Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Megan Eguchi Date

Predictors of Drug Resistance or Negative Culture at Baseline: Identifying Ineligible Patients in a Clinical Trial for Tuberculosis Treatment

By

Megan Eguchi
MPH

Epidemiology

_____

Anne Spaulding, MD, MPH
Committee Chair

_____

Erin Bliven-Sizemore, MPH
Committee Member

Predictors of Drug Resistance or Negative Culture at Baseline: Identifying Ineligible Patients in a Clinical Trial for Tuberculosis Treatment

By

Megan Eguchi

BS
University of California at San Diego
2008

Thesis Committee Chair: Anne Spaulding, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2011

Abstract

Predictors of Drug Resistance or Negative Culture at Baseline: Identifying Ineligible Patients in a Clinical Trial for Tuberculosis Treatment

By Megan Eguchi

Setting: Tuberculosis (TB) control continues to be hindered by sluggish diagnostic tests. Delays prevent patients from receiving appropriate treatment if infected with drug-resistant TB or organisms other than TB. In research, eligibility often requires drug-sensitive TB infection, and delays in identifying ineligible patients consume study resources. New rapid diagnostics are available, but high cost often renders universal testing difficult. A risk-based approach to prioritize testing may be more feasible.

Objective: Identify predictors associated with drug-resistance or a negative culture. Determine the time required to identify these conditions.

Design: This is a secondary analysis of 432 patients enrolled domestically and internationally in a Phase 2, randomized clinical trial testing a novel intensive phase TB regimen. Upon enrollment, sputum specimens were cultured and tested for drug resistance. Participants with resistance or negative cultures were deemed ineligible. Patient information collected at enrollment was analyzed to identify associations.

Results: In univariate analyses, presence of nausea and Hispanic ethnicity were associated with resistance in the US. Only Hispanic ethnicity (p-value 0.025) predicted resistance in multivariate analyses. Age 30 years or older (p-value 0.025) was the only predictor outside the US. Negative cultures were found among US non-Hispanic participants only. Univariate predictors included increasing age, race, and presenting less than two of the three symptoms cough, sweats, and loss of appetite. In multivariate analyses, Blacks and Asian/Pacific Islanders were less likely to have negative cultures than whites (p-value 0.0077 and 0.027, respectively). Participants with less than two of the three symptoms were more likely to produce negative cultures (p-value 0.017). Average time to determine ineligibility was 65 days.

Conclusion: The average time required to identify resistance or negative cultures went beyond the duration of intensive phase therapy (54 days). Hispanic patients in the US and patients 30 years or older outside the US had higher levels of resistance. Patients with negative cultures tended to be older non-Hispanic whites living in the US, with less than two of the three symptoms cough, sweats, and loss of appetite. Clinical trial sites should consider taking advantage of new rapid diagnostics by prioritizing testing for persons with these risk factors.

Predictors of Drug Resistance or Negative Culture at Baseline: Identifying Ineligible Patients in a Clinical Trial for Tuberculosis Treatment

By

Megan Eguchi

BS
University of California at San Diego
2008

Thesis Committee Chair: Anne Spaulding, MD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2011

TABLE OF CONTENTS

BACKGROUND

Introduction

Tuberculosis (TB) continues to be a major public health concern worldwide, with over 1.7 million deaths attributed to TB in 2009 (1). Although worldwide incidence rates have been decreasing since 2004, the World Health Organization (WHO) estimated that there were still 9.4 million incident cases of TB (137 per 100 000 persons) in 2009 (1). The control and elimination of TB has been greatly hindered by the HIV co-epidemic and emergence of drug-resistant strains of TB (1).

The WHO recommendation for the treatment of drug susceptible TB involves six months of antimicrobial medications taken daily under directly observed therapy (DOT). The standard treatment for drug-sensitive TB involves a combination of four drugs: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) during the first two months of TB treatment, followed by four months of INH and RIF (2). However, this standard treatment is not appropriate in all cases, and inappropriate treatment can contribute to treatment failure, recurrence, acquired resistance to additional drugs, and further transmission of drug resistant TB (3-7). Tuberculosis is often treated empirically with standard therapy upon suspicion of TB disease before culture confirmation and drug susceptibility test results are available. However, in TB suspects found not to have TB disease or those determined to have drug resistant TB the standard treatment would be inappropriate. Characterizing the predictors and clinical characteristics of drug resistance and culture-negativity among TB suspects may help practitioners recognize those most likely to require alternative or no treatment. Armed with this information, practitioners can then request additional rapid diagnostic tests that may allow them to initiate an appropriate regimen earlier in the course of treatment for these patients.

The data for this study were taken from a clinical trial conducted by the Tuberculosis Trials Consortium (TBTC), Study 28. Patients were ineligible for the study if they were found to have baseline drug resistance to INH, RIF, PZA or any fluoroquinolone (FQ), or if they were

found to be uninfected with TB, as indicated by a negative sputum culture grown in either solid or liquid media. In addition to selection of a more appropriate treatment regimen, earlier identification of ineligible subjects will reduce the resources and funds that are unnecessarily spent on their study treatment during the clinical trial.

The main objective of this study is to identify risk factors for and clinical characteristics of ineligibility due to resistance or negative culture results. Patients are enrolled in a variety of locations, both US sites and international sites, which may produce different results than previous single-site studies. A secondary objective is to investigate the total time required to determine ineligibility in the setting of a clinical trial, which has rarely been examined in the literature. We hope that practitioners can use these risk factors to identify those most likely to require alternative treatment regimens. The knowledge gained by this study will help prioritize the use of more advanced diagnostic tools that are capable of determining the presence of TB infection and drug resistance in a timely manner.

Current Diagnostics

The accuracy and speed of diagnostic tools greatly impact the time required to determine ineligibility for TBTC clinical trials. The current US diagnostic standards endorsed by the American Thoracic Society (ATC) and the Centers for Disease Control and Prevention (CDC) require preliminary smear microscopy, supplemental nucleic acid amplification tests, cultivation of the specimen, identification of the cultured mycobacterial species, and drug susceptibility testing (8). The international recommendations put forth by the WHO currently require only smear microscopy, but aim to eventually use culture or molecular tests to diagnose all cases (1). All laboratories participating in the TBTC Study 28 completed sputum smear microscopy, cultivation on solid and liquid media, identification of mycobacterial species, and drug susceptibility testing for each patient upon enrollment.

Smear microscopy is the first test used to detect acid-fast bacilli (AFB) in sputum specimens from patients with suspected pulmonary TB infections. Because of their cell wall composition, mycobacteria retain dye even after treatment with acid solutions and can be identified microscopically by their stained appearance (8). Results should be available within 24 hours of receipt in the laboratory (9). Smear microscopy is still used in many countries as the primary diagnostic test because of its rapid turnaround, feasibility in low-resource settings, and ability to identify the most advanced and infectious cases (10).

A microbiologically-confirmed diagnosis can only be achieved by culturing the specimen. In addition to increased sensitivity compared to smear microscopy, growth in culture is used to identify the exact mycobacterial species and determine the drug susceptibility profile. The three common types of media are solid egg-based media (Lowenstein-Jensen), solid agar-based media (Middlebrook 7H10 or 7H11), and liquid media (Middlebrook 7H12 and other broths) (8). Because of the slow-growing nature of mycobacteria, culture on solid media can take 3-8 weeks (11, 12). Culture using liquid media is more rapid but growth still takes 1-3 weeks (11, 12). Because growth is detected by turbidity instead of individual colonies, it is more difficult to detect mixed cultures and contamination in liquid media. ATS and CDC recommend that the detection of growth by culture occur within 14 days of specimen collection (9).

Previously, *Mycobacterium tuberculosis* was distinguished from nontuberculous mycobacterium (NTM) using colony morphology and biochemical testing, which required 3-6 additional weeks (13). More recently, molecular tests such as nucleic acid hybridization, high-performance liquid chromatography, restriction fragment length polymorphism analysis, and DNA sequencing have been used to identify species more quickly (13). Nucleic acid hybridization uses molecular probes to identify *M. tuberculosis*, *M. avium* complex, *M. intracellulare*, *M. kansasii*, and *M. gordonae* within several hours. HPLC and RFLP can be used to identify a wider range of species, but equipment costs limit their use. These tests usually require pure cultures and a large number of organisms, so are performed only after the culture has

sufficient growth. The ATS and CDC recommend that identification of cultured mycobacteria be completed within 21 days from the date of specimen collection (9).

Confirmational drug susceptibility testing (DST) can only be done after isolation of pure cultures, but preliminary tests can be performed directly on smear-positive clinical specimens. The two tests used in the US are the agar and radiometric BACTEC proportion methods. Again, both methods require sufficient time for mycobacterial growth, but the liquid BACTEC system produces results more quickly than the solid agar method and can be used to test all four first line drugs (8). However, the cost of liquid media DST is too high for many settings, so the solid media proportion method is still widely used. Drug susceptibility results for first-line drugs should be complete within 30 days from specimen collection, and results for second-line drugs should be available within four weeks from the date requested (9).

In addition to the time required for the completion of laboratory tests, there are often additional delays in clinical settings. In a study conducted in South Korea, the hospital received culture results a median of 20 days after initiation of patient treatment, but clinicians did not receive the results for 37 days (14). During this time, patients could potentially be treated with ineffective antibiotics if the patient has NTM, or unnecessary drugs if the patient does not have TB disease. DST results were received by the hospital within 67 days, while clinicians received the DST results after 80.5 days. Again, the patient could be undergoing inappropriate treatment during this interval if the drug regimen does not properly address any antibiotic resistance. The South Korean study identified areas for improvement in the process, such as automation of requests for tests and direct reporting from the laboratory to the requesting physician. However, because the laboratory was using solid media for culture and DST instead of the more expensive but rapid liquid media, researchers estimated that the interval for receipt of DST results would be no less than eight weeks even with process improvements.

Survival analysis will be used to examine the time required to determine ineligibility for the study due to drug resistance or lack of MTB infection. This will provide insight into whether

the current diagnostics provide the relevant information in accordance with ATS/CDC recommendations for clinical settings.

Drug Resistance and Risk Factors

Drug resistance has become a major obstacle to the control of TB. In addition to single drug resistance, multidrug resistant (MDR-TB) strains and extensively drug-resistant (XDR-TB) strains have emerged worldwide. MDR-TB strains are resistant to at least INH and RIF, and XDR-TB strains are MDR-TB strains that are also resistant to a FQ and at least one of the second-line injectable agents, amikacin, kanamycin and/or capreomycin) (15). WHO estimates that 440,000 new cases and 150,000 deaths due to MDR-TB occurred in 2008. MDR-TB comprises approximately 3.6% of all new TB cases, with the highest proportions recorded in Eastern Europe and Central Asia. In 2008, 963 cases of XDR-TB from 33 countries were reported, but many cases likely go unreported because of limited capacity to test for resistance to second-line drugs (15). Although FQs are not used in standard therapy regimens, they are important second-line drugs and prospective first-line drugs, so resistance is of increasing concern.

It is imperative to detect MDR-TB, XDR-TB, and even monoresistant TB because these cases require appropriate drug regimens to address any resistance. When treated with standard short course chemotherapy, patients with drug resistance are more likely to suffer negative outcomes, such as treatment failure, relapse or recurrence (3-6). Among patients with monoresistance to INH or RIF, up to 70% of treatment failures can develop MDR-TB if treated with the standard drug regimen (6). Delay in initiation of appropriate treatment can also extend transmission of drug resistant strains in the community, contributing to the problems facing TB control (7).

However, early identification of drug resistance and early initiation of an appropriate drug regimen can improve treatment outcomes and help control further transmission (16).

Ideally, all patients suspected of TB infection should be tested for drug resistance, but in limited settings, it may be more feasible to prioritize testing for those with increased risk of resistance. Currently, patients with prior treatment for TB (17-22), contact with an MDR-TB case, and in some settings, HIV infection (23-25) are considered to be at high risk for drug resistance and are targeted for DST (26). Other predictors that have been identified are foreign birth, residence in a correctional facility, Asian race, Hispanic ethnicity, and cavitary disease (18, 20, 21, 24, 27). FQ resistance remains relatively low, but previous FQ therapy for other infections has been shown to be associated with FQ-resistant TB (28, 29). This association is of growing concern because FQs are the most prescribed drug in the US and prescriptions continue to increase (30). Using the described risk factors and any additional predictors identified in this study, laboratories with limited resources may be able to prioritize DST and other more rapid but costly tests to detect drug resistance and identify those that require alternative treatment regimens.

Culture Negativity and Risk Factors

The other group of interest in this study is comprised of those patients who produce a positive sputum smear but for whom *M. tuberculosis* does not grow in culture. The two possibilities for a smear positive, culture negative patient are 1) false positive smear, meaning that the specimen truly does not contain *M. tuberculosis*, or 2) false negative culture, meaning that *M. tuberculosis* is truly in the specimen, but failed to grow (31).

In false positive smear cases, there are several consequences to misdiagnosing patients who do not have TB. These include unnecessary treatment with risk of complications, delay in a correct diagnosis and initiation of proper treatment, and emotional stress for the patient if tuberculosis carries stigma in the community (32). In the clinical trial setting, false positive smear cases result in the waste of medications and financial loss for the clinic and study organization.

False positive smears can result from laboratory errors, such as contamination with acid-fast organisms from the environment or other specimens, or improper staining and decoloration techniques (31, 33). Assessments of laboratory networks have found that such errors are common, and should be addressed with improvements in training and quality control (33). Other organisms such as *Legionella micdadei*, spores of *Bacillus subtilis*, and some yeasts are also acid-fast and can cause false positive smears (32, 34). Because these patients are not infected with TB, it is possible that they can be identified by differences in clinical characteristics and targeted for additional testing.

NTM will also appear as acid-fast bacilli on a sputum smear, but are not differentiated from TB until after the specimen has been cultured. NTM infections require treatment strategies different from that for TB (35), so it is important to identify these patients as early as possible. Several species, such as *M. avium-intracellular complex* strains, *M. kansasii*, *M. gordonae*, and *M. xenopi*, can cause pulmonary disease (35). Clinical presentations may be similar to TB, with chest x-rays showing cavities and infiltrates, but NTM patients are less likely to have systemic symptoms such as fever, night sweats, and weight loss (13, 36). Patient risk factors include underlying lung disease, older age, and immunosuppression due to HIV infection, transplants, diabetes, and other immune disorders (35, 37). NTM infections occur more frequently in developed countries (13), and can comprise a significant portion of suspected TB infections (31, 38). This study aims to contribute to the characterization of those patients most likely to be infected with NTM so that additional testing can be prioritized.

False negative cultures can also consume clinical and study resources if repeat diagnostic tests are required or if treatment regimens are altered unnecessarily. Many false negative cultures result from errors such as improper storage, incorrect specimen processing, and inadequate time to allow the culture to show growth (31). These errors should be addressed with proper training and quality control before considering the use of additional diagnostic tests. Even with proper laboratory protocol, false negative cultures can occur because the sensitivities of each culture

method are not 100% (11, 12). However, the use of liquid media and solid media in combination increases sensitivity to detect MTB to approximately 92-95%, depending on the liquid culture method (12). There are also dormant forms of MTB that are nonculturable on solid media, but these have been shown to be reactivated and culturable in liquid media (39). Because each patient provides multiple specimens that are processed on both solid and liquid media, these types of false negative cultures are expected to be rare in this study.

METHODS

Study Design of Tuberculosis Trials Consortium Study 28

TBTC Study 28 was a phase 2, randomized, double-blind, placebo-controlled trial comparing an experimental regimen of moxifloxacin, RIF, PZA, and EMB to the standard regimen of INH, RIF, PZA, and EMB for the first two months of TB treatment. Study 28 enrolled patients from 26 sites located in Brazil, Canada, South Africa, Uganda, and the US. Patients were eligible for participation if they were at least 18 years of age, had suspected pulmonary TB, and produced a sputum specimen positive for acid fast bacilli (AFB) by smear microscopy. Patients were ineligible for Study 28 if they had received more than seven days of antituberculosis treatment in the previous six months, more than seven days of FQ treatment in the previous three months, or were pregnant or breast-feeding. If baseline culture results were negative for *M. tuberculosis* or showed resistance to INH, RIF, PZA, or FQs, the patient was excluded from further participation in Study 28. The current study focuses on these latter two criteria for ineligibility (40).

Data Source

Information on patient histories, symptoms, and blood chemistries was collected at enrollment. Baseline and biweekly specimens were cultured by local study laboratories during the first two months of treatment. DST was performed on baseline isolates by the local study lab and confirmed at the CDC Mycobacteriology Laboratory using the indirect agar proportion method. Patients with cultures that did not grow TB or who had resistance to INH, RIF, PZA or FQs were deemed ineligible and were discontinued from the study. The focus of this study was to identify factors associated with ineligibility for either negative culture or resistance.

After examination of the study protocol, the Emory Institutional Review Board (IRB) ruled that the current study did not require further review and was declared exempt (Appendix).

Statistical Analysis

We evaluated the differences in the baseline demographic and clinical characteristics between ineligible and eligible patients. Ineligibility due to resistance and ineligibility due to culture-negativity were analyzed separately. In both analyses, race was categorized as white, black, or Asian/Pacific Islander (PI) if the patient was enrolled in the US, and white, black, or Brazilian if the patient was enrolled outside of the US. Native Americans were excluded from analyses regarding race because of the small number of patients (N=6). In the culture-negativity analysis, symptoms of cough, sweats and loss of appetite were grouped together as all are common indicators of pulmonary TB (41). For the resistance analysis, demographic factors such as ethnicity and foreign birth were thought to be more applicable to the US population than international sites, and the study population was stratified by enrollment at a site either in the US or outside the US. The culture negativity analysis was restricted to non-Hispanic patients enrolled at US sites, because negative cultures were only reported among this population.

Preliminary univariate analyses were conducted using Pearson's chi-square, Fisher's exact tests, or Student's t-test. The significance level was set at 5%. Odds ratios were calculated using a continuity correction of 0.5 if cells contained zero observations. Variables with a p-value of 0.20 or less and factors documented in the literature to be associated with outcomes of interest were included in a multivariate logistic regression model. Using backwards selection, variables with a p-value of 0.05 or less retained in the final model. Odds ratios were estimated through logistic regression. Kaplan-Meier survival curves were used to examine the time from enrollment to determination of ineligibility. All analyses were done in SAS version 9.2 (Cary, North Carolina).

RESULTS

There were 433 patients enrolled in TBTC Study 28. One patient was excluded from the current analysis because the local laboratory erroneously reported resistance. Of the 432 subjects, 26 (6%) were found to be ineligible due to resistance, and 16 (4%) were found to be ineligible because cultures were negative for TB (Table 1). Case report forms were complete for all but eight patients. Three patients were missing information on race, two patients were missing information on unemployment status within the past 24 months, and one each for ethnicity, education level, injection drug use within the past year, and excess alcohol use within the past year.

Resistance

Of the resistant isolates, 16 were resistant to INH only, four to FQs only, two to RIF only, and one to PZA only. Three isolates showed multidrug resistance, one to INH and RIF, one to INH, RIF, and PZA, and one to INH, RIF, PZA, and EMB (Table 1).

One hundred forty-eight patients were enrolled at US sites, of which, 14 (9.5%) had some form of resistance. In comparison to patients with pan-sensitive TB, patients with resistance were more likely to be of Hispanic or Latino ethnicity. Presenting with nausea was the only clinical characteristic that was significantly associated with resistance. No significant differences were found in regards to patient background or any other clinical characteristics or symptoms (Table 2). Among the 148 patients enrolled at US sites, Hispanic ethnicity was the only significant predictor of resistance in multivariate analyses [Odds Ratio (OR) = 3.70, 95% Confidence Interval (CI): 1.10-12.42, p-value 0.034) (Table 3). All 10 Hispanic patients with resistant isolates were enrolled at US sites, 4 from sites in Texas and 3 from a site in San Diego. Nine of the 10 were born in countries other than the US (6 from Mexico, and 1 each from Guatemala, El Salvador, and Honduras).

Two hundred eighty-four patients were enrolled in sites outside the US. Of these, 12 (4.2%) had resistant isolates (Table 2). The only variable that possessed any relationship to negative cultures was age. Those with resistant isolates had a mean age of 34.9 (SD 9.3) years and those with susceptible isolates had a mean age of 30.0 (SD 9.5) years. When the population was divided into those younger than 30 years and those aged 30 years or older, age 30 years or older was positively associated with resistance (Table 2), although no patients with resistant isolates were older than 60. In multivariate analysis, the only significant predictor of resistance was age 30 years or older (OR = 4.05, 95% CI: 1.08-15.46, p-value 0.025) (Table 3).

Culture negativity

Sixteen isolates were culture-negative for TB: 2 isolates showed no growth; 9 grew *M. kansasii*; 3 grew *M. avium complex* 1 grew *M. chelonae*; and 1 grew *M. kansasii* and *M. avium complex* (Table 1).

All patients with culture-negative isolates were enrolled at US sites, and none of the 16 was of Hispanic ethnicity. In univariate analysis, race was associated with the culture outcome. Specifically, both black and Asian/PI patients were less likely to be culture-negative than white patients. Loss of appetite was significantly less common among those with negative cultures. The mean age of patients with negative cultures (53.1 years) was significantly higher than those with positive TB cultures (45.6 years) (Table 4). In multivariate analysis, the presence of less than two of the three common TB symptoms (cough, sweats, and loss of appetite) was positively associated with having a culture negative for TB (OR = 5.00, 95% CI: 1.34-18.74, p-value 0.017), while race other than white was negatively associated with having a culture negative for TB (black vs. white: OR = 0.14, 95% CI: 0.03-0.59, p-value 0.0077; Asian/PI vs. white OR = 0.14, 95% CI: 0.02-0.80, p-value 0.027) (Table 5)

Time to determination of ineligibility

The mean time required to determine ineligibility for either reason was 65.4 days (SD 9.8 days) (Figure 1, Table 6), with a range from one day to 246 days. Fifty percent of all ineligible patients had been identified as such within 42.0 days of enrollment. The time to determination of ineligibility was shorter for those with negative cultures than those with resistance (Figure 2). The mean time to identify patients with negative culture was 26.6 days (range 1-57 days), while the mean time to identify patients with resistance was 89.3 days (range 17-246 days).

When stratified by enrollment site in or out of the US, the mean time to identify resistance in patients within the US was 66.9 days (range 17-240 days), whereas it was 115.4 days (range 37-246 days) outside the US (Figure 3). The slope of the survival plot of US patients declines more steeply than the plot for non-US patients in the first 100 days after enrollment, with 50% of US patients identified in 39 days compared to 113 days for non-US patients. However, the slope flattens out for US patients and there is no significant difference in the time to detection of resistance between the two groups (Figure 3, Log Rank p-value = 0.088).

The four patients with resistance to FQ alone were among those with the longest times to determination of resistance (124 days, 189 days, 198 days, and 240 days). In all four cases, FQ resistance was detected through secondary DST performed at the CDC because the local laboratories did not routinely test for FQ resistance. For this reason and because FQs are not currently a first line drug in TB treatment, the times to determination excluding these four patients were thought to be more representative of clinical settings, and are also included in Table 6.

Excluding the patients with FQ resistance, the mean time to determination of resistance was 71.4 days (range 17-246 days) (Table 6, Figure 4). US patients were identified in a mean of 41.5 days (range 17-98 days) while the non-US patients were identified in a mean of 107.2 days. After excluding the four FQ-resistant patients, 50% of US patients were identified within 35 days (range 17-98 days), compared to 102.5 (range 37-246 days) days for non-US patients, and the

difference in the time to determination of resistance in the two groups was statistically significant (Figure 5, Log Rank p-value = 0.0016).

DISCUSSION

In this study, resistance in the US population was predicted only by Hispanic ethnicity in multivariate models. Most of the Hispanic patients were foreign born and many were enrolled in sites that were near the border between the US and Mexico, indicating a higher likelihood of TB exposure within Latin American countries with elevated levels of resistance. No patients with recent TB treatment were enrolled, and few of the enrolled patients had ever undergone previous TB treatment, so these patients were most likely infected with primary resistant strains. These findings support the hypothesis, demonstrated in other studies, that there are higher levels of resistant TB strains circulating in the Hispanic population within the US, especially among those living on the border with Mexico (42-44).

The only predictor of resistance in the study population outside the US was age 30 years or older, but none of the drug resistant patients were older than 60 years, which is consistent with findings that drug resistance is less likely among those aged 65 years and older (20). The elderly were more likely exposed to TB prior to the widespread use of antituberculosis drugs and the emergence of drug-resistant strains. Previous literature is inconsistent on the relationship between age and drug resistance in younger age groups, depending on region and categorization of age (27, 45).

In both populations, there were no significant differences in the clinical presentation of TB disease that could be identified in multivariate models. Nausea was significantly associated with resistance in univariate analyses in the US population, so this may still be a useful tool in identifying those most likely to have resistant isolates. The population studied was largely without previous TB treatment, since patients with treatment for TB in the past six months were excluded from enrollment. This exclusion factor most likely explains the lack of association between previous TB treatment and resistance seen in many other studies. The literature is still inconsistent on the relationship between HIV and drug resistance, and in this study, there was no association present.

Patients with negative cultures were only detected within the US population, reflecting the increase in NTM disease in developed countries (13). There were also no negative cultures among Hispanic/Latino patients, and fewer negative cultures among the black and Asian/Pacific Islander populations. One possible explanation could be that these racial and ethnic groups are more likely to be foreign born, to travel to other countries, or be exposed to populations within the US where TB is present. Non-Hispanic whites may be less likely to have these TB exposures and may be more at risk for NTM infections than other racial and ethnic groups.

Among the US non-Hispanic population, negative TB cultures were also predicted by the presence of less than two of the three symptoms, cough, sweats, and loss of appetite, consistent with previous studies showing that constitutional symptoms such as sweats and loss of appetite are less common in NTM infection than in TB disease (13, 36). Although fever is another typical constitutional symptom of TB, there was no statistical difference in the prevalence of fever between positive and negative TB culture patients. Fever is a common symptom of many infections, while cough, sweats, and loss of appetite seemed to be more unique to those with pulmonary TB. Although not significant in multivariate analyses, increasing age was a predictor of negative culture in univariate analyses, which is consistent with other studies indicating that older age is associated with NTM infection, most likely due to decreased immune capacity.

Overall, the times to determination of ineligibility were found to be fairly high. More than half of ineligible patients had not been identified within a month of enrollment in the study. Those with a negative culture were identified earlier than those with resistance because DST is generally not done until after growth and identification of the organism in culture. Still, it took an average of about 27 days to identify those without TB infection, longer than the ATS/CDC recommended time of 21 days (9).

FQs are not currently used as a first line drug in TB treatment, so many laboratories do not routinely test for FQ susceptibility. For this reason, the statistics including the FQ monoresistant patients were taken to be reflective of the time required to determine the eligibility

status of subjects in the research setting, while the statistics excluding the FQ monoresistant patients were taken to be reflective of the time required to determine the proper treatment regimen for resistant patients in the clinical setting. When including FQ resistance, it took an average of 89 days for resistant isolates to be identified, a very long time for study resources and personnel time to be spent on ineligible patients. Even when excluding FQ resistance, it still took an average of 71 days to identify resistant isolates, much longer than the ATC/CDC recommendation of 30 days (9). In addition to the resources being spent, patients are undergoing treatment regimens that are less than ideal. In the US, half of patients were identified within 35 days, but outside the US, it took 102 days. Patients have already completed the intensive phase of treatment and are well into the continuation phase by 102 days. A few resistant patients were not identified until the entire six month standard course of treatment had been completed.

Reducing the time to identify those patients with resistance and those with infections with organisms other than TB is beneficial both in the research study setting and in the clinical setting. Fewer resources will be wasted and patients will receive appropriate treatment regimens earlier in the course of disease. Traditional solid culture methods can take up to 3-8 weeks, and even the more rapid liquid culture methods can still take 1-3 weeks (11, 12). DST results may not be available for several weeks after detection of TB in culture, resulting in the long times to determination of ineligibility seen in this study. Ideally, all patients could be administered new rapid diagnostic tests to identify TB and drug resistance within a day, but the equipment and tests are often too expensive. A possible alternative could be risk-based screening to prioritize patients who are most likely to be either infected with a drug-resistant TB isolate or to be uninfected with TB.

In addition to predictors of resistance previously identified, such as previous TB treatment, this study indicates that Hispanic ethnicity and possibly nausea could be useful indicators of resistance in the US population. Age 30 years or older was found to be a predictor of resistance in the non-US population, but may not be sufficient as the only criteria to prioritize

patients for testing. Further studies may be needed to help identify risk factors for resistance in non-US sites. When trying to identify those without TB infection, this study demonstrates that patients are more likely to be older non-Hispanic white persons living within the US, and more often have none or only one of the symptoms cough, sweats, and loss of appetite. Patients with these characteristics could be prioritized for additional rapid testing upon enrollment in order to identify ineligible patients in need of alternative therapy in a more cost effective and timely manner.

Strengths and Weaknesses

Because this study was conducted using clinical trial data, we believe the data are very thorough, accurate, and complete. Missing information and data inconsistencies were usually resolved through discussion with onsite personnel prior to data analysis. There is some possibility that recall bias affected questions about patient history or that patients were less likely to report undesirable behaviors, but these effects would probably not differ between eligible and ineligible patients. Although cultures were performed by different laboratories for each site, the culture outcomes and DST should be considered very reliable, since both solid and liquid media were required. Most patients had multiple specimens cultured before being designated as culture negative. All baseline isolates were sent to the CDC to confirm the results of DST.

Patients were enrolled in many sites worldwide, so the findings are applicable to a fairly large population. However, some sites enrolled only a few patients, so site location could only be grouped into two categories: within the US and outside the US, which limited the ability to detect differences between more specific regions. Because patient enrollment was limited to adults with suspected pulmonary TB and positive AFB smears, and without recent treatment for TB or with FQs, the findings should be generalized to this population only. Enrollment also required patient consent, which has the possibility of introducing selection bias, but seems unlikely to differ between eligible and ineligible patients in this study.

The study may also have lacked the statistical power to detect some associations. TBTC is in the process of conducting Study 29, which has a similar protocol and exclusion criteria. Further studies could benefit from combining the study populations of both Study 28 and Study 29.

Future Directions

Future research should also include evaluation of the literature regarding new rapid diagnostic tests that can reduce the time required to identify TB infection and drug resistance. One possibility is the Xpert MTB/RIF, which has recently been endorsed by the WHO for global implementation (46) after it was shown to be useful in clinical settings for rapidly identifying TB and RIF resistance (47). However, a more extensive susceptibility profile is required for the research study criteria, since many of the ineligible cases showed resistance to other drugs. Additional research is needed to determine the best test to implement for supplementary rapid testing, but this study provides some basis for selecting which patients should undergo the chosen test. The identified risk factors could be incorporated into a screening algorithm to be tested and evaluated in a programmatic setting. Such research should evaluate the accuracy and cost-effectiveness of using such a strategy to help guide future plans in the roll-out and implementation of TB rapid diagnostic testing.

REFERENCES

1. World Health Organization. Global tuberculosis control: WHO report 2010. Geneva: World Health Organization; 2010.
2. World Health Organization. Stop TB Dept. Treatment of tuberculosis: guidelines. 4th edition. ed. Geneva: World Health Organization; 2010.
3. Espinal MA, Kim SJ, Suarez PG, et al. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. *JAMA* 2000;283(19):2537-45.
4. Migliori GB, Espinal M, Danilova ID, Punga VV, Grzemska M, Raviglione MC. Frequency of recurrence among MDR-tB cases 'successfully' treated with standardised short-course chemotherapy. *Int J Tuberc Lung Dis* 2002;6(10):858-64.
5. Lew W, Pai M, Oxlade O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. *Ann Intern Med* 2008;149(2):123-34.
6. Seung KJ, Gelmanova IE, Peremitin GG, et al. The effect of initial drug resistance on treatment response and acquired drug resistance during standardized short-course chemotherapy for tuberculosis. *Clin Infect Dis* 2004;39(9):1321-8.
7. Uys PW, Warren R, van Helden PD, Murray M, Victor TC. Potential of rapid diagnosis for controlling drug-susceptible and drug-resistant tuberculosis in communities where Mycobacterium tuberculosis infections are highly prevalent. *J Clin Microbiol* 2009;47(5):1484-90.
8. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med* 2000;161(4 Pt 1):1376-95.

9. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. *Am J Respir Crit Care Med* 2005;172(9):1169-227.
10. Hopewell PC, Pai M, Maher D, Uplekar M, Raviglione MC. International standards for tuberculosis care. *Lancet Infect Dis* 2006;6(11):710-25.
11. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *J Clin Microbiol* 2004;42(5):2321-5.
12. Sorlozano A, Soria I, Roman J, et al. Comparative evaluation of three culture methods for the isolation of mycobacteria from clinical samples. *J Microbiol Biotechnol* 2009;19(10):1259-64.
13. Field SK, Cowie RL. Lung disease due to the more common nontuberculous mycobacteria. *Chest* 2006;129(6):1653-72.
14. Joh JS, Lee CH, Lee JE, et al. The interval between initiation of anti-tuberculosis treatment in patients with culture-positive pulmonary tuberculosis and receipt of drug-susceptibility test results. *J Korean Med Sci* 2007;22(1):26-9.
15. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB) : 2010 global report on surveillance and response. Geneva: World Health Organization; 2010.
16. Mitnick C, Bayona J, Palacios E, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. *N Engl J Med* 2003;348(2):119-28.
17. Cattamanchi A, Dantes RB, Metcalfe JZ, et al. Clinical characteristics and treatment outcomes of patients with isoniazid-monoresistant tuberculosis. *Clin Infect Dis* 2009;48(2):179-85.
18. Hoopes AJ, Kammerer JS, Harrington TA, Ijaz K, Armstrong LR. Isoniazid-monoresistant tuberculosis in the United States, 1993 to 2003. *Arch Intern Med* 2008;168(18):1984-92.

19. Melzer M, Gupta N, Petersen I, Cook S, Hall B. Previous treatment in predicting drug-resistant tuberculosis in an area bordering East London, UK. *Int J Infect Dis* 2010;14(8):e717-22.
20. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax* 2006;61(2):158-63.
21. Espinal MA, Laserson K, Camacho M, et al. Determinants of drug-resistant tuberculosis: analysis of 11 countries. *Int J Tuberc Lung Dis* 2001;5(10):887-93.
22. Andrews JR, Shah NS, Weissman D, Moll AP, Friedland G, Gandhi NR. Predictors of multidrug- and extensively drug-resistant tuberculosis in a high HIV prevalence community. *PLoS One* 2010;5(12):e15735.
23. Suchindran S, Brouwer ES, Van Rie A. Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review. *PLoS One* 2009;4(5):e5561.
24. Sandman L, Schluger NW, Davidow AL, Bonk S. Risk factors for rifampin-monoresistant tuberculosis: A case-control study. *Am J Respir Crit Care Med* 1999;159(2):468-72.
25. Li J, Munsiff SS, Driver CR, Sackoff J. Relapse and acquired rifampin resistance in HIV-infected patients with tuberculosis treated with rifampin- or rifabutin-based regimens in New York City, 1997-2000. *Clin Infect Dis* 2005;41(1):83-91.
26. World Health Organization. Multidrug-resistant tuberculosis (MDR-TB) indicators: a minimum set of indicators for the programmatic management of MDR-TB in national tuberculosis control programmes. Geneva: World Health Organization; 2010.
27. Moniruzzaman A, Elwood RK, Schulzer M, FitzGerald JM. A population-based study of risk factors for drug-resistant TB in British Columbia. *Int J Tuberc Lung Dis* 2006;10(6):631-8.
28. Ginsburg AS, Hooper N, Parrish N, et al. Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. *Clin Infect Dis* 2003;37(11):1448-52.

29. Devasia RA, Blackman A, Gebretsadik T, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis: the effect of duration and timing of fluoroquinolone exposure. *Am J Respir Crit Care Med* 2009;180(4):365-70.

30. Linder JA, Huang ES, Steinman MA, Gonzales R, Stafford RS. Fluoroquinolone prescribing in the United States: 1995 to 2002. *Am J Med* 2005;118(3):259-68.

31. Lipsky BA, Gates J, Tenover FC, Plorde JJ. Factors affecting the clinical value of microscopy for acid-fast bacilli. *Rev Infect Dis* 1984;6(2):214-22.

32. Toman K, Frieden TR, World Health Organization. Toman's tuberculosis : case detection, treatment, and monitoring : questions and answers. 2nd ed. Geneva: World Health Organization; 2004.

33. Kusznierz GF, Latini OA, Sequeira MD. Quality assessment of smear microscopy for acid-fast bacilli in the Argentine tuberculosis laboratory network, 1983-2001. *Int J Tuberc Lung Dis* 2004;8(10):1234-41.

34. Hilton E, Freedman RA, Cintron F, Isenberg HD, Singer C. Acid-fast bacilli in sputum: a case of Legionella micdadei pneumonia. *J Clin Microbiol* 1986;24(6):1102-3.

35. Katoch VM. Infections due to non-tuberculous mycobacteria (NTM). *Indian J Med Res* 2004;120(4):290-304.

36. Kendall BA, Varley CD, Choi D, et al. Distinguishing tuberculosis from nontuberculous mycobacteria lung disease, Oregon, USA. *Emerg Infect Dis* 2011;17(3):506-9.

37. McGrath EE, Blades Z, McCabe J, Jarry H, Anderson PB. Nontuberculous mycobacteria and the lung: from suspicion to treatment. *Lung* 2010;188(4):269-82.

38. Lee JS, Kim EC, Joo SI, et al. The incidence and clinical implication of sputum with positive acid-fast bacilli smear but negative in mycobacterial culture in a tertiary referral hospital in South Korea. *J Korean Med Sci* 2008;23(5):767-71.

39. Shleeva MO, Mukamolova GV, Telkov MV, et al. [Formation of nonculturable Mycobacterium tuberculosis and their regeneration]. *Mikrobiologiia* 2003;72(1):76-83.

40. Dorman SE, Johnson JL, Goldberg S, et al. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. *Am J Respir Crit Care Med* 2009;180(3):273-80.

41. Centers for Disease Control and Prevention. Signs and Symptoms of TB Disease. (http://www.cdc.gov/tb/topic/basics/signsandsymptoms.htm). (Accessed 1 April 2011).

42. Taylor JP, Suarez L. Prevalence and risk factors of drug-resistant tuberculosis along the Mexico-Texas border. *Am J Public Health* 2000;90(2):271-3.

43. Schneider E, Laserson KF, Wells CD, Moore M. Tuberculosis along the United States-Mexico border, 1993-2001. *Rev Panam Salud Publica* 2004;16(1):23-34.

44. El Sahly HM, Teeter LD, Pawlak RR, Musser JM, Graviss EA. Drug-resistant tuberculosis: a disease of target populations in Houston, Texas. *J Infect* 2006;53(1):5-11.

45. Green E, Obi CL, Nchabeleng M, et al. Drug-susceptibility patterns of Mycobacterium tuberculosis in Mpumalanga province, South Africa: possible guiding design of retreatment regimen. *J Health Popul Nutr* 2010;28(1):7-13.

46. World Health Organization. WHO Endorses New Rapid Tuberculosis Test. (http://www.who.int/tb/strategy/en/). (Accessed 15 February 2011).

47. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010;363(11):1005-15.

TABLES

Table 1: Summary of Ineligible Patients for Tuberculosis Trials Consortium Study 28

	Number (n = 432)	**Percent**
Total ineligible due to resistance	**26**	**6.02%**
Isoniazid monoresistant	16	3.70%
Fluoroquinolone monoresistant	4	0.93%
Rifampin monoresistant	2	0.46%
Pyrazinamide monoresistant	1	0.23%
Multidrug resistant	3	0.69%
Total ineligible due to negative TB culture	**16**	**3.70%**
No growth on culture media	2	0.46%
M. kansasii	9	2.08%
M. avium complex	3	0.69%
M. chelonae	1	0.23%
M.kansasii and *M. avium complex*	1	0.23%

TB = tuberculosis
Multidrug resistant: Resistant to at least isoniazid and rifampin, with or without resistance to other drugs

Table 2: Univariate analyses for patients with resistant isolates, by site location

	US Sites (n = 148)				Non-US Sites (n = 284)			
	Resistant (n = 14)	**Susceptible (n = 134)**	**Odds Ratio (95% CI)**	**p-value**	**Resistant (n = 12)**	**Susceptible (n = 272)**	**Odds Ratio (95% CI)**	**p-value**
Demographic Factors								
Female	5 (36%)	42 (31%)	1.21 (0.38-3.85)	0.77	3 (25%)	72 (26%)	0.93 (0.24-3.52)	1.00
Completed high school	6 (43%)	75 (56%)	0.59 (0.19-1.79)	0.35	3 (25%)	52 (20%)	1.40 (0.37-5.37)	0.71
Foreign born	12 (86%)	81 (60%)	3.92 (0.84-18.25)	0.063	1 (8%)	10 (4%)	2.38 (0.28-20.29)	0.38
Hispanic/Latino ethnicity	10 (71%)	54 (40%)	3.70 (1.10-12.42)	0.025	0 (0%)	3 (1%)	3.07 (0.15-62.69)	1.00
Age mean (SD)	40.8 (15.0)	43.7 (14.6)		0.48	34.9 (9.3)	30.0 (9.5)		0.08
Age ≥ 30 years					9 (75%)	115 (42%)	4.05 (1.08-15.46)	0.025
Race (Overall Type 3 p-value)[†]				0.47				0.31
White	10 (71%)	70 (52%)	1[‡]		1 (8%)	17 (6%)	1[‡]	
Black	2 (14%)	36 (27%)	0.39 (0.08-1.87)		9 (75%)	236 (87%)	0.65 (0.08-5.42)	
Asian/Pacific Islander	2 (14%)	22 (18%)	0.64 (0.13-3.13)		-	-	-	
Brazilian	-	-	-		2 (17%)	15 (6%)	2.27 (0.19-27.58)	
Patient Background								
Homeless in past year	0 (0%)	14 (10%)	0.29 (0.02-5.06)	0.36	0 (0%)	3 (1%)	3.08 (0.15-62.92)	1.00
Unemployed in past two years	3 (21%)	39 (29%)	0.66 (0.17-2.49)	0.76	3 (25%)	54 (20%)	1.34 (0.35-5.12)	0.72
Non-injecting drug use in past year	1 (7%)	24 (18%)	0.35 (0.04-2.83)	0.47	1 (8%)	13 (5%)	1.81 (0.22-15.11)	0.46
Excess alcohol use in past year	1 (7%)	32 (24%)	0.25 (0.03-1.95)	0.19	1 (8%)	21 (8%)	1.08 (0.13-8.79)	1.00
History of cigarette smoking	6 (43%)	86 (64%)	0.42 (0.14-1.28)	0.12	5 (42%)	84 (31%)	1.60 (0.49-5.18)	0.53
Previous TB treatment	1 (7%)	6 (5%)	1.64 (0.18-14.70)	0.51	0 (0%)	5 (2%)	1.95 (0.10-37.17)	1.00
Clinical Factors								
HIV positive	2 (14%)	8 (6%)	2.63 (0.50-13.79)	0.24	2 (17%)	37 (14%)	1.27 (0.27-6.03)	0.67

Extrapulmonary TB	0 (0%)	3 (2%)	1.30 (0.06-26.35)	1.00	1 (8%)	32 (12%)	0.68 (0.08-5.46)	1.00
Concomitant diagnoses	6 (43%)	54 (40%)	1.11 (0.36-3.38)	1.00	10 (83%)	229 (84%)	0.94 (0.20-4.44)	1.00
Liver disease	0 (0%)	7 (5%)	0.59 (0.03-10.80)	1.00	0 (0%)	3 (1%)	3.08 (0.15-62.92)	1.00
Diabetes	3 (21%)	20 (15%)	1.55 (0.40-6.07)	0.46	0 (0%)	6 (2%)	1.64 (0.09-30.77)	1.00
Chest X-ray								
Cavitation	10 (71%)	101 (75%)	0.82 (0.24-2.78)	0.75	10 (83%)	198 (73%)	1.87 (0.40-8.73)	0.52
Infilitrates	14 (100%)	125 (93%)	2.20 (0.12-39.71)	1.00	12 (100%)	269 (99%)	0.32 (0.02-6.63)	1.00
Adenopathy	1 (7%)	13 (10%)	0.72 (0.09-5.92)	1.00	0 (0%)	13 (5%)	0.66 (0.04-13.69)	1.00
Pleural disease	4 (29%)	38 (28%)	1.01 (0.30-3.42)	1.00	1 (8%)	27 (10%)	0.82 (0.10-6.64)	1.00
Symptoms								
Fever	7 (50%)	65 (49%)	1.06 (0.35-3.19)	0.92	7 (58%)	168 (62%)	0.87 (0.27-2.80)	1.00
Sweats	8 (57%)	70 (52%)	1.22 (0.40-3.70)	0.73	8 (67%)	176 (65%)	1.09 (0.32-3.72)	1.00
Cough	12 (86%)	126 (94%)	0.38 (0.07-2.00)	0.24	12 (100%)	270 (99%)	0.23 (0.01-5.07)	1.00
Rash	0 (0%)	6 (5%)	0.68 (0.04-12.63)	1.00	0 (0%)	6 (2%)	1.64 (0.09-30.77)	1.00
Itching	0 (0%)	11 (8%)	0.37 (0.02-6.62)	0.60	0 (0%)	11 (4%)	0.91 (0.05-16.33)	1.00
Nausea	4 (29%)	10 (7%)	4.96 (1.32-18.69)	0.029	0 (0%)	10 (4%)	1.00 (0.05-18.05)	1.00
Vomiting	2 (14%)	12 (9%)	1.69 (0.34-8.48)	0.62	1 (8%)	22 (8%)	1.03 (0.13-8.38)	1.00
Diarrhea	1 (7%)	12 (9%)	0.78 (0.09-6.51)	1.00	1 (8%)	17 (6%)	1.36 (0.17-11.19)	0.55
Loss of appetite	5 (36%)	53 (40%)	0.84 (0.27-2.64)	0.76	5 (42%)	112 (41%)	1.02 (0.32-3.30)	1.00
Altered taste	1 (7%)	19 (14%)	0.47 (0.06-3.77)	0.69	1 (8%)	14 (5%)	1.68 (0.20-13.91)	0.49
Vision problems	2 (14%)	17 (13%)	1.15 (0.24-5.57)	1.00	0 (0%)	10 (4%)	0.99 (0.06-17.91)	1.00
Numbness/tingling in extremities	2 (14%)	15 (11%)	1.32 (0.27-6.49)	0.66	1 (8%)	14 (5%)	1.68 (0.20-13.91)	0.49
Headache	3 (21%)	23 (17%)	1.32 (0.34-5.09)	0.71	2 (17%)	23 (8%)	2.17 (0.45-10.48)	0.29
Dizziness	4 (29%)	16 (12%)	2.95 (0.83-10.52)	0.10	1 (8%)	19 (9%)	1.21 (0.15-9.88)	0.59
Insomnia	2 (14%)	26 (19%)	0.69 (0.15-3.28)	1.00	0 (0%)	18 (7%)	0.55 (0.03-9.66)	1.00
Joint pain	1 (7%)	21 (16%)	0.41 (0.05-3.34)	0.69	0 (0%)	29 (11%)	0.33 (0.02-5.72)	0.62

CI = confidence interval; SD = standard deviation; TB = tuberculosis

[†]Race categorized as white, black, or Asian/Pacific Islander in the US, and white, black, or Brazilian outside the US. Two participants that did not report race and six patients reporting Native American race were excluded from analyses regarding race because of small numbers.

[‡]Reference category for calculating odds ratios.

Table 3: Significant predictors of resistance in multivariate logistic regression, by site location

	Odds Ratio (95% CI)	**p-value**
US Sites (n = 148)		
Hispanic/Latino ethnicity	3.70 (1.10-12.42)	0.025
Non-US Sites (n = 284)		
Age ≥ 30 years	4.05 (1.08-15.46)	0.025

CI: confidence interval

Table 4: Univariate analyses for patients with cultures that were negative for tuberculosis, all patients and US non-Hispanic patients only

	All Patients (n = 432)				US, Non-Hispanic Patients Only (n = 84)			
	Culture Negative for TB (n = 16)	**Culture Positive for TB (n = 416)**	**Odds Ratio (95% CI)**	**p-value**	**Culture Negative for TB (n = 16)**	**Culture Positive for TB (n = 68)**	**Odds Ratio (95% CI)**	**p-value**
Demographic Factors								
Female	5 (31%)	117 (28%)	1.16 (0.40-3.42)	0.78	5 (31%)	21 (31%)	1.02 (0.31-3.30)	1.00
Completed high school	13 (81%)	123 (30%)	10.29 (2.88-36.74)	<0.0001	13 (81%)	51 (75%)	1.44 (0.37-5.69)	0.75
Enrolled at US site	16 (100%)	132 (32%)	70.86 (4.22-1189.98)	<0.0001	-	-	-	-
Hispanic/Latino	0 (0%)	67 (16%)	0.16 (0.01-2.64)	0.15	-	-	-	-
Foreign born	4 (25%)	100 (24%)	1.05 (0.33-3.34)	1.00	4 (25%)	33 (49%)	0.35 (0.10-1.21)	0.088
Age mean (SD)	53 (10.0)	34.0 (12.7)		<0.0001	53.1 (17.7)	45.6 (13.6)		0.04
Race (overall Type 3 p-value) [†]				0.0024				0.014
White	9 (56%)	89 (22%)	1[‡]		9 (56%)	12 (18%)	1[‡]	
Black	4 (25%)	279 (69%)	0.14 (0.04-0.47)		4 (25%)	32 (49%)	0.17 (0.04-0.64)	
Asian/Pacific Islander	3 (19%)	22 (5%)	1.35 (0.34-5.4)		3 (19%)	21 (32%)	0.19 (0.04-0.84)	
Patient Background								
Homeless in past year	2 (13%)	15 (4%)	3.82 (0.80-18.33)	0.13	2 (13%)	10 (15%)	0.83 (0.16-4.21)	1.00
Unemployed in past two years	3 (20%)	96 (23%)	0.83 (0.23-3.00)	1.00	3 (20%)	26 (38%)	0.40 (0.10-1.57)	0.18
Non-injecting drugs in past year	1 (6%)	38 (9%)	0.66 (0.09-5.16)	1.00	1 (6%)	14 (21%)	0.23 (0.03-2.12)	0.28
Excess alcohol use in past year	2 (13%)	53 (13%)	0.98 (0.22-4.41)	1.00	2 (13%)	19 (28%)	0.37 (0.08-1.78)	0.34
History of cigarette smoking	14 (88%)	167 (40%)	10.44 (2.34-46.52)	0.0002	14 (88%)	49 (72%)	2.71 (0.56-13.09)	0.34
Previous TB treatment	1 (6%)	11 (3%)	2.45 (0.30-20.27)	0.37	1 (6%)	3 (4%)	1.44 (0.14-14.87)	0.58
Clinical Factors	3 (19%)	46 (11%)	1.86 (0.51-6.76)	0.41				
HIV positive	0 (0%)	36 (9%)	0.32 (0.02-5.37)	0.38	3 (19%)	4 (6%)	3.69 (0.74-18.50)	0.12

Extrapulmonary TB	3 (19%)	296 (71%)	0.09 (0.03-0.33)	<0.0001	0 (0%)	1 (1%)	1.36 (0.05-35.01)	1.00
Concomitant Diagnoses	0 (0%)	10 (2%)	1.17 (0.07-20.89)	1.00	3 (19%)	26 (38%)	0.37 (0.10-1.43)	0.14
Liver disease	0 (0%)	29 (7%)	0.40 (0.02-6.80)	0.61	0 (0%)	5 (7%)	0.35 (0.02-6.65)	0.58
Diabetes					0 (0%)	9 (13%)	0.19 (0.01-3.43)	0.20
Chest X-ray	9 (56%)	310 (75%)	0.44 (0.16-1.21)	0.14				
Cavitation	14 (88%)	406 (98%)	0.17 (0.03-0.86)	0.068	9 (56%)	55 (81%)	0.31 (0.10-0.97)	0.052
Infilitrates	2 (13%)	25 (6%)	2.23 (0.48-10.38)	0.26	14 (88%)	62 (91%)	0.68 (0.12-3.72)	0.64
Adenopathy	6 (38%)	64 (15%)	3.30 (1.16-9.40)	0.031	2 (13%)	7 (10%)	1.24 (0.23-6.65)	0.68
Pleural disease					6 (38%)	14 (21%)	2.31 (0.72-7.46)	0.19
Symptoms	6 (37%)	241 (58%)	0.44 (0.16-1.22)	0.11				
Fever	5 (31%)	257 (62%)	0.28 (0.10-0.82)	0.014	6 (38%)	32 (47%)	0.68 (0.22-2.07)	0.49
Sweats	14 (88%)	406 (98%)	0.17 (0.03-0.86)	0.068	5 (31%)	37 (54%)	0.38 (0.12-1.21)	0.10
Cough	1 (6%)	11 (3%)	2.45 (0.30-20.22)	0.37	14 (88%)	66 (97%)	0.21 (0.03-1.64)	0.16
Rash	1 (6%)	21 (5%)	1.25 (0.16-9.95)	0.57	1 (6%)	1 (1%)	4.47 (0.26-75.52)	0.35
Itching	1 (6%)	23 (6%)	1.14 (0.14-9.00)	0.61	1 (6%)	5 (7%)	0.84 (0.09-7.73)	1.00
Nausea	0 (0%)	37 (9%)	0.31 (0.02-5.21)	0.38	1 (6%)	3 (4%)	1.44 (0.14-14.87)	0.58
Vomiting	1 (6%)	30 (7%)	0.86 (0.11-6.72)	1.00	0 (0%)	3 (4%)	0.57 (0.03-11.53)	1.00
Diarrhea	2 (13%)	173 (42%)	0.20 (0.04-0.89)	0.02	1 (6%)	4 (6%)	1.07 (0.11-10.25)	1.00
Loss of appetite	4 (25%)	31 (7%)	4.14 (1.26-13.60)	0.033	2 (13%)	29 (43%)	0.19 (0.04-0.91)	0.025
Altered taste	2 (13%)	27 (7%)	2.05 (0.44-9.48)	0.29	4 (25%)	7 (11%)	2.90 (0.73-11.50)	0.21
Vision problems	1 (6%)	31 (7%)	0.83 (0.11-6.48)	1.00	2 (13%)	7 (10%)	1.24 (0.23-6.65)	0.68
Numbness/tingling	1 (6%)	50 (12%)	0.49 (0.06-3.77)	0.71	1 (6%)	10 (15%)	0.39 (0.05-3.26)	0.68
Headache	1 (6%)	39 (9%)	0.64 (0.08-5.01)	1.00	1 (6%)	11 (16%)	0.35 (0.04-2.89)	0.45
Dizziness	1 (6%)	45 (11%)	0.55 (0.07-4.26)	1.00	1 (6%)	11 (16%)	0.35 (0.04-2.89)	0.45
Insomnia	1 (6%)	50 (12%)	0.49 (0.06-3.77)	0.71	1 (6%)	15 (22%)	0.34 (0.03-1.93)	0.29
Joint pain					1 (6%)	10 (15%)	0.39 (0.05-3.26)	0.68

Combination of symptoms: cough, sweat, loss of appetite					
None of the three		2 (13%)	2 (3%)	4.71 (0.61-36.37)	0.16
Less than two of the three		11 (69%)	20 (29%)	5.28 (1.62-17.16)	0.0033
Less than three of the three		14 (88%)	50 (74%)	2.52 (0.52-12.19)	0.34

CI = confidence interval; SD = standard deviation; TB = tuberculosis

† Two participants that did not report race and six patients reporting Native American race were excluded from analyses regarding race because of small numbers.

‡Reference category for calculating odds ratios.

Table 5: Significant predictors of negative culture in multivariate logistic regression

US non-Hispanics (n = 84)	Odds Ratio (95% CI)	p-value
Less than two of three symptoms (cough, sweats, loss of appetite)	5.00 (1.34-18.74)	0.017
Race (overall Type 3 p-value)		0.012
Blacks compared to whites	0.14 (0.03-0.59)	0.0077
Asians/PIs compared to whites	0.14 (0.02-0.80)	0.027

CI: confidence interval; PI: Pacific Islander

Table 6: Times to determination of ineligibility

	All Ineligible Patients					Excluding FQ Monoresistant Patients				
	Mean (Days)	**SD (Days)**	**Median (Days)**	**Range (Days)**	**Log Rank p-value**	**Mean (Days)**	**SD (Days)**	**Median (Days)**	**Range (Days)**	**Log Rank p-value**
All ineligible	65.4	9.8	42.0	1-246		52.5	8.1	37.0	1-246	
Reason for Ineligibility										
Negative Culture	26.6	4.5	25.5	1-57	<0.0001	26.6	4.5	25.5	1-57	0.0004
Resistance	89.3	14.7	57.5	17-246		71.4	12.2	50.0	17-246	
Resistant Patients										
US sites	66.9	18.2	39.0	17-240	0.088	41.5	6.5	35.0	17-98	0.0016
Non-US	115.4	18.6	113.0	37-246		107.2	20.9	102.5	37-246	

SD: standard deviation

FIGURES

Figure 1: Times to determination of ineligibility for all ineligible patients

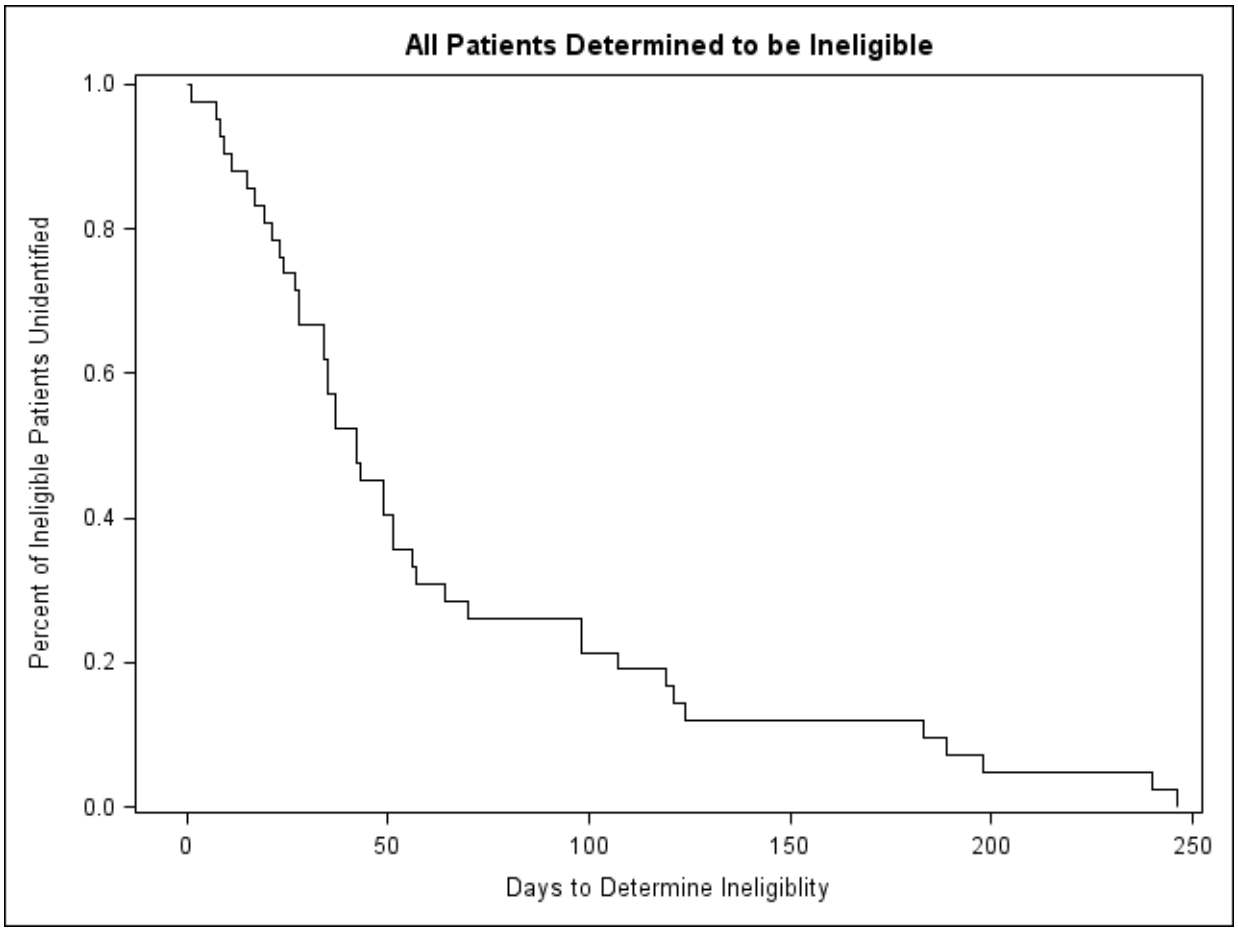


Figure 2: Times to determination of ineligibility by reason: culture-negative for tuberculosis or drug resistant

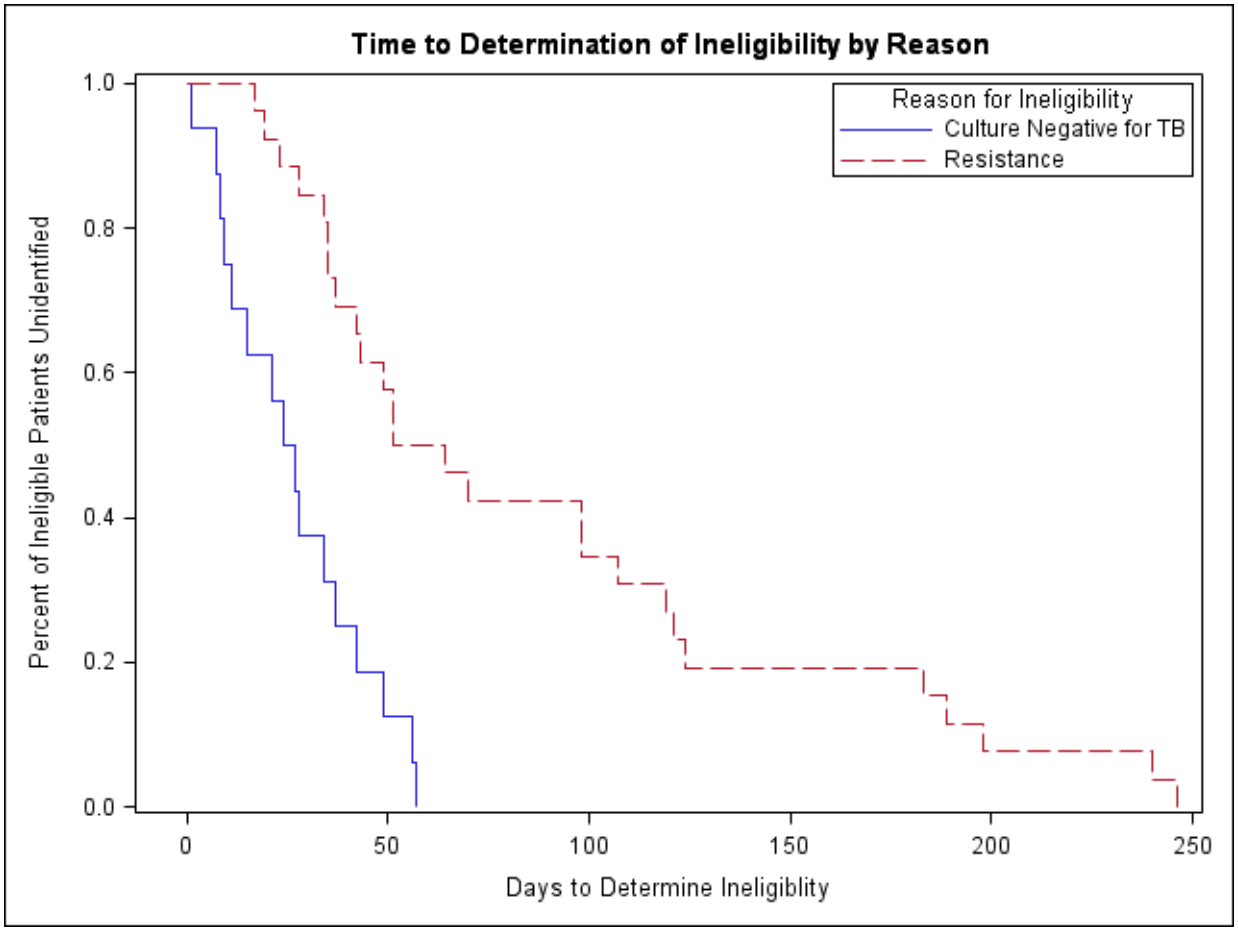


Figure 3: Times to determination of resistance by site location

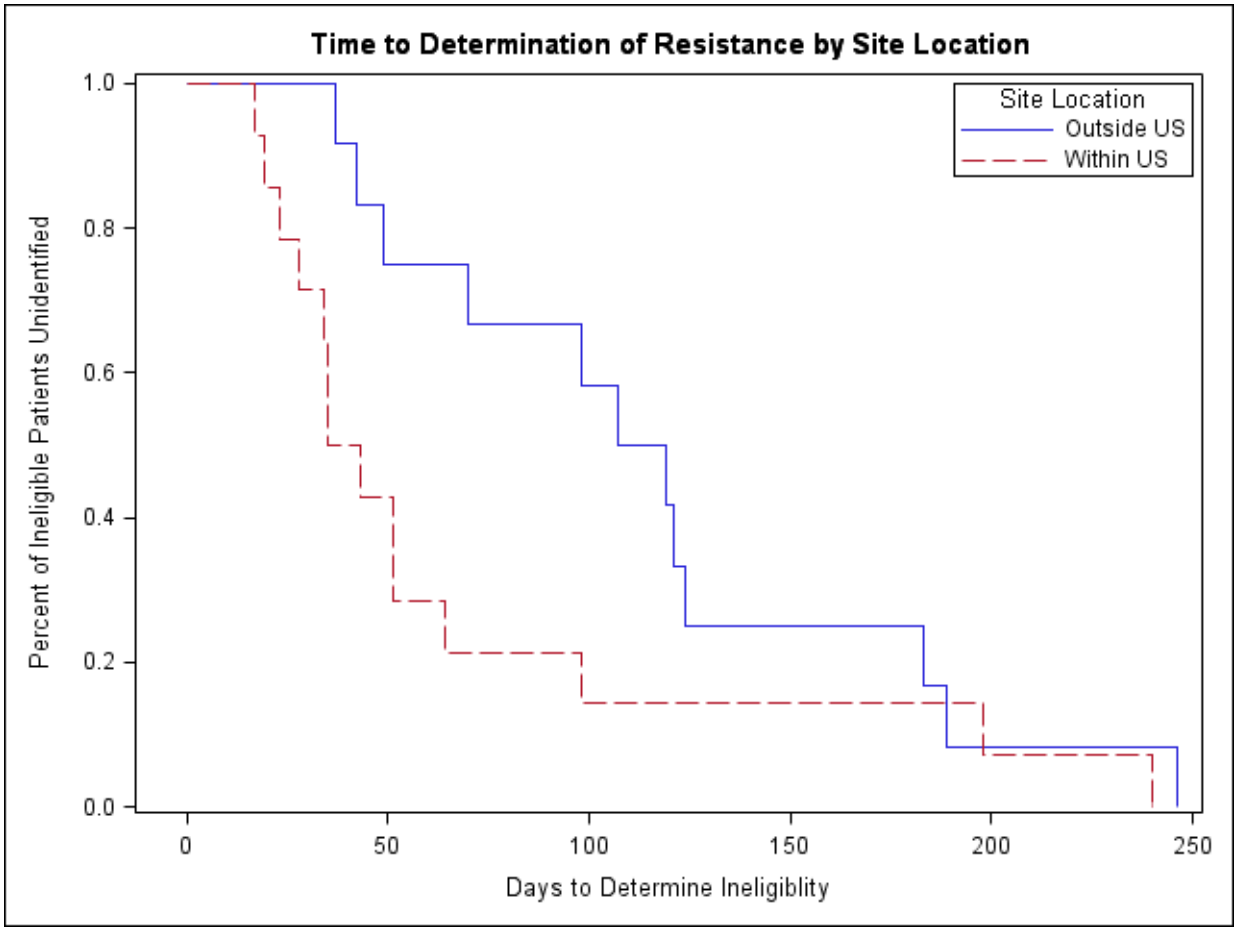


Figure 4: Times to determination of ineligibility for all ineligible patients, excluding those with fluoroquinolone monoresistance

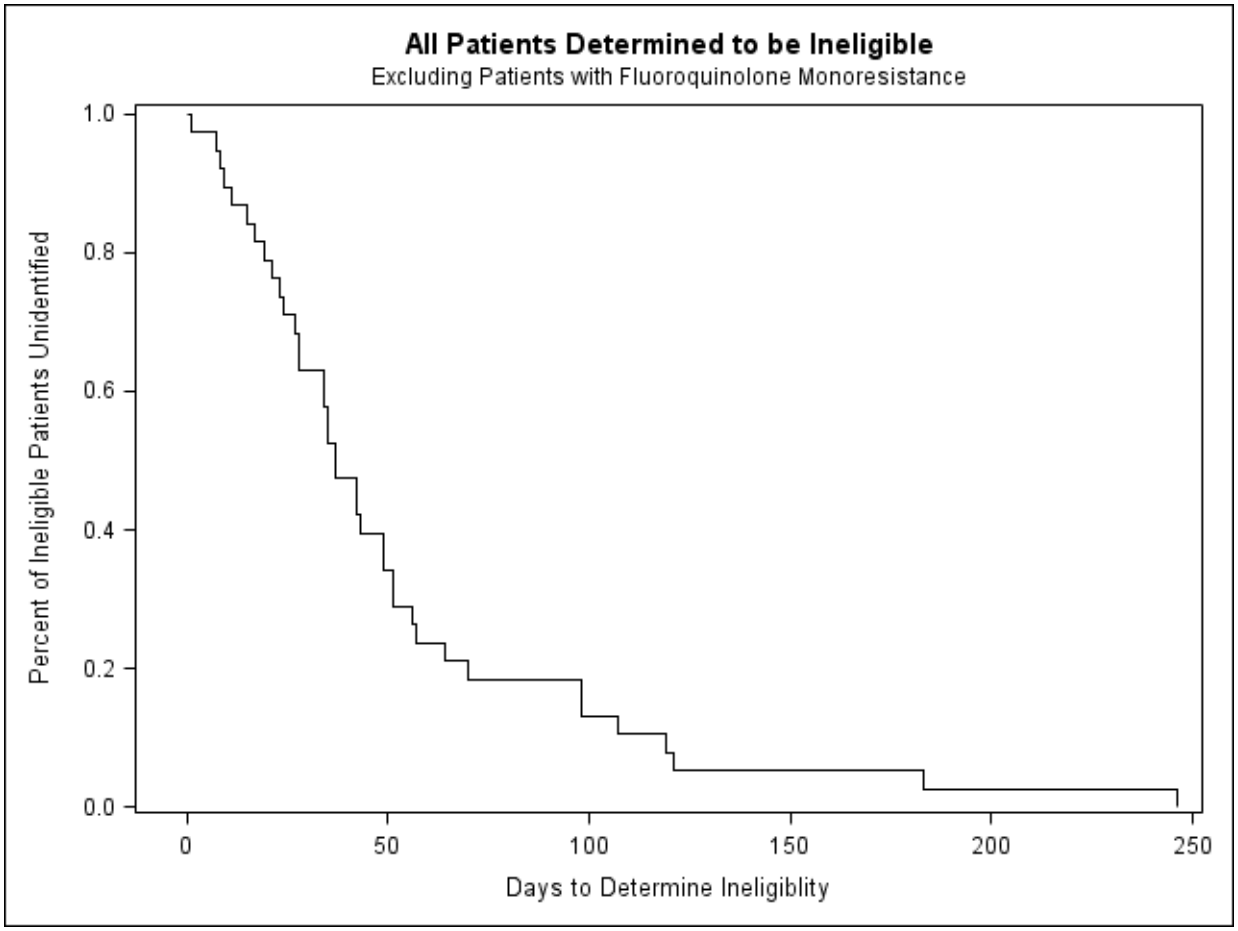


Figure 5: Times to determination of ineligibility by reason, excluding those with fluoroquinolone monoresistance

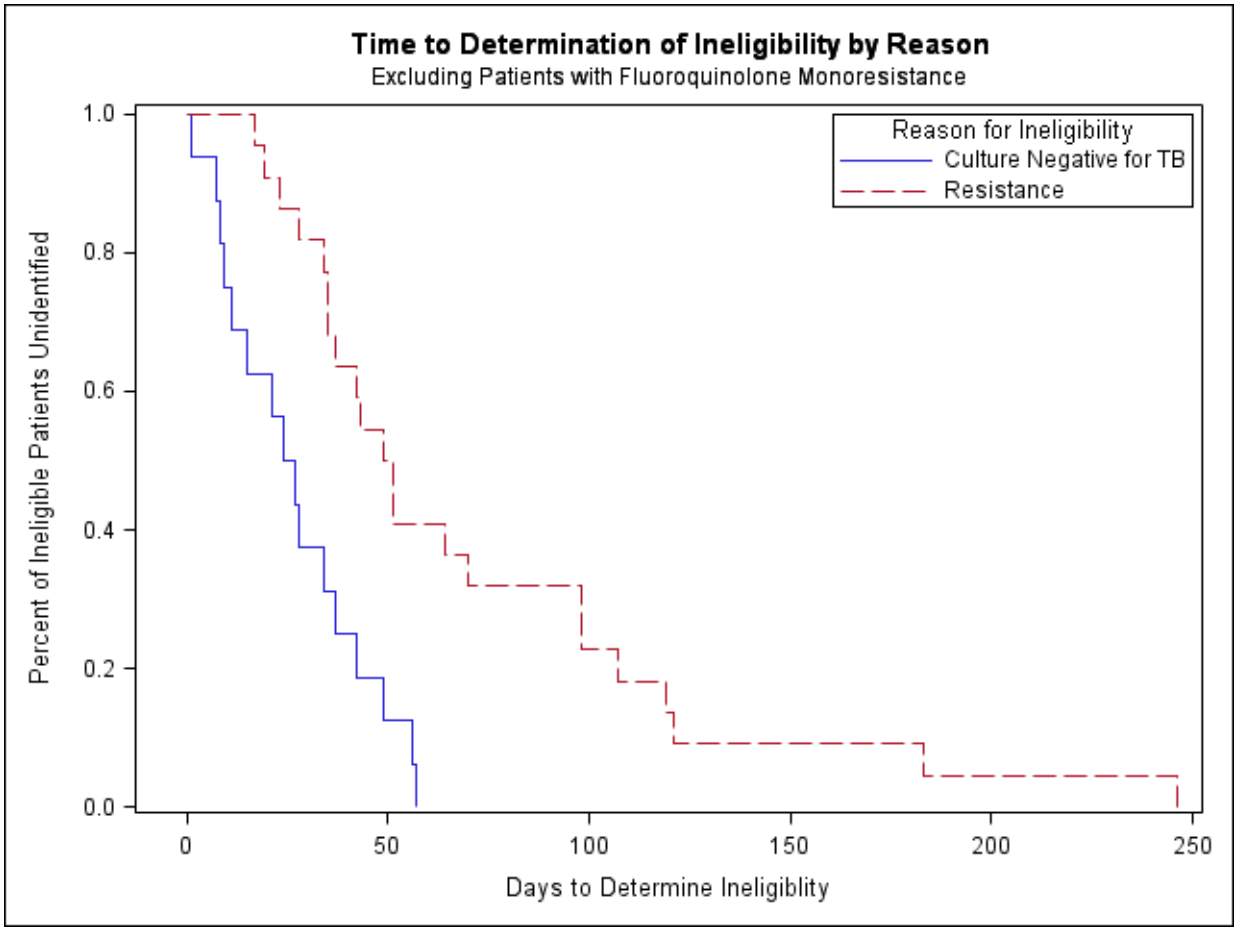
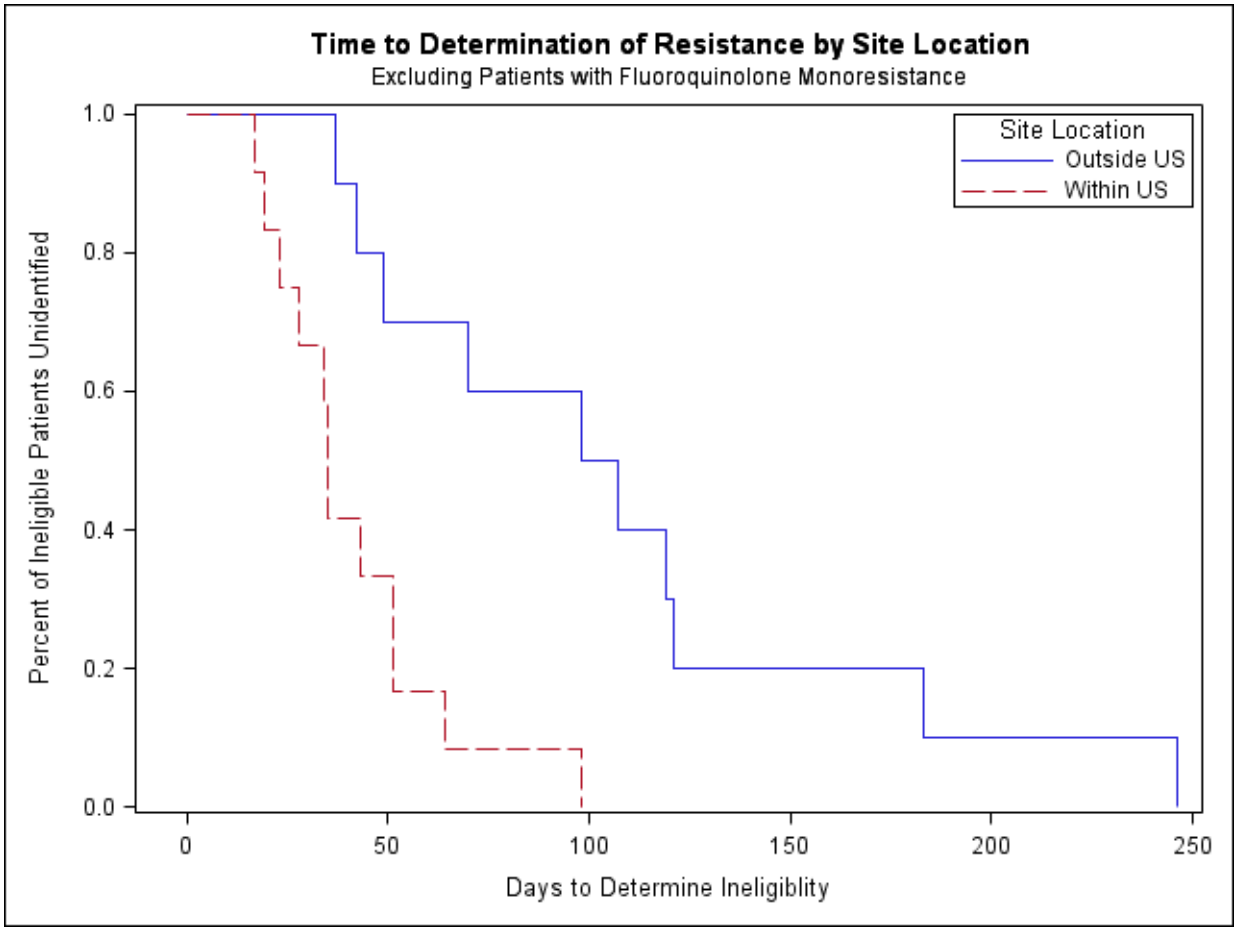


Figure 6: Times to determination of resistance by site location, excluding patients with fluoroquinolone monoresistance



APPENDIX

IRB Exemption Letter

EMORY UNIVERSITY

Institutional Review Board

February 22, 2011

RE: Determination: No IRB Review Required
Title: Ineligibility due to drug resistance or negative cultures in a tuberculosis treatment clinical trial
PI: Megan Eguchi

Dear Ms. Eguchi:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition(s) of "research" involving "human subjects" or the definition of "clinical investigation" as set forth in Emory policies and procedures and federal rules, if applicable. Specifically, in this project, you will be conducting a secondary analysis of non identifiable data collected using the during a clinical trial at the CDC.

This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Andrea Goosen, MPH
Research Protocol Analyst
This letter has been digitally signed