<.001
Pulmonary only	4,754 (37.69%)	39,486 (89.25%)	
Both pulmonary and extrapulmonary disease	2,157 (17.10%)	10,457 (23.64%)	
Provider type			<.001
Health Department	8,523 (67.57%)	28,076 (63.46%)	
All other	3,004 (23.81%)	11,658 (26.35%)	
Both	1,072 (8.50%)	4,444 (10.05%)	
Unknown or Missing	15 (0.12%)	62 (0.14%)	
Chest radiograph			<.001
Abnormal with no cavitation	9,476 (75.12%)	26,543 (60.00%)	

[g] Any reported alcohol use, injection drug use or non-injection drug use in the 12 months prior to diagnostic evaluation for TB.

[h] Risk factors include non-U.S.–born status, homelessness or substance use in the 12 months prior to diagnostic evaluation for TB, being a contact of a TB patient but had been missed in a contact investigation, or having contact with an infectious TB patient within 2 years of diagnosis. “Some risk factors” is indicated for patients who meet at least one criterion.

Abnormal with cavitation	1,665 (13.20%)	13,646 (30.85%)	
Normal	936 (7.42%)	1,997 (4.51%)	
Not done	459 (3.64%)	1,679 (3.80%)	
Missing	78 (0.62%)	375 (0.85%)	
Non-sputum culture result			<.001
Negative	2,229 (17.67%)	1,715 (3.88%)	
Positive	3,716 (29.46%)	11,195 (25.31%)	
Not done	6,642 (52.66%)	31,115 (70.33%)	
Unknown	27 (0.21%)	215 (0.49%)	
TST result			<.001
Negative	1,049 (8.32%)	3,292 (7.44%)	
Positive	4,174 (33.09%)	11,686 (26.42%)	
Not done	7,273 (57.66%)	28,676 (64.82%)	
Unknown	118 (0.94%)	586 (1.32%)	
IGRA result			<.001
Negative	1,529 (12.12%)	2,905 (6.57%)	
Positive	5,825 (46.18%)	19,247 (43.51%)	
Not done	4,880 (38.69%)	20,532 (46.41%)	
Unknown	380 (3.01%)	1,556 (3.52%)	
NAAT result			<.001
Negative	4,338 (34.39%)	3,285 (7.43%)	
Positive	1,916 (15.19%)	27,558 (62.29%)	
Not done	6,309 (50.02%)	13,211 (29.86%)	
Unknown	51 (0.40%)	186 (0.42%)	
Sputum smear result			<.001
Negative	11,664 (92.47%)	15,231 (34.43%)	
Positive	868 (6.88%)	28,688 (64.85%)	
Not done	81 (0.64%)	308 (0.70%)	
Unknown	1 (0.01%)	13 (0.03%)	

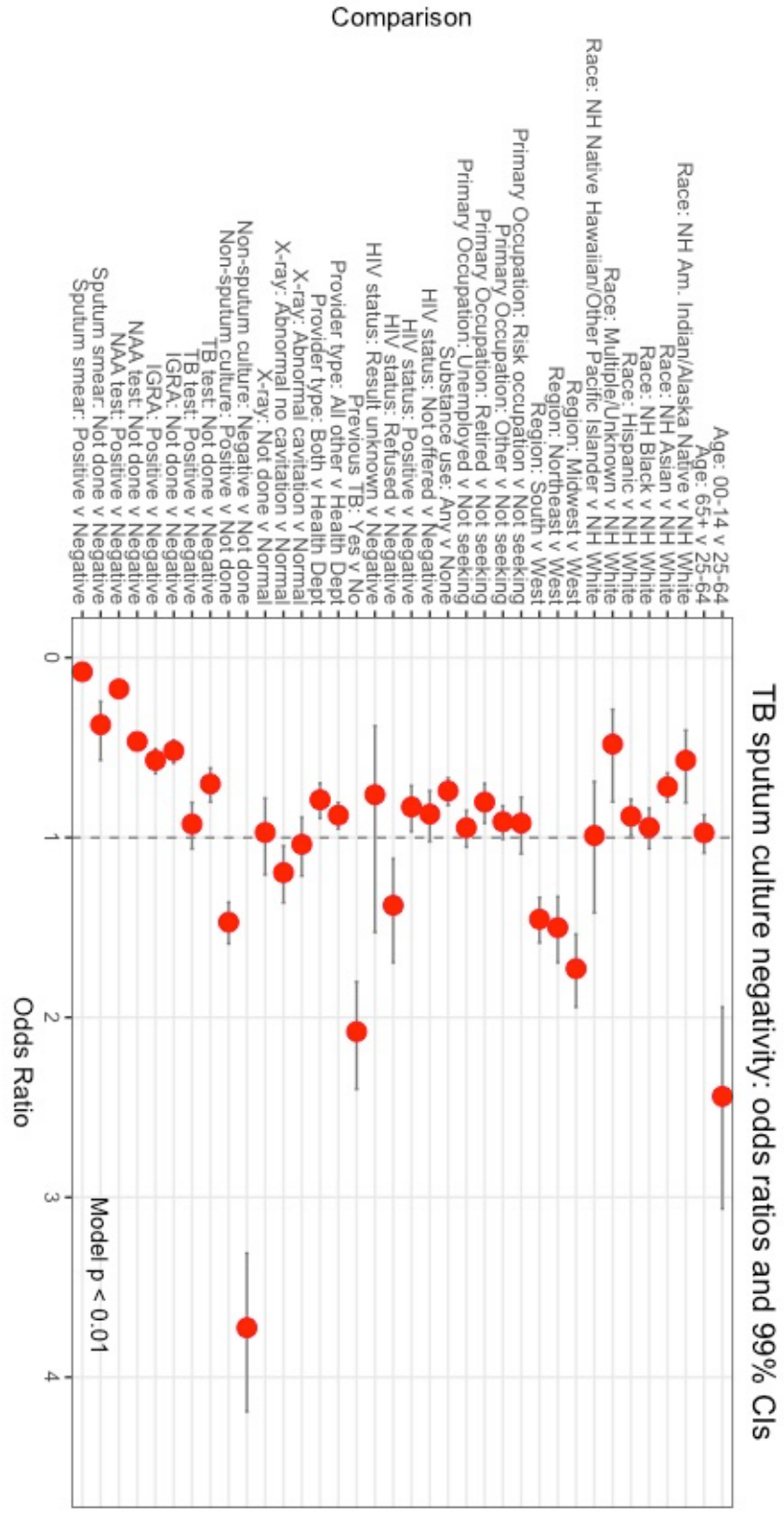
Table 5: Crude and adjusted ORs for sputum culture negativity by patient characteristic, 2011–2019

	Total (N = 56,337)				
	Sputum culture not completed	Bivariate analysis		Multivariable analysis	
	N (%)	cOR (99% CI)	*P*-value	aOR (99% CI)	*P*-value
Total cases	11,349 (22.30%)				
Demographic characteristics					
Sex					
Female	4,426 (39.00%)	1.20 (1.13, 1.27)	<.001		
Male	6,923 (61.00%)	Reference	-		
Age					
0-14	549 (4.84%)	5.18 (4.35, 6.17)	<.001	2.44 (1.94, 3.06)	<.001
15-64	8,260 (72.78%)	Reference	-	Reference	-
65+	2,540 (22.38%)	0.94 (0.88, 1.00)	.010	0.97 (0.87, 1.09)	.536
Origin					
Non-U.S.–born	7,563 (66.64%)	0.99 (0.93, 1.05)	.553		
U.S.-born	3,786 (33.36%)	Reference	-		
Race/Ethnicity					
NH American Indian/ Alaska Native	100 (0.88%)	0.49 (0.37, 0.66)	<.001	0.57 (0.40, 0.81)	<.001
NH Asian	3,564 (31.40%)	0.87 (0.80, 0.95)	<.001	0.72 (0.64, 0.80)	<.001
NH Black	2,689 (23.69%)	1.10 (1.01, 1.21)	.207	0.94 (0.84, 1.06)	.207
Hispanic or Latino	3,119 (27.48%)	0.83 (0.76, 1.21)	.004	0.88 (0.79, 0.99)	.004
NH Native Hawaiian/ Other Pacific Islander	122 (1.07%)	1.13 (0.85, 1.50)	.936	0.99 (0.69, 1.42)	.936
NH White	1,712 (15.09%)	Reference	-	Reference	-
Multiple races or unknown	43 (0.38%)	0.53 (0.35, 0.82)	.000	0.48 (0.29, 0.80)	<.001
Region					
Midwest	1,595 (14.05%)	1.69 (1.55, 1.85)	<.001	1.73 (1.54, 1.94)	<.001
Northeast	1,306 (11.51%)	1.50 (1.36, 1.65)	<.001	1.5 (1.33, 1.70)	<.001
South	5,179 (45.63%)	1.39 (1.30, 1.48)	<.001	1.45 (1.33, 1.59)	<.001
West	3,269 (28.80%)	Reference	-	Reference	-
Primary occupation					
Occupations with TB risk	587 (5.17%)	0.77 (0.67, 0.88)	<.001	0.92 (0.78, 1.09)	.208
Not seeking	2,495 (21.98%)	Reference	-	-	-
Retired	1,640 (14.45%)	0.65 (0.59, 0.71)	<.001	0.80 (0.70, 0.92)	<.001
Unemployed	2,849 (25.10%)	0.76 (0.70, 0.83)	<.001	0.95 (0.85, 1.05)	.180
Other	3,778 (33.29%)	0.67 (0.62, 0.72)	<.001	0.91 (0.82, 1.01)	.019
Risk factors					
Long-term care facility					
No	11,157 (98.31%)	Reference	-		
Yes	192 (1.69%)	1.07 (0.87, 1.33)	.409		
Corrections					
No	10,761 (94.82%)	Reference	-		
Yes	588 (5.18%)	1.33 (1.17, 1.51)	<.001		
Substance use					
No substance use	9,904 (87.27%)	Reference	-	Reference	-

Any substance use	1,445 (12.73%)	0.62 (0.57, 0.67)	<.001	0.74 (0.67, 0.82)	<.001
Homeless					
No	10,757 (94.78%)	Reference	-		
Yes	592 (5.22%)	0.78 (0.69, 0.88)	<.001		
HIV Status					
Negative	9,792 (86.28%)	Reference	-	Reference	-
Positive	639 (5.63%)	1.07 (0.95, 1.21)	.154	0.83 (0.71, 0.97)	.002
Not offered	530 (4.67%)	1.05 (0.92, 1.19)	.383	0.87 (0.74, 1.02)	.027
Refused	364 (3.21%)	1.80 (1.53, 2.14)	<.001	1.38 (1.12, 1.70)	<.001
Result unknown/ Indeterminate	24 (0.21)	0.84 (0.47, 1.52)	.457	0.76 (0.38, 1.53)	.314
Risk factors for infection					
No risk factors	8,131 (71.65%)	Reference	-		
Some risk factors	3,218 (28.35%)	0.78 (0.74, 0.83)	<.001		
Previous TB					
No	10,528 (92.77%)	Reference	-	Reference	-
Yes	821 (7.23%)	1.54 (1.38, 1.72)	<.001	2.08 (1.80, 2.40)	<.001
Clinical and laboratory characteristics					
Disease site					
Pulmonary only	9,403 (82.85%)	Reference	-		
Both pulmonary and extrapulmonary disease	1,946 (17.15%)	1.74 (1.61, 1.88)	<.001		
Provider type					
Health Department	7,859 (69.25%)	Reference	-	Reference	-
All other	2,535 (22.34%)	0.87 (0.81, 0.93)	<.001	0.88 (0.81, 0.95)	<.001
Both	955 (8.41%)	0.78 (0.71, 0.86)	<.001	0.79 (0.70, 0.89)	<.001
Chest radiograph					
Abnormal with no cavitation	8,614 (75.90%)	0.77 (0.69, 0.86)	<.001	1.20 (1.05, 1.36)	.001
Abnormal with cavitation	1,499 (13.21%)	0.26 (0.23, 0.29)	<.001	1.04 (0.89, 1.21)	.537
Normal	849 (7.48%)	Reference	-	Reference	-
Not done	387 (3.41%)	0.58 (0.48, 0.69)	<.001	0.97 (0.78, 1.21)	.732
Non-sputum culture result					
Negative	2,020 (17.80%)	6.19 (5.63, 6.80)	<.001	3.73 (3.31, 4.19)	<.001
Positive	3,315 (29.21%)	1.55 (1.46, 1.65)	<.001	1.47 (1.36, 1.59)	<.001
Not done	6,014 (52.99%)	Reference	-	Reference	-
TST result					
Negative	926 (8.16%)	Reference	-	Reference	-
Positive	3,849 (33.91%)	1.13 (1.02, 1.26)	<.001	0.93 (0.81, 1.06)	.148
Not done	6,574 (57.93%)	0.81 (0.73, 0.90)	<.001	0.70 (0.61, 0.80)	<.001
IGRA result					
Negative	1,424 (12.55%)	Reference	-	Reference	-
Positive	5,452 (48.04%)	0.57 (0.52, 0.63)	<.001	0.57 (0.51, 0.64)	<.001
Not done	4,473 (39.41%)	0.45 (0.41, 0.50)	<.001	0.52 (0.46, 0.59)	<.001
NAAT result					
Negative	3,898 (34.35%)	Reference	-	Reference	-
Positive	1,704 (15.01%)	0.05 (0.05, 0.06)	<.001	0.17 (0.16, 0.19)	<.001
Not done	5,754 (50.64%)	0.37 (0.34, 0.40)	<.001	0.47 (0.43, 0.51)	<.001
Sputum smear result					

Negative	10,521 (92.70%)	Reference	-	Reference	-
Positive	773 (6.81%)	0.04 (0.04, 0.04)	<.001	0.08 (0.07, 0.09)	<.001
Not done	55 (0.48%)	0.32 (0.22, 0.47)	<.001	0.37 (0.24, 0.57)	<.001

Figure 2: Odds ratios and 99% CIs, factors associated with negative TB sputum culture, 2011–2019



CHAPTER 4: PUBLIC HEALTH SIGNIFICANCE

Summary of research

Tuberculosis disease continues to be a public health problem in the U.S. Early and accurate case identification will be critical in helping us pursue our ultimate goal of elimination. The most sensitive diagnostic test we have for TB is the sputum culture, which is the gold standard for diagnosis. From 2011–2019, 70% of verified TB cases were confirmed via positive sputum culture, 20% had a negative result for their sputum culture, and 10% had no sputum culture completed. The 30% of cases for which sputum culture was negative or not completed were verified with another laboratory test or based on clinical criteria.

Non-completion of sputum cultures indicates either that a sputum specimen could not be obtained from the patient or that the sputum culture was not ordered, both of which could suggest reliance on other specimens or tests for diagnosis. This could mean lower sensitivity and result in missing TB cases. Analyzing factors associated with sputum culture non-completion could give insights about populations in which culturing rates are lowest and in which cases could be missed. We found sputum culture non-completion to be associated with pediatric and elderly age, residence in a long-term care facility, having a care provider that was not a public health department, and having a non-sputum specimen culture completed.

A negative result for sputum culture indicates that MTBC could not be isolated from the specimen, which in TB patients may result from paucibacillary or early-stage disease or a poor specimen. Paucibacillary and early-stage TB disease may be underrecognized and underdiagnosed. Examination of patients with negative sputum culture results can help us understand in which patients standard laboratory tests are more likely to be negative, warranting consideration of clinical and epidemiologic evidence in supporting a diagnosis. We found sputum culture negativity to be associated with pediatric age, having had previous TB, and less frequent cavitation compared with sputum culture-positive patients.

Recommendations and future directions

Sputum culture remains the gold standard in TB diagnosis due to its high sensitivity and usefulness in drug susceptibility testing. Results from our study suggest that TB was not always considered on a differential diagnosis. Sputum culturing rates were especially low in the elderly and in residents of long-term care facilities, many of whom were foreign-born; this indicates an underrecognition of basic TB risk factors and underprioritization of TB on the differential diagnosis. This seems to indicate a waning general awareness of tuberculosis epidemiology often seen in low-burden countries with decreasing incidence. Clinicians should familiarize themselves with the epidemiology of and risk factors associated with TB, some of the most critical being foreign-born status, experience in congregate settings like LTCFs, and co-morbidities and underlying medical conditions.

Primary care physicians especially should have a higher suspicion of TB. We observed many incidental TB diagnoses via culturing of specimens such as bronchial fluid and biopsies; obtaining a sputum sample should be the first step in ruling out TB before obtaining other specimens. If a patient is unable to expectorate, an induced sputum sample should be considered as the first alternative for culturing, as it is a less invasive procedure compared with the bronchial lavage or lung biopsy. Following this protocol will minimize invasiveness, infection control risks, and could result in fewer missed TB cases.

Conclusion

Our analyses show sputum cultures are often not completed in children, probably owing to low sensitivity and difficulty obtaining a sputum specimen. Culturing of a sputum specimen should be considered if the patient is able to expectorate, as it can provide critical drug sensitivity information and contributes to molecular surveillance. In very young patients who are unable to expectorate, other specimens like gastric aspirates may be useful for culturing. However, confirmation via positive non-sputum culture after a negative sputum culture is not always successful in children. Due to decreased

sensitivity in this age group, all laboratory tests should be interpreted with caution and considered in clinical and epidemiologic context. Lab tests should never rule out TB in children, as risk of a false negative result is higher in children than adults. Physicians should have a higher suspicion for TB and should be aware of the often-atypical presentation of pediatric TB disease.

In addition to children, patients in which sputum culture may be less reliable due to an increased risk of false negative include previous TB patients and patients whose x-ray results are abnormal but without cavitation. Our findings prompt further research to better understand these patients and presentation associated with sputum culture-negative disease. Further investigation may inform guidelines for diagnosis protocol in populations more likely to have sputum culture-negative disease. Improvement of the general awareness of TB risk factors and presentations, including atypical presentations, will help clinicians recognize TB and understand in which populations clinical criteria should be prioritized in making a diagnosis.

Other non-sputum cultures may be an alternative in patients with extrapulmonary involvement, but may still not be successful in all TB cases, especially in children and patients with solely pulmonary disease. More research is needed on the use of diagnostic tools by different types of care providers to understand where differences in diagnostic protocols are. Future analyses with more symptomological data are needed to better understand and recognize atypical TB presentation, specifically among patients whose sputum cultures are more likely to be negative. This will give physicians better confidence in diagnoses grounded in clinical evidence where laboratory tests are not sensitive enough for diagnosis. As patients with paucibacillary disease, such as young children, are more likely to progress to life-threatening conditions if untreated, being better able to identify these cases and initiate treatment could prevent avoidable morbidity and mortality. In patients with early-stage TB, prompt diagnosis and treatment before infectiousness increases will help us reduce TB transmission and will help us achieve TB elimination in the U.S.