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Prevalence of pyrazinamide resistance and Wayne assay performance analysis in a tuberculosis cohort in Lima, Peru

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Abstract

Rationale—Multidrug-resistant (MDR) tuberculosis (TB) regimens often contain pyrazinamide, despite the absence of confirmed sensitivity to the drug. This gap is due to limited availability and reliability of pyrazinamide susceptibility testing.

Objectives—To estimate the prevalence of pyrazinamide resistance by the Wayne assay among TB patients in Lima, Peru; to describe characteristics associated with pyrazinamide resistance; and to compare Wayne performance to BACTEC MGIT 960.

Methods—In patients diagnosed with culture-positive pulmonary TB from September 2009 to August 2012, we tested pyrazinamide susceptibility using the Wayne assay. We evaluated factors associated with pyrazinamide resistance. In a convenience sample, we compared Wayne assay performance to MGIT 960.

Results—Pyrazinamide resistance prevalence was 6.6% (95% CI: 5.8%–7.5%) among 3,277 patients and 47.7% (95% CI: 42.7%–52.6%) among the subset of 405 MDR-TB patients. In multivariable analysis, MDR-TB (OR=86.0; 95% CI: 54.0–136.9) and LAM lineage (OR=3.40; 95% CI: 2.33–4.96) were associated with pyrazinamide resistance. The Wayne assay agreed with MGIT 960 in 83.9% of samples (Kappa: 0.66; 95% CI: 0.56–0.76).

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Conclusion—Resistance to pyrazinamide was detected by the Wayne assay in nearly half of MDR-TB patients in Lima. This test can inform selection and composition of regimens, especially those dependent on additional resistance.

Keywords

Pyrazinamide; drug resistance; multidrug-resistant tuberculosis; Wayne assay; pyrazinamidase assay; Peru

Introduction

Multidrug-resistant (MDR) tuberculosis (TB), defined as TB resistant to at least isoniazid and rifampin, was estimated to have occurred in 480,000 patients with TB in 2015.^[1] In Peru, the 2015 TB incidence was estimated at 27,300 cases.^[2] The proportion of MDR-TB is increasing and was last estimated to occur in 5.3% of new TB cases and 23.6% of previously treated cases.^[3]

Treatment of MDR-TB relies on second-line antituberculous drugs, as well as pyrazinamide, an essential element in treatment of drug-susceptible TB.^[4] Valued for its sterilizing activity and for its synergy with other antituberculous drugs, pyrazinamide was key to shortening the duration of standard TB treatment^[5] and may permit MDR-TB treatment shortening.^[6] Questions have emerged about the utility of pyrazinamide in MDR-TB treatment when sensitivity to the drug is not confirmed.^[7] The inclusion of pyrazinamide when *M. tuberculosis* bacilli are pyrazinamide resistant has been associated with higher risk of death,^[8] and may be associated with unnecessary toxicity. Consequently, current WHO guidelines recommend that additional drugs should be included in MDR-TB regimens if the effectiveness of pyrazinamide or other core drugs may be compromised by resistance.^[9]

Pyrazinamide susceptibility testing, however, is infrequently performed because of challenges in testing and interpretation of results as well as the high cost of the gold standard, the BACTEC MGIT 960 system (MGIT 960).^[5] An alternative, the Wayne assay,^[10] was adopted by the Peruvian National Reference Laboratory (NRL).^[11] Compared to MGIT 960, this assay is less expensive, easier to implement and can be performed in any laboratory that conducts mycobacterial culture.^[10]

Here, we estimate the prevalence of pyrazinamide resistance detected by the Wayne assay and identify risk factors for pyrazinamide resistance in a cohort of TB patients in Lima, Peru. Further, we evaluate the performance characteristics of the Wayne assay using MGIT 960 as the reference assay.

Methods

This is a sub-study of a prospective cohort study of household TB transmission conducted between September 1, 2009 and August 29, 2012 in Lima, Peru. The primary objectives of the parent study were to measure the transmissibility of drug-resistant TB compared to drug-sensitive TB and to identify host and environmental factors associated with development of TB infection and disease. The study population comprised pulmonary TB patients 16 years

of age or older diagnosed by the Peruvian National TB Strategy in outpatient public health centers in 23 districts in two regions of Lima.

In this study, we included all patients enrolled in the parent study who had baseline drug-susceptibility testing (DST). We defined baseline DST as the first results from sputum samples collected between 60 days before and up to 30 days after initiation of antituberculous therapy. We excluded patients whose baseline culture results were negative, missing, or contained insufficient growth to perform DST. The characteristics of this cohort have been previously described.^[12,13] Baseline demographic and clinical information (age, sex, incarceration in the last 5 years, educational level, alcohol and smoking status, type of housing, region of residence, self-reported diabetes, HIV infection, previous TB treatment for more than 30 days, and presence of cavities on chest X-rays) and all lab results were prospectively double-entered in OpenMRS version 1.6.2 (OpenMRS Inc., Eldoret, Kenya).^[14]

Ziehl-Neelsen acid-fast staining and mycobacterial culture on Löwenstein-Jensen (LJ) medium was performed at the Socios En Salud (SES) Laboratory located in Lima.^[15,16] DST for isoniazid, rifampin, ethambutol, and streptomycin was performed using the proportion method.^[17] Pyrazinamide susceptibility was tested using the Wayne assay as recommended by the Peruvian NRL.^[10,18] Briefly, 0.5 mL of cells from a fresh LJ culture performed on sputum were inoculated on Dubos agar containing 100 mg/L of pyrazinamide. Cultures were incubated for 4 days at 37 degrees Celsius. Then, we poured 1 mL of freshly prepared 1% ferrous ammonium sulfate into each tube. The Wayne assay determines pyrazinamide susceptibility by assessing, in the presence of pyrazinamide, the activity of the pyrazinamidase enzyme in *M. tuberculosis*. When an isolate is susceptible to pyrazinamide, pyrazinamidase converts pyrazinamide to pyrazinoic acid. The Wayne assay detects the presence of pyrazinoic acid through a reaction with ferrous ammonium sulfate; this is indicated by the appearance of a pink band on the surface of the medium.^[10]

In a subset of isolates, we also performed pyrazinamide DSTs in MGIT 960, using a critical concentration of 100 mg/L of pyrazinamide.^[19] At the National Jewish Health Laboratory (NJHL, Denver, Colorado), this was performed in 143 isolates—23 strains randomly selected for quality assurance and 120 that were undergoing MIC testing and whole-genome sequencing as a sub-study of the parent study. An additional 80 isolates were tested by MGIT 960 at the study laboratory. These were the subset of study isolates that had been subcultured between January and September 2014 to be sent for molecular fingerprinting (performed for all parent study isolates). Both laboratories passed annual MGIT 960 pyrazinamide proficiency testing with the College of American Pathologists (CAP, Northfield, Illinois). The study laboratory began annual CAP testing in 2012. NJHL is an ISO15189 and CAP accredited laboratory. We defined pan-susceptible TB as an *M. tuberculosis* isolate susceptible to all first-line drugs: isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin; monoresistant TB as an isolate resistant to exactly one of these; drug-resistant TB as an isolate resistant to at least one of these; MDR-TB as resistance to at least isoniazid and rifampin; and polyresistant TB as resistance to more than one first-line drug, exclusive of MDR-TB. We genotyped *M. tuberculosis* isolates with 24-loci mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-

VNTR) using standard methods.^[20] We assigned *M. tuberculosis* lineages using the MIRU-VNTR^{plus} web application (<http://www.miru-vntrplus.org>) with no categorical distance cutoff.^[21]

The primary outcome in this study was prevalence of pyrazinamide resistance by the Wayne assay. We calculated *p* values using the chi-square or Fisher's exact test for baseline characteristics. We calculated the performance characteristics (including positive and negative predictive values) of the Wayne assay and the Kappa statistic of interrater agreement between this assay and MGIT 960. The Kappa statistic was interpreted using Landis and Koch criteria.^[22] As a sensitivity analysis for potential selection bias, we calculated performance characteristics in the subpopulation of MDR isolates.

We evaluated potential covariates of pyrazinamide resistance using univariate and multivariable logistic regression. We considered age, sex, history of incarceration in the last 5 years, type of housing, region of residence, previous TB treatment, cavitory disease, MDR-TB, and strain lineage as potential confounders. We selected age, sex, and factors associated with pyrazinamide resistance with a *p*<0.1 on univariate analysis for inclusion in the multivariable model. All statistical tests were two-sided with significance set at $\alpha=0.05$. We performed all analyses using Stata/SE version 14.1 (StataCorp LP, College Station, Texas).

The study was approved by the Research Ethics Committee of the Peruvian National Institute of Health (Lima, Peru) and the Committee on Human Studies at Harvard Medical School (Boston, Massachusetts). Written informed consent was obtained from all subjects and from the parents or guardians of subjects who were under 18 years old.

Results

We enrolled 4,500 individuals with newly diagnosed, active pulmonary TB across 92 participating health centers in Lima Ciudad and Lima Este. Among these, 3,277 (72.8%) were age 16, had positive cultures for *M. tuberculosis* and baseline DST results, and were included in the study (Figure 1). The majority were 44 years (2,558 [78.1%]), male (2,045 [62.4%]), residents of Lima Ciudad (2,894 [88.3%]), and had not received previous TB treatment (2,652 [80.9%]). We found baseline pyrazinamide resistance in 217 (6.6%) subjects, including in 193 (47.7%) of 405 subjects with MDR-TB (Table 1). Nearly all pyrazinamide resistance (98.6%) occurred among participants with isolates resistant to at least one other drug (Table 2). We performed MIRU-VNTR on 3,266 (99.7%) participant strains. Of 3,247 (99.1%) evaluable strains, 974 (30.0%) of lineages were LAM (Table 1).

In univariate analysis (Table 3), baseline MDR-TB was associated with higher odds of pyrazinamide resistance (OR=108.0; 95% CI: 69.1–168.8). Other risk factors for pyrazinamide resistance included residence in Lima Este (OR=2.17; 95% CI: 1.54–3.07), previous TB treatment (OR=2.80; 95% CI: 2.09–3.75), and infection with a LAM strain (OR=5.46; 95% CI: 4.06–7.34). In multivariate analysis, only MDR-TB (OR=86.0; 95% CI: 54.0–136.9) and LAM lineage (OR=3.40; 95% CI: 2.33–4.96) remained significantly associated with higher odds of pyrazinamide resistance; cavitory disease (OR=0.61; 95% CI: 0.40–0.93) was associated with lower odds of pyrazinamide resistance.

Table 4 shows the comparison between the Wayne assay and MGIT 960. Among 223 isolates tested by both methods, 187 (83.9%) had concordant results (Kappa: 0.66; 95% CI: 0.56–0.76). Sensitivity of Wayne assay was 75.6% (95% CI: 65.4%–84.0%); and specificity was 89.5% (95% CI: 83.0%–94.1%). In the subset of 153 MDR-TB isolates tested by both methods, the agreement was slightly lower (Kappa: 0.57; 95% CI: 0.44–0.70), due to a decrease in specificity (80.6%; 95% CI: 69.1%–89.2%) (data not shown). Among MDR-TB patients, the prevalence of pyrazinamide resistance was 47.7% and the positive predictive value of the Wayne assay was 86.8%. For non MDR-TB patients, in whom the observed prevalence of pyrazinamide resistance was 0.8%, the negative predictive value of the Wayne assay was 99.8% (Figure 2). Relative to isolates not submitted to paired resistance testing, isolates tested by both methods were less likely to be fully susceptible (22.9% vs. 70.1%, $p<0.001$), more likely to have MDR-TB (68.6% vs. 8.3%, $p<0.001$) and more likely to have characteristics associated with MDR-TB: LAM lineage (50.7% vs. 28.2%, $p<0.001$) and originating from patients who were more likely to reside in Lima Este (19.3% vs. 11.1%, $p<0.001$) (data not shown).

Discussion

In a cohort of newly diagnosed TB patients in Lima, Peru, we detected pyrazinamide resistance by the Wayne assay in 47.7% of MDR-TB patient isolates. This high prevalence of pyrazinamide resistance among MDR-TB patients is not unique to Peru.^[23] Prior studies have generally used MGIT 960 to test for pyrazinamide resistance; our results using the Wayne assay were similar. Caution is warranted about the use of MDR-TB regimens that rely on pyrazinamide in Lima and in South Africa or Rio de Janeiro where 52.1%^[24] and 56.6%^[25] of MDR-TB patients are reported to have isolates resistant to pyrazinamide, respectively.

The results from the present, large, representative cohort study in Lima, home to more than two-thirds of the MDR-TB in Peru,^[3] have important implications for clinical and policy guidance for the treatment of MDR-TB. The effectiveness of pyrazinamide in MDR-TB regimens influences treatment outcomes. A recent meta-analysis reported an adjusted odds of treatment success of 1.9 when pyrazinamide is likely effective and is included in the regimen.^[26] In contrast, the risk of mortality is nearly tripled when patients with pyrazinamide-resistant MDR-TB receive a regimen containing four likely effective drugs plus pyrazinamide that is not likely effective.^[8] Since efficacy of treatment with pyrazinamide-containing regimens is compromised when pyrazinamide resistance is documented, pyrazinamide DST, even if imperfect, should guide regimen selection for patients with MDR-TB.^[27] This is relevant to the current standard of care, including the recently endorsed shortened regimen and the conventional regimen,^[9] as well as to new regimens under evaluation.

Conventional regimen design in Lima is guided by prevalence estimates of resistance to isoniazid, rifampin, ethambutol, and streptomycin obtained between 2007 and 2009. Guidelines call for the inclusion of pyrazinamide in all empirical and individualized regimens for MDR-TB.^[28] In light of the high prevalence of pyrazinamide resistance among MDR-TB patients, consideration should be given to adapting conventional regimens

according to current WHO recommendations: if a regimen containing five effective drugs cannot be composed using four core drugs and pyrazinamide, it is advised to use new or repurposed drugs such as bedaquiline, delamanid, clofazimine, and linezolid.^[9] This should result in increased use of new or repurposed drugs and in refined screening criteria to determine which patients will require changes to their regimens.

The high prevalence of pyrazinamide resistance among MDR-TB patients in Lima suggests that the new, shortened regimen could be compromised there. This regimen is recommended only for MDR-TB patients without any of the following: prior treatment with second-line drugs, resistance to fluoroquinolones, second-line injectables, and “documented or likely resistance to medicines in the regimen,” including pyrazinamide.^[9] Accordingly, any plan for introduction of the shortened regimen should include methods for screening out patients with resistance to pyrazinamide or other drugs in the regimen.^[4] Lastly, novel pyrazinamide-containing regimens, for example, the moxifloxacin-bedaquiline-pyrazinamide regimen being tested in the STAND trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02342886) NCT02342886), will require rapid pyrazinamide-resistance screening strategies.

A possible tool to inform decision-making among various MDR-TB regimens, the Wayne assay may be performed by labs with basic culture capacity; its use was feasible in Lima for a large volume of samples in a routine setting. This assay uses simple reagents, minimizes manipulation of the inoculum, and provides results relatively quickly after culture of *M. tuberculosis*.^[29] Relying on pyrazinamidase as the indicator of susceptibility, the Wayne assay avoids the inherent conflict in optimal conditions in culture-based pyrazinamide susceptibility testing: pyrazinamide activity requires an acidic environment (pH of 5.0–6.0) while growth of *M. tuberculosis* is supported by a more neutral environment.^[5]

In a sub-sample, the Wayne assay showed substantial agreement (Kappa: 0.66) with MGIT 960. In populations with high pyrazinamide resistance, *i.e.*, greater than 40%, the positive predictive value is over 80%. In populations with pyrazinamide resistance of 5% or less, the negative predictive value is greater than 98%. This strong distinction suggests that the assay may be used in representative surveys to guide selection of empiric regimens for MDR-TB and to confirm ongoing utility of pyrazinamide in regimens for other forms of TB.

For individual patient treatment decisions, however, the Wayne assay may have limited value. An indirect test, requiring culture and therefore significant delay to results, it is unlikely to provide patient-level information to guide initial regimen selection. And, its sensitivity, 75.6%, was lower than expected.^[23] Low assay sensitivity has previously been speculated to be the result of mechanisms for pyrazinamide resistance that do not impact pyrazinamidase activity.^[30] It could also be a consequence of the aforementioned challenge to growth of *M. tuberculosis* in culture with sufficiently low pH to induce pyrazinamide activity, which could result in classification of sensitive strains as resistant in MGIT 960.^[27] Previous attempts to optimize Wayne assay performance have met with variable success.^[31,32] Since the specificity reached nearly 90% in the present study, the primary concern is that pyrazinamide resistance prevalence might have been underestimated.

Other limitations include a possible selection bias; this study was performed in patients from two regions in Lima, in which the epidemiology of MDR-TB may differ from others. Although there is no evidence of variability of pyrazinamide resistance (independent of MDR-TB), the clear differences in the prevalence of MDR-TB in Lima support characterization of patterns in smaller geographical areas.^[21] Disproportionate representation of MDR-TB in the sample used in the paired diagnostic comparison could result in biased estimates of Wayne assay performance; the Kappa result recorded (0.66) may represent a lower bound of agreement if this assay is used on isolates with a more heterogeneous distribution of resistance. This can be inferred from the sensitivity analysis that revealed worse concordance (and specificity) when the comparison was made exclusively in MDR-TB isolates.

In multivariable analysis, the LAM lineage remained significantly associated with pyrazinamide resistance. Our findings may represent a propensity for development of pyrazinamide resistance in LAM strains, mediated by low pyrazinamidase activity, as has been previously described for other lineages.^[33] The negative association between cavitory disease and pyrazinamide resistance could support the previously reported theory that pyrazinamide resistance induces a fitness cost^[34] or could be an artifact of a survival effect in patients with pyrazinamide-resistant TB.^[35] These findings require further investigation.

In summary, the Wayne assay, implemented in Lima under routine program conditions for a large sample volume, detected pyrazinamide resistance in nearly 50% of MDR-TB patients. Wayne may be a helpful complement in settings where cost or other challenges preclude universal use of the faster, more sensitive MGIT 960. Although possibly underestimating burden, the Wayne assay has adequate predictive value to inform population-level decisions. Early clinical decision-making remains unguided by individual pyrazinamide susceptibility results since there is considerable delay with any culture-based method and there is no widely available, validated rapid molecular test.^[27]

Treatment decisions that are dependent on the activity of pyrazinamide demand enhanced information about pyrazinamide susceptibility. While rapid, reliable assays are developed and deployed, wider introduction of existing tools, including the Wayne assay, will be essential to detect pyrazinamide resistance and inform decision-making. Interpretation of pyrazinamide susceptibility test results in the epidemiological context in which they occur is key to the appropriate use of pyrazinamide-containing regimens for treatment of drug-resistant TB.

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References

1. World Health Organization. Global Tuberculosis Report 2016. Geneva, Switzerland: 2016.

2. Ministerio de Salud del Peru, Dirección General de Epidemiología. Análisis de la Situación Epidemiológica de la Tuberculosis en el Perú, 2015. Lima, Peru: 2016.
3. Asencios L, Quispe N, Mendoza-Ticona A, et al. Vigilancia nacional de la resistencia a medicamentos antituberculosos, Perú 2005–2006. *Rev Peru Med Exp Salud Publica*. 2009; 26(3): 278–287.
4. Domínguez J, Boettger EC, Cirillo D, et al. Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a TBNET/RESIST-TB consensus statement. *Int J Tuberc Lung Dis*. 2016; 20(1):24–42.
5. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. *Int J Tuberc Lung Dis*. 2003; 7(1):6–21. [PubMed: 12701830]
6. Nuernberger E, Tyagi S, Tasneen R, et al. Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin, and pyrazinamide in a murine model of tuberculosis. *Antimicrob Agents Chemother*. 2008; 52(4):1522–1524. [PubMed: 18285479]
7. Yuen CM, Kurbatova EV, Tupasi T, et al. Association between Regimen Composition and Treatment Response in Patients with Multidrug-Resistant Tuberculosis: A Prospective Cohort Study. *PLoS Med*. 2015; 12(12):e1001932. [PubMed: 26714320]
8. Franke MF, Becerra MC, Tierney DB, et al. Counting pyrazinamide in regimens for multidrug-resistant tuberculosis. *Ann Am Thorac Soc*. 2015; 12(5):674–679. [PubMed: 25664920]
9. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. Geneva, Switzerland: 2016.
10. Wayne LG. Simple pyrazinamidase and urease tests for routine identification of mycobacteria. *Am Rev Respir Dis*. 1974; 109(1):147–151. [PubMed: 4203284]
11. Shin SS, Yagui MJA, Asencios L, et al. Scale-up of multidrug-resistant tuberculosis laboratory services, Peru. *Emerg Infect Dis*. 2008; 14(5):701–708. [PubMed: 18439349]
12. Huang C-C, Tchetgen ET, Becerra MC, et al. The effect of HIV-related immunosuppression on the risk of tuberculosis transmission to household contacts. *Clin Infect Dis*. 2014; 58(6):765–774. [PubMed: 24368620]
13. Odone A, Calderon RI, Becerra MC, et al. Acquired and Transmitted Multidrug Resistant Tuberculosis: The Role of Social Determinants. *PLoS ONE*. 2016; 11(1):e0146642. [PubMed: 26765328]
14. Fraser HSF, Thomas D, Tomaylla J, et al. Adaptation of a web-based, open source electronic medical record system platform to support a large study of tuberculosis epidemiology. *BMC Med Inform Decis Mak*. 2012; 12(1):125. [PubMed: 23131180]
15. Pan American Health Organization. Manual para el diagnóstico bacteriológico de la tuberculosis. Normas y guía técnica. Parte 1 Baciloscopia. Washington, D.C: 2008.
16. Pan American Health Organization. Manual para el diagnóstico bacteriológico de la tuberculosis. Normas y guía técnica. Parte 2 Cultivo. Washington, D.C: 2008.
17. Canetti G, Froman S, Grosset J, et al. Mycobacteria: Laboratory Methods for Testing Drug Sensitivity and Resistance. *Bull World Health Organ*. 1963; 29(5):565–578. [PubMed: 14102034]
18. Leo E, Vásquez L, Asencios L, et al. Determinación de la susceptibilidad de *Mycobacterium tuberculosis* a la pirazinamida mediante la prueba de la pirazinamidasa, Perú - 1999. *Rev Peru Med Exp Salud Publica*. 2003; 20(2):105–106.
19. Siddiqi, SH., Rüsch-Gerdes, S. MGIT™ Procedure Manual. Geneva, Switzerland: Foundation for Innovative New Diagnostics (FIND); 2006.
20. Supply P, Allix C, Lesjean S, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2006; 44(12):4498–4510. [PubMed: 17005759]
21. Zelner JL, Murray MB, Becerra MC, et al. Identifying Hotspots of Multidrug-Resistant Tuberculosis Transmission Using Spatial and Molecular Genetic Data. *J Infect Dis*. 2016; 213(2): 287–294. [PubMed: 26175455]
22. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977; 33(1):159–174. [PubMed: 843571]

23. Chang K-C, Yew WW, Zhang Y. Pyrazinamide susceptibility testing in *Mycobacterium tuberculosis*: a systematic review with meta-analyses. *Antimicrob Agents Chemother*. 2011; 55(10):4499–4505. [PubMed: 21768515]
24. Mphahlele M, Syre H, Valvatne H, et al. Pyrazinamide resistance among South African multidrug-resistant *Mycobacterium tuberculosis* isolates. *J Clin Microbiol*. 2008; 46(10):3459–3464. [PubMed: 18753350]
25. Fonseca L de S, Marsico AG, Vieira GB de O, Duarte RDS, Saad MHF, Mello F de CQ. Correlation between resistance to pyrazinamide and resistance to other antituberculosis drugs in *Mycobacterium tuberculosis* strains isolated at a referral hospital. *J Bras Pneumol*. 2012; 38(5): 630–633. [PubMed: 23147056]
26. Bastos ML, Hussain H, Weyer K, et al. Treatment Outcomes of Patients With Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis According to Drug Susceptibility Testing to First- and Second-line Drugs: An Individual Patient Data Meta-analysis. *Clin Infect Dis*. 2014; 59(10): 1364–1374. [PubMed: 25097082]
27. Velásquez GE, Calderon RI, Mitnick CD, et al. Pyrazinamide resistance assays and two-month sputum culture status in patients with multidrug-resistant tuberculosis. *Antimicrob Agents Chemother*. 2016; 60(11):6766–6773. [PubMed: 27600032]
28. Ministerio de Salud, Dirección General de Salud de las Personas, Estrategia Sanitaria Nacional para la Prevención y Control de la Tuberculosis. Norma técnica de salud para la atención integral de las personas afectadas por la tuberculosis. NTS N° 104-MINSA/DGSP-V.01. Lima, Peru: 2013. Available from: ftp://ftp2.minsa.gob.pe/normaslegales/2013/RM715_2013_MINSA.pdf
29. Meinzen C, Proaño A, Gilman RH, et al. A quantitative adaptation of the Wayne test for pyrazinamide resistance. *Tuberculosis (Edinb)*. 2016; 99:41–46. [PubMed: 27450003]
30. Jonmalung J, Prammananan T, Leechawengwongs M, Chaiprasert A. Surveillance of pyrazinamide susceptibility among multidrug-resistant *Mycobacterium tuberculosis* isolates from Siriraj Hospital, Thailand. *BMC Microbiol*. 2010; 10(1):223. [PubMed: 20727143]
31. Morlock GP, Crawford JT, Butler WR, et al. Phenotypic characterization of *pncA* mutants of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*. 2000; 44(9):2291–2295. [PubMed: 10952570]
32. Singh P, Wesley C, Jadaun GPS, et al. Comparative evaluation of Löwenstein-Jensen proportion method, BacT/ALERT 3D system, and enzymatic pyrazinamidase assay for pyrazinamide susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2007; 45(1):76–80. [PubMed: 17093022]
33. Kurbatova EV, Cavanaugh JS, Dalton T, Click ES, Cegielski JP. Epidemiology of pyrazinamide-resistant tuberculosis in the United States, 1999–2009. *Clin Infect Dis*. 2013; 57(8):1081–1093. [PubMed: 23840002]
34. Hertog den AL, Sengstake S, Anthony RM. Pyrazinamide resistance in *Mycobacterium tuberculosis* fails to bite? *Pathog Dis*. 2015; 73(6):ftv037. [PubMed: 25994506]
35. Velásquez GE, Becerra MC, Gelmanova IY, et al. Improving outcomes for multidrug-resistant tuberculosis: aggressive regimens prevent treatment failure and death. *Clin Infect Dis*. 2014; 59(1): 9–15. [PubMed: 24729493]

Figure 1.

Flow of patient inclusion, classification by resistance group (MDR-TB, pan-susceptible TB, or DR-TB other than MDR-TB) and prevalence of resistance to PZA assessed by the Wayne assay. DR=drug-resistant; PZA=pyrazinamide; MDR=multidrug-resistant; TB=tuberculosis.

Figure 2.

Positive and negative predictive values of pyrazinamide susceptibility testing by the Wayne assay versus prevalence of pyrazinamide resistance. The vertical, dashed lines show the prevalence of pyrazinamide resistance in Lima among non MDR-TB patients and among MDR-TB patients. MDR=multidrug-resistant; NPV=negative predictive value; PPV=positive predictive value; PZA=pyrazinamide; PZA-R, pyrazinamide resistance; TB=tuberculosis.

Table 1

Baseline characteristics of subjects in a TB cohort study in Lima, by baseline pyrazinamide resistance.

Characteristic	Total (N=3277)		PZA susceptible (n=3060)		PZA resistant (n=217)		P value*
	No.	%	No.	%	No.	%	
Age categories							0.240
16 to 24	1267	38.7	1176	38.4	91	41.9	
25 to 44	1291	39.4	1203	39.3	88	40.6	
45 to 64	521	15.9	497	16.2	24	11.1	
65 and older	198	6.0	184	6.0	14	6.5	
Sex							0.125
Female	1232	37.6	1161	37.9	71	32.7	
Male	2045	62.4	1899	62.1	146	67.3	
Incarceration in the past 5 years							0.848
Yes	171	5.2	159	5.2	12	5.5	
No	3071	93.7	2867	93.7	204	94	
Unknown	35	1.1	34	1.1	1	0.5	
Highest educational attainment							0.295
Preschool	257	7.8	242	7.9	15	6.9	
Elementary school	902	27.5	843	27.5	59	27.2	
High school	1672	51.0	1550	50.7	122	56.2	
University	364	11.1	347	11.3	17	7.8	
Unknown	82	2.5	78	2.5	4	1.8	
Alcohol use [†]							0.686
Heavy	344	10.5	321	10.5	23	10.6	
Light	1046	31.9	972	31.8	74	34.1	
Non-Drinker	1747	53.3	1638	53.5	109	50.2	
Unknown	140	4.3	129	4.2	11	5.1	
Smoking status [‡]							0.380 [§]
Heavy	59	1.8	55	1.8	4	1.8	
Light	33	1.0	29	0.9	4	1.8	

Characteristic	Total (N=3277)		PZA susceptible (n=3060)		PZA resistant (n=217)		P value*
	No.	%	No.	%	No.	%	
Non-Smoker	3111	94.9	2909	95.1	202	93.1	
Unknown	74	2.3	67	2.2	7	3.2	
Type of housing							0.403
Substandard housing	405	12.4	382	12.5	23	10.6	
Standard housing	2844	86.8	2651	86.6	193	88.9	
Unknown	28	0.9	27	0.9	1	0.5	
Region							<0.001
Lima Ciudad	2894	88.3	2723	89.0	171	78.8	
Lima Este	383	11.7	337	11.0	46	21.2	
Self-reported diabetes							0.968
Yes	182	5.6	170	5.6	12	5.5	
No	3059	93.3	2855	93.3	204	94.0	
Unknown	36	1.1	35	1.1	1	0.5	
HIV infection							0.504
Yes	110	3.4	101	3.3	9	4.1	
No	3120	95.2	2915	95.3	205	94.5	
Unknown	47	1.4	44	1.4	3	1.4	
Previous TB treatment							<0.001
Yes	616	18.8	535	17.5	81	37.3	
No	2652	80.9	2516	82.2	136	62.7	
Unknown	9	0.3	9	0.3	0	0	
No. of previous TB treatments							<0.001 [§]
None	2652	80.9	2516	82.2	136	62.7	
One	460	14.0	407	13.3	53	24.4	
Two	81	2.5	65	2.1	16	7.4	
Three	16	0.5	16	0.5	0	0	
Four	9	0.3	5	0.2	4	1.8	
Unknown	59	1.8	51	1.7	8	3.7	
Cavitary disease							0.089

Characteristic	Total (N=3277)		PZA susceptible (n=3060)		PZA resistant (n=217)		P value [*]
	No.	%	No.	%	No.	%	
Yes	952	29.1	900	29.4	52	24.0	
No	2269	69.2	2108	68.9	161	74.2	
Unknown	56	1.7	52	1.7	4	1.8	
MDR-TB							<0.001
Yes	405	12.4	212	6.9	193	88.9	
No	2872	87.6	2848	93.1	24	11.1	
LAM lineage							<0.001
Yes	974	29.7	830	27.1	144	66.4	
No	2273	69.4	2203	72.0	70	32.3	
Unknown	30	0.9	27	0.9	3	1.4	

HIV=human immunodeficiency virus; MDR-TB=multidrug-resistant tuberculosis; PZA=pyrazinamide; TB=tuberculosis.

^{*} Boldface indicates $P < 0.05$. All tests of significance exclude unknown categories. The chi-squared test was performed unless otherwise noted.

[†] Heavy alcohol use defined as 40 grams or 3 drinks per day, light alcohol use defined as <40 grams or <3 drinks per day.

[‡] Heavy smoking defined as >1 cigarette per day, light smoking defined as one cigarette per day.

[§] Fisher's exact test.

Table 2

Baseline prevalence of pyrazinamide resistance among subjects in a TB cohort study in Lima, by baseline resistance pattern.

Resistance pattern	Total No.	PZA resistance No.	% PZA resistance % (95% CI)
Pan-susceptible to all FLD	2191	0	0 (0–0.2) *
Monoresistant	466	3	0.6 (0.1–1.9)
Polyresistant to FLD, not MDR-TB	213	21	9.9 (6.2–14.7)
Resistant to two FLD	187	9	4.8 (2.2–8.9)
Resistant to three or more FLD	26	12	46.2 (26.6–66.6)
MDR-TB	405	193	47.7 (42.7–52.6)
Incomplete DST to FLD †	2	0	0 (0–84.2) *
Total	3277	217	6.6 (5.8–7.5)

DST=drug susceptibility testing; FLD=first-line drugs (isoniazid, rifampin, ethambutol, pyrazinamide, and streptomycin); MDR-TB=multidrug-resistant tuberculosis; PZA=pyrazinamide.

* One-sided, 97.5% confidence interval.

† One patient had missing DST to rifampin and streptomycin, and another patient had missing DST to streptomycin.

Table 3

Univariate and multivariate analysis of pyrazinamide resistance by the Wayne assay in subjects in a TB cohort study in Lima.

Variable	Unadjusted OR (95% CI)*	Adjusted OR (95% CI)*
Age	0.99 (0.99–1.00)	1.00 (0.99–1.02)
Sex		
Male	Reference	Reference
Female	0.80 (0.59–1.07)	0.78 (0.53–1.16)
Incarceration in the past 5 years (<i>n</i> =3,242)		
No	Reference	
Yes	1.06 (0.58–1.94)	N/A
Type of housing (<i>n</i> =3,249)		
Standard housing	Reference	
Substandard housing	0.83 (0.53–1.29)	N/A
Region		
Lima Ciudad	Reference	Reference
Lima Este	2.17 (1.54–3.07)	1.34 (0.82–2.20)
Previous treatment (<i>n</i> =3,268)		
No	Reference	Reference
Yes	2.80 (2.09–3.75)	1.38 (0.92–2.08)
Cavitary disease (<i>n</i> =3,221)		
No	Reference	Reference
Yes	0.76 (0.55–1.04)	0.61 (0.40–0.93)
MDR-TB		
No	Reference	Reference
Yes	108.0 (69.1–168.8)	86.0 (54.0–136.9)
LAM lineage (<i>n</i> =3,247)		
No	Reference	Reference
Yes	5.46 (4.06–7.34)	3.40 (2.33–4.96)

CI=confidence interval; MDR-TB=multidrug-resistant tuberculosis; PZA=pyrazinamide; OR=odds ratio.

* Boldface indicates $P < 0.05$. The multivariate model uses $n=3,187$ (97.3%) observations with complete data.

Table 4

Performance characteristics of the Wayne assay for pyrazinamide susceptibility testing in 223 clinical isolates, using BACTEC™ MGIT 960 as the reference standard.

Wayne assay	BACTEC™ MGIT 960				
	Total No.	Resistant No. (%)	Susceptible No. (%)	Sensitivity (95% CI)	Specificity (95% CI)
Resistant	82	68 (82.9)	14 (17.1)		
Susceptible	141	22 (15.6)	119 (84.4)	75.6% (65.4–84.0)	89.5% (83.0–94.1)
Total	223	90 (40.4)	133 (59.6)		

CI=confidence interval; MGIT=mycobacteria growth indicator tube.