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Epidemiologic Correlates of Pyrazinamide-Resistant *Mycobacterium tuberculosis* in New York City

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Pyrazinamide (PZA) has important sterilizing activity in tuberculosis (TB) chemotherapy. We describe trends, risk factors, and molecular epidemiology associated with PZA-resistant (PZA^r) *Mycobacterium tuberculosis* in New York City (NYC). From 2001 to 2008, all incident culture-positive TB cases reported by the NYC Department of Health and Mental Hygiene (DOHMH) were genotyped by IS*6110*-based restriction fragment length polymorphism and spoligotype. Multidrug-resistant (MDR) isolates underwent DNA sequencing of resistance-determining regions of *pncA*, *rpoB*, *katG*, and *fabG1*. Demographic and clinical information were extracted from the NYC DOHMH TB registry. During this period, PZA^r doubled (1.6% to 3.6%) overall, accounting for 44% (70/159) of the MDR population and 1.4% (75/5511) of the non-MDR population. Molecular genotyping revealed strong microbial phylogenetic associations with PZA^r. Clustered isolates and those from acid-fast bacillus (AFB) smear-positive cases had 2.7 (95% confidence interval [CI] = 1.71 to 4.36) and 2.0 (95% CI = 1.19 to 3.43) times higher odds of being PZA^r, respectively, indicating a strong likelihood of recent transmission. Among the MDR population, PZA^r was acquired somewhat more frequently via primary transmission than by independent pathways. Our molecular analysis also revealed that several historic *M. tuberculosis* strains responsible for MDR TB outbreaks in the early 1990s were continuing to circulate in NYC. We conclude that the increasing incidence of PZA^r, with clear microbial risk factors, underscores the importance of routine PZA drug susceptibility testing and *M. tuberculosis* genotyping for the identification, control, and prevention of increasingly resistant organisms.

Pyrazinamide (PZA) is a first-line antituberculosis (anti-TB) drug and the cornerstone of modern short-course chemotherapy. PZA acts synergistically with other TB drugs to accelerate culture conversion and reduce the risk of relapse among patients with drug-susceptible TB (1). In patients with multidrug-resistant (MDR) TB, defined as resistance to at least isoniazid (INH) and rifampin (RIF), inclusion of PZA is recommended to reduce the treatment duration (2, 3), while optimizing MDR TB treatment regimens based on PZA susceptibility may improve clinical outcomes (4). Due to its well-documented sterilizing capability, PZA has been included in several new TB drug regimens (5–7).

PZA is a prodrug that requires activation to pyrazinoic acid by the pyrazidamidase of *M. tuberculosis* under acidic conditions (8). While at least one other *M. tuberculosis* gene has been associated with PZA resistance (PZA^r) (9), mutations in *pncA*, which encodes the pyrazidamidase, account for the majority of PZA^r *in vitro* (10, 11). Most genes associated with drug resistance in *M. tuberculosis*, such as *katG* (INH) and *rpoB* (RIF), have clear mutational hot spot regions 7 to 66 nucleotides (nt) in length. In contrast, mutations observed in *pncA* span a region of ~600 nt, comprising the entire gene and the putative promoter region (10, 12, 13). Growth-based assays of *M. tuberculosis* PZA susceptibility are the standard but are not always performed routinely, except in large referral laboratories, because they are technically challenging (14, 15). However, *pncA* mutations in clinical *M. tuberculosis* isolates have generally been found to correlate with phenotypic PZA drug susceptibility testing (DST) results, supporting the value of *pncA* sequence analysis as an alternative means to establish PZA resistance (13, 16).

Genetic markers have been used to confirm or refute TB outbreaks and to estimate the proportion of recent transmission in a population (17). For instance, due to the wide diversity of *pncA* mutations, these sequences can provide a genetic marker to confirm or resolve genotypic clusters, where the presence of identical *pncA* mutations in genotypically clustered strains is supportive of primary transmission while genotypic clusters with diverse *pncA* mutations suggest acquired (*de novo*) PZA^r, indicating these cases may not be epidemiologically related (17).

Despite the unique role PZA plays in modern TB chemotherapy, few studies have investigated the epidemiology of PZA^r in both general and MDR TB populations (18). Using TB case records and surveillance data provided by the New York City (NYC) Department of Health and Mental Hygiene (DOHMH) from 2001 to 2008, we conducted a population-based study of *M. tuberculosis* isolates from 6,260 culture-positive TB cases to examine PZA^r in terms of clinical, microbial, and demographic risk factors. Due to the clinical and epidemiologic importance of MDR TB, we also performed a case-control study to identify PZA^r risk factors and examine PZA^r acquisition and clustering among the MDR population.

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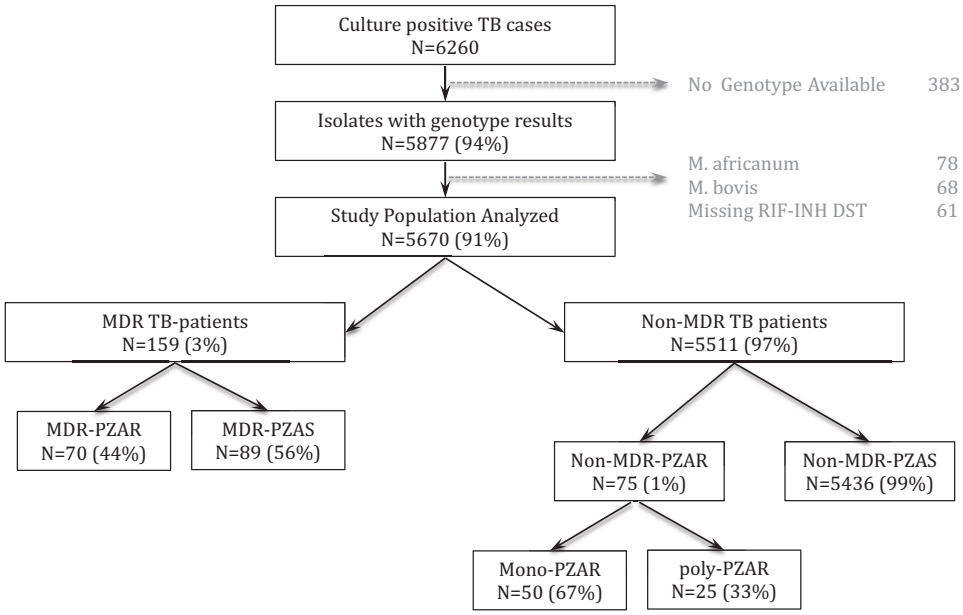

FIG 1 Study schema. MDR, resistant to at least isoniazid and rifampin; PZAR, PZA resistant; PZAS, PZA susceptible; mono-PZAR, resistant only to PZA and no other drug; MDR-PZAR, resistant to at least isoniazid, rifampin, and PZA; poly-PZAR, resistant to PZA and at least one other drug, excluding MDR-PZAR.

MATERIALS AND METHODS

All incident culture-positive TB cases reported and verified by the NYC DOHMH between January 2001 and December 2008 (n = 6,260) were included in the study (Fig. 1). Routine genotyping was performed by the Public Health Research Institute (PHRI) Tuberculosis Center at Rutgers University (IS*6110*-restriction fragment length polymorphism [RFLP]) and New York State Wadsworth Center (spoligotype) (19). All strains with genotyping results available (n = 5,877) were assigned a molecular lineage using the taxonomic designation previously described by Gagneux and Small (20) and a strain code following a nomenclature system of the PHRI TB Center that has been described previously (21, 22). Strains identified as *Mycobacterium bovis* (n = 68) or *Mycobacterium africanum* (n = 78) (23) were omitted from our analysis. Clusters were defined as two or more strains sharing identical IS*6110*-RFLPs and spoligotypes. DST was performed at the NYC DOHMH Public Health Laboratories and the New York State Wadsworth Center (24) reference laboratories, which utilized the Bactec 460TB system (Becton, Dickinson and Company, Franklin Lakes, NJ) until 2003 and the BD Bactec MGIT 960 mycobacterial detection system thereafter. DST results were available for culture-positive TB cases for all first-line anti-TB agents: INH, RIF, ethambutol (EMB), and PZA. DST results for second-line drugs (SLD), including kanamycin, capreomycin, amikacin, fluoroquinolones, and ethionamide, were avail-

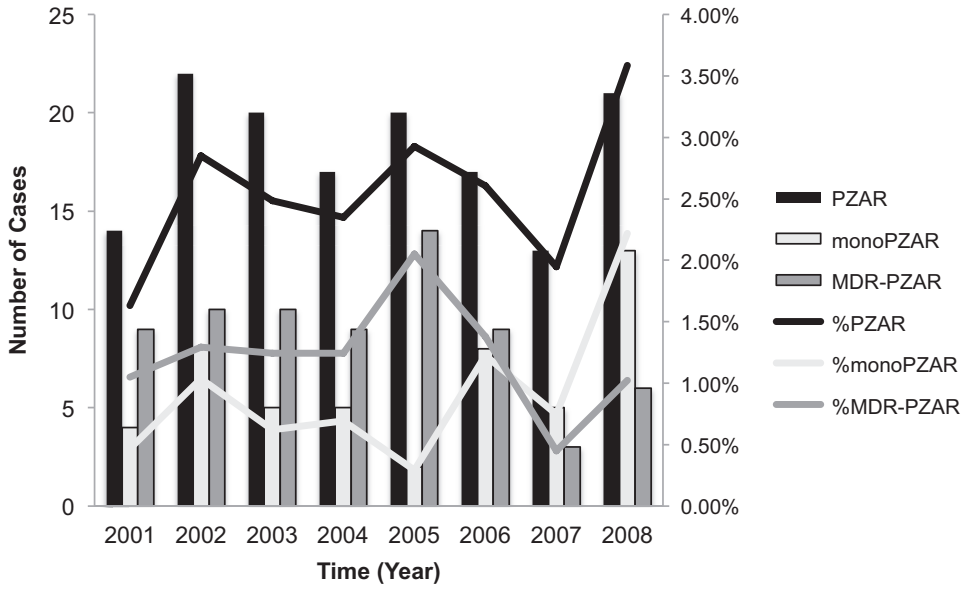

FIG 2 Distribution of PZA^r, mono-PZA^r, and MDR-PZA^r TB in NYC from 2001 to 2008.

TABLE 1 Epidemiologic characteristics associated with pyrazinamide resistance

Characteristic	PZA^r ($n = 145$)	PZA^s ($n = 5,525$)	Unadjusted OR	95% CI	P value[a]
Median age, yr (IQR)	40 (30–50)	42 (30–57)	0.99	0.98–1.00	0.0118
Sex[b]					
M	89 (61.38%)	3,469 (62.79%)	0.94	0.67–1.32	0.7292
F	56 (38.62%)	2,056 (37.21%)			
U.S. born[c]					
Yes	47 (32.64%)	1,598 (29.03%)	1.18	0.83–1.69	0.3472
No	97 (67.36%)	3,906 (70.97%)			
History of TB					
Yes	8 (5.52%)	139 (2.52%)	2.26	1.09–4.71	0.0248
No	137 (94.48%)	5,386 (97.48%)			
History of LTBI					
Yes	5 (3.45%)	320 (5.79%)	0.58	0.24–1.43	0.2308
No	140 (96.55%)	5,205 (94.21%)			
History of homelessness					
Yes	18 (12.41%)	518 (9.38%)	1.37	0.83–2.26	0.2171
No	127 (87.59%)	5,007 (90.62%)			
History of substance abuse					
Yes	19 (13.29%)	494 (9.15%)	1.52	0.93–2.49	0.0924
No	124 (86.71%)	4,903 (90.85%)			
History of alcohol abuse					
Yes	24 (16.78%)	875 (16.20%)	1.04	0.67–1.63	0.8513
No	119 (83.22%)	4,527 (83.80%)			
History of Rikers treatment					
Yes	3 (2.07%)	106 (1.92%)	1.08	0.34–3.44	0.8964
No	142 (97.93%)	5,419 (98.08%)			
HIV serostatus					
Positive	31 (21.38%)	825 (14.93%)	1.39	0.92–2.11	0.1226
Negative	85 (58.62%)	3,145 (56.92%)		Reference	
Unknown	29 (20.00%)	1,555 (28.14%)	0.69	0.45–1.06	0.0877
TB infection site					
Pulmonary	114 (78.62%)	3,882 (70.26%)		Reference	
Extrapulmonary	14 (9.66%)	986 (17.85%)	0.48	0.28–0.85	0.0109
Both	17 (11.72%)	657 (11.89%)	0.88	0.53–1.48	0.6309
Respiratory AFB smear status					
Positive	104 (74.82%)	2,967 (57.77%)	2.17	1.48–3.2	<0.0001
Negative	35 (25.18%)	2,169 (42.23%)			
Abnormal chest X-ray					
Yes	59 (93.65%)	2,091 (88.87%)	1.85	0.67–5.13	0.2311
No	4 (6.35%)	262 (11.13%)			
Final culture conversion					
Yes	106 (86.18%)	3,563 (85.92%)	1.02	0.61–1.72	0.9345
No	17 (13.82%)	584 (14.08%)			
Any cavitation					
Yes	32 (25.00%)	1,010 (21.62%)	1.21	0.81–1.81	0.3606
No	96 (75.00%)	3,661 (78.38%)			
Any death[d]					
Yes	23 (17.83%)	582 (11.18%)	1.72	1.09–2.70	0.0187
No	106 (82.17%)	4,623 (88.82%)			

(Continued on following page)

TABLE 1 (Continued)

Characteristic	PZA^r (n = 145)	PZA^s (n = 5,525)	Unadjusted OR	95% CI	P value[a]
Drug resistance[e]					
Mono	50 (34.48%)	461 (59.87%)		Reference	
Poly	25 (17.24%)	220 (28.57%)	1.05	0.63–1.74	0.8568
MDR	70 (48.28%)	89 (11.56%)	7.25	4.73–11.13	<0.0001
Molecular epidemiology					
Clustered					
Yes	83 (57.24%)	2,414 (43.69%)	1.73	1.24–2.41	0.0012
No	62 (42.76%)	3,111 (56.31%)			
Phylogenetic lineage					
1	18 (12.50%)	490 (8.90%)	1.91	1.13–3.22	0.0159
2	44 (30.56%)	878 (15.95%)	2.60	1.78–3.81	<0.0001
3	9 (6.25%)	347 (6.31%)	0.67	0.67–2.71	0.4063
4	73 (50.69%)	3,788 (68.84%)		Reference	

[a] Calculated using chi-square.
[b] M, male; F, female.
[c] Includes Puerto Rico.
[d] Excludes patients who refused treatment or who were lost to follow-up.
[e] Mono, resistance to any 1 drug only (isoniazid, rifampin, ethambutol, or second-line [fluoroquinolone, kanamycin, capreomycin, amikacin, or ethionamide)]; Poly, resistance to any combination of drugs excluding MDR; MDR, multidrug resistant only.

able for all cases with reduced susceptibility to any first-line agent. *M. tuberculosis* isolates were classified as MDR if they were resistant to at least INH and RIF (3). Isolates were classified as poly-PZA^r if they were resistant to PZA and at least one other drug, excluding MDR isolates (Fig. 1).

Data collection. Demographic and clinical information was provided by the NYC DOHMH TB Registry, which contains information for each reported TB patient obtained by interview and medical-record abstraction performed by trained Bureau of Tuberculosis Control (BTBC) staff, using standard data collection forms. Demographic variables included age at TB diagnosis, sex, birthplace (United States or foreign born with country of birth), number of years since arrival in the United States for foreign-born patients, and race/ethnicity. Sociodemographic variables included reported homelessness; substance use (injection drug use), noninjection crack cocaine use, or noninjection drug use (consolidated into yes or no); alcohol abuse; and history of TB treatment at Rikers Island Prison Complex. Clinical variables included initial chest radiography results (normal/abnormal and absence/presence of cavities), the anatomical site of TB disease, respiratory acid-fast bacillus (AFB) smear status, final culture conversion (final conversion from positive to negative culture), HIV status (infected, uninfected, or unknown); and death from any cause (yes or no, excluding patients who refused treatment or were lost to follow-up).

All MDR isolates (n = 159) were subjected to PCR amplification (primers, 5′-ATGCGGCGTTGATCATCG-3′ and 5′-CAGGAGCTGCA AACCAACTCG-3′), followed by standard capillary sequencing of *pncA* promoter and coding DNA sequence (CDS), as previously described (12). Mutations were identified by alignment of nucleotide sequences to the *M. tuberculosis* H37Rv reference strain (NCBI accession number AL123456) (25) using ClustalW2 (26). All MDR isolates were also analyzed for the presence of known drug resistance-conferring mutations in the gene targets *katG*, *fabG1* (promoter), and *rpoB*, using the IBIS platform (Ibis Biosciences Carlsbad, CA), which is based on PCR followed by electrospray ionization mass spectrometry, as described previously (27).

Statistical analysis. All statistical analysis was performed using SAS (Cary, NC) 9.3. To examine aggregate patient risk factors associated with PZA^r, unadjusted odds ratios (OR) were estimated using standard two-by-two contingency table univariate analysis with Fisher's exact test at a 0.05 significance level. We compared demographic and clinical characteristics of TB patients infected with PZA^r *M. tuberculosis* to those of patients infected with PZA-sensitive (PZA^s) *M. tuberculosis*, together with the microbial features (phylogenetic lineage) of the infecting isolates. Trend analysis was performed using the least-squares method, and R^2 values are reported. The analysis of PZA susceptibility was stratified by MDR. In the case-control study, we compared the characteristics of MDR-PZA^r cases to those of MDR-PZA^s controls. A multivariate analysis by logistic regression was performed, using *a priori* variables reported in the literature to be associated with drug resistance, including age and HIV status. Variables with P values of ≤0.2 in the univariate analysis were included in the multivariate models. We further examined genotypic clustering of MDR-PZA^r *M. tuberculosis* strains using *pncA* sequence data to support primary PZA^r transmission events (defined by identical *pncA* mutations in genotypically clustered strains) and to distinguish independently acquired PZA^r (IS*6110*-RFLP/spoligotype-based clusters showing diverse *pncA* mutations) within the MDR population (17). Finally, we evaluated the overall agreement between PZA susceptibility defined by phenotype (DST) and genotype (*pncA* sequence) among MDR isolates using a kappa statistic.

Ethical approval for this study was obtained from the Institutional Review Boards of the NYC DOHMH and Rutgers University (Newark, NJ).

TABLE 2 Adjusted[a] epidemiologic characteristics of pyrazinamide resistance

Characteristic	OR	95% CI	P value
Age (yr)	0.99	0.98–1.01	0.2007
HIV positivity	1.39	0.80–2.43	0.2467
TB infection site			
Extrapulmonary vs pulmonary	0.84	0.33–2.14	0.7192
Both vs pulmonary	0.81	0.42–1.55	0.5272
AFB smear positivity	2.02	1.19–3.43	0.0094
History of TB	0.93	0.28–3.08	0.9032
History of substance abuse	1.29	0.70–2.40	0.4164
Any death	1.77	0.89–3.52	0.1033
Clustered	2.73	1.71–4.36	<0.0001
Lineage 1 vs 4	3.45	1.72–6.95	0.0005
Lineage 2 vs 4	5.01	3.13–8.03	<0.0001
Lineage 3 vs 4	3.30	1.33–8.19	0.0101

[a] Adjusted for known TB risk factors, including age, HIV, and any univariate variable with a P value of <0.2.

TABLE 3 Epidemiologic characteristics associated with pyrazinamide resistance stratified by MDR

Characteristic	Non-MDR TB Cases					MDR TB cases					Breslow-Day test for homogeneity	
	PZA^r (n = 75)	PZA^s (n = 5,436)	OR	95% CI	P value	PZA^r (n = 70)	PZA^s (n = 89)	OR	95% CI	P value	χ^2	P value
Median age, yr (IQR)	40 (31–51)	42 (30–57)	0.99	0.98–1.01	0.262	40 (28–49)	42 (29–53)	0.98	0.96–1.01	0.120	NA[a]	
Sex[b]												
M	49 (65.33%)	3,425 (63.01%)	1.11	0.69–1.79	0.678	40 (57.14%)	44 (49.44%)	1.36	0.73–2.56	0.334	0.2679	0.6047
F	26 (34.67%)	2,011 (36.99%)				30 (42.86%)	45 (50.56%)					
U.S. born												
Yes	17 (22.67%)	1,563 (28.86%)	0.72	0.42–1.24	0.239	30 (43.48%)	35 (39.33%)	1.19	0.63–2.25	0.599	1.3523	0.2449
No	58 (77.33%)	3,852 (71.14%)				39 (56.52%)	54 (60.67%)					
History of TB												
Yes	1 (1.33%)	132 (2.43%)	0.54	0.07–3.94	0.539	7 (10.00%)	7 (7.87%)	1.30	0.43–3.9	0.637	0.5967	0.4399
No	74 (98.67%)	5,304 (97.57%)				63 (90.00%)	82 (92.13%)					
History of LTBI												
Yes	3 (4.00%)	313 (5.76%)	0.68	0.21–2.18	0.516	2 (2.86%)	7 (7.87%)	0.34	0.07–1.71	0.175	0.4622	0.4966
No	72 (96.00%)	5,123 (94.24%)				68 (97.14%)	82 (92.13%)					
History of homelessness												
Yes	6 (8.00%)	506 (9.31%)	0.85	0.37–1.96	0.698	12 (17.14%)	12 (13.48%)	1.33	0.56–3.17	0.522	0.533	0.4654
No	69 (92.00%)	4,930 (90.69%)				58 (82.86%)	77 (86.52%)					
History of substance abuse												
Yes	5 (6.67%)	481 (9.06%)	0.72	0.29–1.79	0.473	14 (20.59%)	13 (15.12%)	1.46	0.63–3.35	0.375	1.2787	0.2581
No	70 (93.33%)	4,830 (90.94%)				54 (79.41%)	73 (84.88%)					
History of alcohol abuse												
Yes	11 (14.67%)	853 (16.05%)	0.90	0.47–1.71	0.747	13 (19.12%)	22 (25.58%)	0.69	0.32–1.49	0.273	0.6013	0.737
No	64 (85.33%)	4,463 (83.95%)				55 (80.88%)	64 (74.42%)					
History of Rikers treatment												
Yes	0 (0.00%)	104 (1.91%)				3 (4.29%)	2 (2.25%)	1.95	0.32–11.99	0.465	2.2788	0.1312
No	75 (100.00%)	5,332 (98.09%)				67 (95.71%)	87 (97.75%)					
HIV serostatus												
Positive	11 (14.67%)	803 (14.77%)	0.94	0.49–1.83	0.859	20 (28.57%)	22 (24.72%)	0.18	0.57–2.46	0.655	0.4642[c]	0.4957[c]
Negative	45 (60.00%)	3,093 (56.90%)		Reference		40 (57.14%)	52 (58.43%)		Reference			
Unknown	19 (25.33%)	1,540 (28.33%)	0.85	0.49–1.46	0.549	10 (14.29%)	15 (16.85%)	0.87	0.35–2.13	0.755		
TB infection site												
Pulmonary	55 (73.33%)	3,816 (70.20%)		Reference		59 (84.29%)	66 (74.16%)		Reference		1.4359[c]	0.2308[c]
Extrapulmonary	8 (10.67%)	976 (17.95%)	0.57	0.27–1.20	0.138	5 (7.14%)	10 (11.24%)	0.67	0.23–1.96	0.466		
Both	12 (16.00%)	644 (11.85%)	1.29	0.69–2.43	0.424	5 (7.04%)	13 (14.61%)	0.43	0.15–1.28	0.129		

Respiratory AFB smear status												
Positive	50 (70.42%)	2914 (57.74%)	1.74	1.04–2.91	0.032	54 (79.41%)	53 (59.55%)	2.62	1.27–5.41	0.008	0.8126	0.3673
Negative	21 (29.58%)	2,133 (42.26%)				14 (20.59%)	36 (40.45%)					
Abnormal chest X-ray												
Yes	31 (91.18%)	2,056 (88.81%)	1.30	0.4–4.29	0.664	28 (96.55%)	35 (92.11%)	2.40	0.24–24.35	0.447	0.2147	0.6431
No	3 (8.82%)	259 (11.19%)				1 (3.45%)	3 (7.89%)					
Final culture conversion												
Yes	54 (88.52%)	3,498 (85.90%)	1.27	0.57–2.8	0.559	52 (83.87%)	65 (86.67%)	0.80	0.31–2.07	0.645	0.532	0.4658
No	7 (11.48%)	574 (14.10%)				10 (16.13%)	10 (13.33%)					
Any cavitation												
Yes	13 (19.70%)	992 (21.60%)	0.89	0.48–1.64	0.709	19 (30.65%)	18 (23.08%)	1.47	0.69–3.13	0.313	1.0368	0.3086
No	53 (80.30%)	3,601 (78.40%)				43 (69.35%)	60 (76.92%)					
Any death												
Yes	9 (12.50%)	568 (11.07%)	1.15	0.57–2.32	0.701	14 (24.56%)	14 (19.18%)	1.37	0.60–3.15	0.459	0.1021	0.7493
No	63 (87.50%)	4,564 (88.93%)				43 (75.44%)	59 (80.82%)					
Drug resistance[d]												
INH												
Yes	12 (40.00%)	345 (57.02%)	0.50	0.24–1.06	0.089							
No	18 (60.00%)	260 (42.98%)										
INH-EMB												
Yes	3 (10.00%)	28 (4.63%)	2.29	0.65–8.00	0.175							
No	27 (90.00%)	577 (95.37%)										
INH-EMB-SLD												
Yes	1 (3.33%)	6 (0.99%)	3.44	0.40–29.54	0.289							
No	29 (96.67%)	599 (99.01%)										
EMB												
Yes						22 (32.35%)	22 (25.00%)	1.43	0.71–2.89	0.371		
No						46 (67.65%)	66 (75.00%)					
EMB-SLD												
Yes						36 (52.94%)	23 (26.14%)	3.18	1.62–6.23	<0.0001		
No						32 (47.06%)	65 (73.86%)					
Molecular epidemiology												
Clustered												
Yes	38 (50.67%)	2,363 (43.47%)	1.34	0.85–2.11	0.212	45 (64.29%)	51 (57.30%)	1.34	0.7–2.56	0.372	0.0001	0.9918
No	37 (49.33%)	3,073 (56.53%)				25 (35.71%)	38 (42.70%)					
Phylogenetic lineage												
1	17 (22.97%)	488 (9.01%)	3.10	1.75–5.49	<0.0001	1 (1.43%)	2 (2.25%)	0.79	0.07–9.09	0.850	10.8398[c]	0.0010[c]
2	9 (12.16%)	846 (15.63%)	0.95	0.46–1.95	0.883	35 (50.00%)	32 (35.96%)	1.73	0.90–3.34	0.103		
3	6 (8.11%)	341 (6.30%)	1.57	0.67–3.71	0.308	3 (4.29%)	6 (6.74%)	0.79	0.18–3.39	0.7515		
4	42 (56.76%)	3,739 (69.06%)		Reference		31 (44.29%)	49 (55.06%)		Reference			

[a] NA, not applicable.
[b] M, male; F, female.
[c] Cochran-Mantel-Haenszel nonzero correlation statistic.
[d] Drug resistance analysis excludes drug-susceptible *M. tuberculosis* strains and is independent of PZA^r. The INH category excludes strains with additional RIF^r, EMB^r, or SLD^r. INH-EMB excludes strains with additional RIF^r or SLD^r. INH-EMB-SLD excludes strains with additional RIF^r. The EMB category excludes strains with additional SLD^r.

RESULTS

PZA resistance trends and associated risk factors among NYC TB cases. Culture-positive TB cases in New York City steadily declined from 950 cases in 2001 to 649 in 2008 ($R^2 = 0.9$). During this time, a total of 6,260 culture-positive TB cases were reported, 5,670 (91%) of which were due to *M. tuberculosis* infection and had genotype and DST results available (Fig. 1). Of these, 145 (2.6%) cases involved infection with PZA^r *M. tuberculosis*. The annual *M. tuberculosis* PZA^r prevalence was between 1.6% and 3.6%, resulting in an average of 18 cases per year. The burden of PZA^r fluctuated considerably, peaking in 2002, 2005, and 2008 (Fig. 2). From 2001 to 2005, MDR-PZA^r (resistance to PZA, INH, and RIF only) accounted for an average of 60% of all PZA^r in NYC, whereas mono-PZA^r accounted for an average of 24%. Between 2006 and 2008, there was a shift in the relative proportions of mono-PZA^r and MDR-PZA^r. While the overall proportion of PZA^r remained relatively constant during the study period ($R^2 = 0.2$), by 2008, mono-PZA^r accounted for 60% of PZA^r in NYC, whereas MDR-PZA^r accounted for less than 30% of PZA^r.

The univariate analysis of epidemiologic risk factors associated with PZA^r is presented in Table 1. Predictors of PZA^r included AFB smear positivity, history of TB, death, and strain clustering. Among drug-resistant strains ($n = 915$), MDR was strongly associated with PZA^r (OR = 7.25; 95% confidence interval [CI] = 4.73 to 11.13) compared to any monoresistance. In the multivariate analysis (Table 2), AFB smear positivity (OR = 2.02; 95% CI = 1.19 to 3.43) and clustering (OR = 2.73; 95% CI = 1.71 to 4.36) maintained significant associations with PZA^r TB caused by PZA^r *M. tuberculosis*.

PZA resistance trends and risk factors among the NYC MDR population. PZA resistance was high among patients with MDR TB, accounting for 44% (70/159) of all MDR cases, while only 1.4% (75/5511) of the non-MDR population was PZA^r. Table 3 shows the odds ratios for PZA^r according to clinical and demographic characteristics, stratified by patients with MDR and non-MDR TB. Factors independently associated with PZA^r among patients with MDR TB in the stratified analysis were EMB-SLD resistance and AFB smear positivity. AFB smear positivity was the only significant characteristic among patients with non-MDR TB (Table 3). In the multivariate MDR TB analysis, only EMB-SLD resistance (OR = 3.48; 95% CI = 1.57 to 7.69) maintained significance (Table 4), while a history of latent *M. tuberculosis* infection (LTBI) trended toward significance (OR = 0.12; 95% CI = 0.01 to 1.07).

Molecular epidemiology of PZA resistance. Overall, PZA^r isolates accounted for 3.5% of lineage 1 (18/508), 4.8% of lineage 2 (44/922), 2.5% of lineage 3 (9/356), and 1.9% of lineage 4 (73/3861). Significant lineage-specific PZA^r associations were identified in the univariate analysis, where lineage 1 and lineage 2 *M. tuberculosis* isolates were approximately twice as likely to be PZA^r as lineage 4 isolates (Table 1). These associations maintained significance in the multivariate model and indicate a phylogenetic *M. tuberculosis* lineage association with PZA^r: lineage 1 (OR = 3.45; 95% CI = 1.72 to 6.95), lineage 2 (OR = 5.01; 95% CI = 3.13 to 8.03), and lineage 3 (OR = 3.30; 95% CI = 1.33 to 8.19) compared to lineage 4 (Table 2). Among the non-MDR population, phylogenetic lineage 1 was the only PZA^r predictor that maintained significance in the multivariate model (OR = 3.27; 95% CI = 1.64 to 6.51) (Table 4).

MDR TB case-control study. To examine PZA^r risk factors among the 159 MDR cases, we performed a case-control study.

TABLE 4 Adjusted[a] epidemiologic characteristics of pyrazinamide resistance among MDR and non-MDR TB cases

Characteristic	OR	95% CI	*P* value
MDR			
Age (yr%)	0.98	0.95–1.01	0.1518
HIV positivity	1.38	0.57–3.37	0.4724
TB infection site			
Extrapulmonary vs pulmonary	0.83	0.16–4.23	0.8211
Both vs pulmonary	0.44	0.12–1.60	0.2149
AFB smear	1.79	0.66–4.87	0.2556
History of LTBI	0.12	0.01–1.07	0.0572
EMB-SLD	3.48	1.57–7.69	0.0021
Non-MDR			
Age (yr)	0.99	0.98–1.01	0.5188
HIV positivity	0.95	0.46–1.98	0.8891
TB infection site			
Extrapulmonary vs pulmonary	0.89	0.28–2.81	0.8379
Both vs pulmonary	1.37	0.63–2.97	0.4256
AFB smear	1.82	0.93–3.58	0.0820
Lineage 1	3.27	1.64–6.51	0.0008

[a] Adjusted for known TB risk factors, including age, HIV, and any univariate variable with a *P* value of <0.2.

Sixty percent of MDR isolates (96/159) were considered genotypically clustered (identical IS*6110*-RFLPs and spoligotypes), with clusters ranging in size from 2 to 21 members. In contrast, only 43.6% (2,401/5,511) of the non-MDR strains were genotypically clustered (Table 3). Forty-four percent of MDR isolates (70/159) were PZA^r based on DST, 64% (45/70) of which belonged to a genotypic cluster. The genetic markers *pncA*, *katG*, and *rpoB* were used to further resolve clustering within the MDR population. Sequence data for the *fabG1* promoter did not provide additional cluster resolution and are not shown.

Complete sequence data (*katG*, *rpoB*, and *pncA*) were available for 88% (140/159) of MDR *M. tuberculosis* isolates (Fig. 1), while *pncA* sequence data were available for 143 isolates. Analysis of the *pncA* CDS-promoter region of MDR isolates identified 83 (58%) mutants. In total, there were 37 unique *pncA* mutations, which included insertions, deletions, and nonsynonymous substitutions. Some discordances between PZA^r determined by DST and the presence of *pncA* mutations (PZA^r-*pncA*) were observed (109/143; kappa = 0.54). However, the majority of these were accounted for by a single family of Beijing strains (termed W; $n = 54$) (29/54; kappa = 0.17), many of which carried a specific *pncA* mutation known to exhibit discordance with phenotypic PZA^r (16, 28), as discussed below. Concordance between the *pncA* sequence and PZA DST results was significantly higher when W strains were excluded and the remaining 89 strains were evaluated (80/89; kappa = 0.79).

Table 5 shows the resolution of genotypic clusters, using DNA sequence data to distinguish primary transmission from independent acquisition of MDR TB. Identical sequences for *katG*, *rpoB*, and *pncA* confirmed the genotyping assignment of seven clusters ($n = 38$). Conversely, diverse *pncA* mutations were identified within four genotypic clusters possessing identical IS*6110*-RFLPs, spoligotypes, and mutations in *katG* and *rpoB*, while a fifth cluster with identical *katG* and *pncA* sequences (P strain; spoligotype S00086) was resolved by different *rpoB* mutations.

TABLE 5 MDR cluster analysis using *katG*, *rpoB*, and *pncA* sequence data

	Genotypic cluster			*katG-rpoB*		*pncA*		
Infection	RFLP (spoligotype)	Lineage (spoligotype)	Count	Cluster	Count	Cluster	Count	Reference
Primary transmission	W (S00034)	2 (Beijing, ST523, ST623)	22	S315T_H526Y	21[a]	ACC(T)47GCC(A)	21[a]	28, 37
	W665 (S00034)	2 (Beijing, ST523, ST623)	3	S315T_H526Y	3	ACC(T)47GCC(A)	3	
	P1 (S00086)	4 (X, Harlem, LAM, Uganda)	2	S315T_H526Y	2	CTG(L)85CCG(P)	2	40, 41
	BW900 (S00005)	4 (X, Harlem, LAM, Uganda)	5	WT_H526D	5	WT[b]	5	
	C (S00030)	4 (X, Harlem, LAM, Uganda)	2	WT_S531L	2	WT	2	38
	DK22 (S00245)	4 (X, Harlem, LAM, Uganda)	2	S315T_L511P	2	WT	2	
	BW230 (S00241)	4 (X, Harlem, LAM, Uganda)	2	S315T_S531L	2	WT	2	39
Independent acquisition	W283 (S00034)	2 (Beijing, ST523, ST623)	3	S315T_S531L	2	290 Δ G	2	
				S315_WT	1	AGG(R)154GGG(G)	1	
	W148 (S00034)	2 (Beijing, ST523, ST623)	3	S315T_S531L	3	WT	1	54
						CAT(H)71CGT(R)	1	
						GGA(G)108CGA(R)	1	
	W1 (S00034)	2 (Beijing, ST523, ST623)	2	S315T_H526Y	2	ACC(T)47GCC(A)	1	28, 43
						ACC(T)47AGC(S)	1	
	W33 (S00034)	2 (Beijing, ST523, ST623)	2	S315T_H526Y	2	ACC(T)47GCC(A)	1	
						ACC(T)47AGC(S)	1	
	P (S00086)	4 (X, Harlem, LAM, Uganda)	3	S315T_D516V	1	CTG(L)85CCG(P)	3	40, 41
				S315T_H526Y	1			
				S315T_S531L	1			
Both	H (S00009)	4 (X, Harlem, LAM, Uganda)	11	S315T_S531L	9	70 Δ G	10	40, 41
				WT_WT	1			
				WT_L511P	1	WT	1	
Unresolved	W738 (S00034)	2 (Beijing, ST523, ST623)	3	S315T_WT	2	TCG(S)67CCG(P)	1[a]	
				S315T_S531L	1	WT	1	
	AB (S00145)	4 (X, Harlem, LAM, Uganda)	2	S315T_V146F	2	WT	1[a]	40
	W269 (S00034)	2 (Beijing, ST523, ST623)	2	WT_S531L	1[a]	ACG(T)142ATG(M)	1[a]	
	GD265 (S00474)	3 (CAS)	2	S315T_S531L	1[a]	CAG(Q)141CCG(P)[c]	2	

[a] One bad sequence.
[b] WT, wild type.
[c] Also contains a lineage-specific synonymous mutation (TCC[S]65TCT[S]).

DISCUSSION

The prevalence of PZA^r has been reported to range from 0.8 to 10% among patients with non-MDR TB and from 10 to 85% among patients with MDR TB worldwide (4, 29, 30). During the study period, TB caused by PZA^r *M. tuberculosis* in NYC was 50% higher than the national average and 20% higher among patients with MDR TB (31). Moreover, the MDR TB burden in NYC was 2-fold higher than national estimates. Thus, the high prevalence of MDR TB could be an explanation for the high proportion of PZA^r we observed. However, the MDR TB incidence in NYC declined over this period (32), while the incidence of PZA^r TB cases increased, indicating that additional factors were contributing to PZA^r in this population. To explain the national increase in PZA^r, Kurbatova and colleagues proposed that a higher proportion of foreign-born TB patients may reflect international programmatic variation in TB treatment protocols, leading to increased levels of PZA^r TB imported to the United States (31). While we observed high proportions of foreign-born TB patients annually (70 to 80%), we did not find being born outside the United States to be a statistically significant risk factor for PZA^r in NYC. In contrast to our findings in NYC, a recent analysis of PZA^r TB trends over 2 decades in San Francisco found rates similar to the national average and no significant association between PZA^r and MDR TB (33).

Our study found AFB smear positivity, clustering, and death to be independently associated with PZA^r. Together, these risk factors suggest that patients infected with PZA^r strains were infectious, transmitting, and not responding well to treatment. A history of TB was also independently associated with PZA^r, perhaps suggesting that a proportion of patients experienced relapses of drug-resistant TB, though relapse data were not available for this analysis (34, 35). Given the clinical importance of MDR TB and the high proportion of PZA^r among these patients, we sought to determine PZA^r risk factors in the MDR TB population. Concurrent resistance to EMB and SLDs was the only PZA^r risk factor that maintained statistical significance in the adjusted case-control MDR model. While a strong association between MDR-PZA^r and EMB^r has been associated with the inappropriate use of standard short-course therapy in patients with MDR TB (36), we do not have sufficient evidence here to address this question. A larger proportion of MDR-PZA^r among clustered versus nonclustered strains suggests that primary transmission was responsible for more PZA^r than acquired resistance within the MDR TB population.

Based on our molecular examination of MDR strains, we were able to further refine our cluster analysis. In particular, we identified MDR TB clusters with IS*6110*-RFLP; spoligotype; and *katG*, *rpoB*, and *pncA* mutations identical to those previously described

in a number of historic NYC drug-resistant outbreak strains (28, 37), e.g., strain C (S00030) (38), strain H (S00009), strain BW (S00241) (39), and strain P (S00086) (40, 41). The sequence-based confirmation of MDR genotypic clusters strongly suggests primary transmission and shows evidence that these historic strains were continuing to circulate and reactivate within NYC during the study period. For example, a 21-member cluster of the W (S00034; Beijing family) MDR-PZA^r strain contained genetic markers identical to those of a highly clonal strain that was responsible for a large NYC MDR outbreak in the early 1990s (28, 37). Smaller clusters of historic W variant strains previously described (28, 37, 42, 43) were also identified. Our data suggest that PZA^r also appeared independently in MDR TB (i.e., not as a result of primary transmission) (44). For example, the P strain was likely transmitted as a *katG-pncA* mutant prior to becoming MDR, which indicates PZA^r was at times acquired prior to MDR development, as seen in other strains (45, 46).

In addition to clustering, further microbial phylogenetic lineage effects were observed. In the general NYC TB population, PZA^r was associated with lineage 1 (East African/Indo-Oceanic), lineage 2 (East Asian/Beijing), and lineage 3 (East Africa/Central Asia) compared to lineage 4 (Euro-American). PZA^r associations with lineages 1 and 2 were consistent with lineage effects reported in the national study conducted by the CDC (31). Our data indicate that PZA^r among patients with MDR TB was associated with lineage 2, while PZA^r was associated with lineage 1 among patients with non-MDR TB. Phylogenetic *M. tuberculosis* lineages are strongly associated with geographic locations. Therefore, the observed phylogenetic associations in this study are curious and may be a proxy for the importation of PZA^r from specific non-U.S. locations. Additional studies would be needed to examine these microbial associations in light of social networks and neighborhood level effects.

Limitations of this study include moderate amounts of HIV data (70%), which is a well-established risk factor for drug-resistant TB. In addition, many demographic variables were self-reported, including a history of TB/LTBI, alcohol or substance abuse, and homelessness, which may have been subject to information bias and misclassification, though it is likely nondifferential. Furthermore, due to the technical complications of PZA-DST (8, 18), our findings may have been subject to some PZA^r misclassification. However, *pncA* sequence data have been shown to be a useful tool to confirm PZA^r-DST results (47). The most common *pncA*-PZA^r-DST inconsistency we observed was that of W strains with a *pncA* mutation in codon 47 (ACC [Thr]→GCC [Ala]). This specific mutation has been shown to correlate poorly with PZA-DST in several previous studies (12, 16, 28, 48, 49), suggesting that the mutation confers borderline resistance at PZA concentrations used routinely in PZA-DST (16). Also, the molecular data suggest that IS*6110*-RFLP analysis and spoligotyping may have slightly overestimated the extent of clustering and primary transmission.

Despite a steady decline in the total number of TB cases in NYC from 2001 to 2008, the incidence of PZA^r increased. Patients with PZA^r TB were more infectious and actively transmitting and had poor clinical outcomes compared to patients with TB caused by PZA^s *M. tuberculosis*. Among the MDR TB population, PZA^r was acquired somewhat more frequently via primary transmission than independently. In addition, concurrent EMB-SLD resistance was the only risk factor for PZA^r among patients with MDR TB. These observations have important clinical and public health implications for control of drug-resistant TB. Specifically, the strength of microbial risk factors for PZA^r highlights the importance of routine PZA-DST, genotyping, and confirmatory sequence analysis for ensuring appropriate drug therapy and disrupting transmission. Finally, as clinical trials of new regimens to shorten treatment of drug-susceptible and MDR TB are expanding, these results support the need to consider PZA^r in trial design (50–53).

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