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Does Resistance to Pyrazinamide Accurately Indicate the Presence of *Mycobacterium bovis*?

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***Mycobacterium bovis* is best identified by screening those isolates of the *Mycobacterium tuberculosis* complex that have any pyrazinamide (PZA) resistance, using a confirmatory test such as spoligotyping, biochemical testing, or genomic deletion analysis. The sensitivity for detection of *M. bovis* is lowered to 82% when only PZA-monoresistant isolates are screened.**

Mycobacterium bovis is intrinsically resistant to pyrazinamide (PZA), and the prevalence of clinical infection with *M. bovis* is low in countries with good bovine tuberculosis control programs. This finding has supported a strategy of using PZA monoresistance as an initial screening tool for *M. bovis*, a strategy that risks missing cases of infection with *M. bovis* strains that have broader resistance. A previous study found that screening for *M. bovis* by using PZA monoresistance had a poor positive predictive value (3), but the study was not able to assess the sensitivity or specificity of PZA resistance screening for the detection of *M. bovis* as it did not include data on the denominator. We therefore sought to assess the efficacy of using either PZA monoresistance or any PZA resistance to identify *M. bovis* in a population-based study. At the same time, we estimated the prevalence of *M. bovis* in San Francisco.

Mycobacterial isolates in all diagnosed cases of tuberculosis in San Francisco were collected in a prospective study (4). The isolates underwent biochemical testing for niacin and nitrate production for identification of species within the *Mycobacterium tuberculosis* complex, as the vast majority of isolates of *M. bovis* are niacin and nitrate negative (6, 8). Susceptibility testing for PZA and other drugs was done with the Bactec method (S. Siddiqi, Bactec 460TB system product and procedure manual, Becton Dickinson and Co., Sparks, MD). Isolates were sent to Stanford University for standard molecular typing using IS*6110* and polymorphic guanine-cytosine-rich sequence (PGRS) restriction fragment length polymorphism (2, 9, 10).

We performed a retrospective cohort study, which included all available isolates from San Francisco from 1991 to 1999 identified as *M. bovis* by nitrate and niacin testing and all isolates that were PZA resistant. Isolates without a PZA susceptibility test result were excluded. For the identification of *M. bovis*, isolates were screened for the absence of the region of difference 4 (RD4) and RD9 (1). All *M. bovis* isolates were screened for the RD1 deletion, which is specific to *M. bovis* bacillus Calmette-Guérin (BCG) (1). Spoligotyping was also performed (5); *M. bovis* has a characteristic pattern consisting of deleted direct repeat spacers 39 to 43, and BCG has a characteristic pattern with three additional deletions. Isolates without available DNA were classified based on the biochemical test results only.

Statistical analysis was done using Stata version 8 (Stata Corporation, College Park, TX). We used the chi-square test of proportions or Fisher's exact test to compare the characteristics of cases of *M. bovis* infection with those of cases of *M. tuberculosis* infection.

Of 2,476 cases of tuberculosis diagnosed and reported in San Francisco from 1991 to 1999, 2,115 (85.4%) were culture positive and 1,526 (72.2%) had a PZA susceptibility test result. The rate of PZA susceptibility testing varied by year, with <20% of isolates tested from 1993 to 1994, 70 to 80% in 1992 and 1995, and >90% of isolates from other years. PZA testing was random, and isolates of *M. bovis* as identified by biochemical testing were not more likely to have been tested than non-*M. bovis* isolates ($P = 0.54$). Our study identified 30 PZA-resistant isolates; 25 had a viable culture and/or DNA available. PZA monoresistance was present in 18 of 30 isolates (Table 1). One additional isolate was pansensitive and was identified as *M. bovis* by biochemical testing but as *M. tuberculosis* by spoligotyping and genomic deletion analysis. Conversely, two PZA-resistant isolates were identified as *M. tuber-*

TABLE 1. Results of PZA susceptibility testing for isolates in culture-positive cases in San Francisco from 1991 to 1999

Resistance profile[a]	No. of resistant isolates/no. of isolates tested (%) of species		OR (95% CI)[b]	*P* value
	M. bovis (including BCG)	*M. tuberculosis*		
PZA resistant	11/11 (100)	19/1,515 (1.3)		<0.001
PZA monoresistant	9/11 (82)	9/19 (47)	5.0 (0.85–30)	0.076
Initially resistant to other drug	2/11 (18)	230/1,515 (15)	1.24 (0.27–5.8)	0.78

[a] PZA susceptibility testing was performed on 11 (85%) of 13 *M. bovis* isolates and 1,515 (72%) of 2,102 *M. tuberculosis* isolates (OR, 2.1; 95% confidence interval, 0.47–9.6; *P*, 0.33).
[b] CI, confidence interval.

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TABLE 2. Clinical and test characteristics of strains identified as *M. bovis* by PZA resistance screening[a] in San Francisco from 1991 to 1999

Case no.	Age (yrs) of patient	Type of infection	Patient's country[b] of birth	Other drug resistance	Identification per biochemical testing	Presence[c] of:			Identification per spoligotyping	No. of IS*6110* copies	PGRS result
						RD1	RD4	RD9			
1	37	Pulmonary	RP	INH	*M. tuberculosis*	P	A	A	*M. bovis*	1	p002
2	33	Pulmonary	MX		*M. bovis*	P	A	A	*M. bovis*	1	p002
3	77	Miliary	US		*M. bovis*	ND	ND	ND	ND	ND	ND
4	32	Miliary	MX		*M. bovis*	P	A	A	*M. bovis*	1	p032
5	71	Genitourinary	US		*M. bovis*	A	A	A	BCG	1	Unique
6	90	Genitourinary	US		*M. bovis*	A	A	A	BCG	1	Unique
7	12	Lymphatic	US		*M. bovis*	P	A	A	*M. bovis*	1	p032
8	24	Pulmonary	MX		*M. bovis*	P	A	A	*M. bovis*	2	p025
9	31	Pulmonary	MX		*M. bovis*	ND	ND	ND	ND	ND	ND
10	32	Pulmonary	MX		*M. bovis*	P	A	A	*M. bovis*	1	ND
11	40	Pulmonary	HK	INH	*M. tuberculosis*	A	ND	A	BCG	1	ND

[a] Data shown are for PZA-resistant isolates.
[b] RP, Philippines; MX, Mexico; US, United States; HK, Hong Kong.
[c] P, present; A, absent; ND, not determined.

culosis by biochemical testing and as *M. bovis* by spoligotyping and genomic deletion analysis (Table 2). The sensitivity for detecting *M. bovis* increased from 81.8% when PZA monoresistance was used to 100% when any PZA resistance was used. Two-thirds of PZA-resistant isolates and half of PZA-monoresistant isolates were *M. tuberculosis*. The positive predictive values of PZA monoresistance versus any PZA resistance were 50.0 and 36.7%, respectively. The specificity and negative predictive value were >98% with both strategies (Table 3).

After excluding two cases of *M. bovis* BCG administration for bladder cancer that resulted in genitourinary disease, we identified nine cases of *M. bovis* infection in San Francisco between 1991 and 1999. These represented 0.6% of all culture-positive cases of tuberculosis with PZA susceptibility test results. One of the nine isolates was identified as *M. bovis* BCG and was resistant to isoniazid (INH) and PZA. This strain was isolated from a 40-year-old Chinese woman with noncavitary pulmonary disease who had not undergone human immunodeficiency virus testing. Patients infected with *M. bovis* versus *M. tuberculosis* were more likely to have been born in Mexico (odds ratio [OR], 33; $P < 0.005$) and were younger (median ages, 32 and 45 years old, respectively; $P = 0.024$).

TABLE 3. Results of PZA susceptibility testing for identification of *M. bovis*

Parameter	Test result[b]	95% CI[a]
Any PZA resistance		
Sensitivity	100 (11/11)	71.5–100
Specificity	98.7 (1,496/1,515)	98.0–99.2
Positive predictive value	36.7 (11/30)	19.9–56.2
Negative predictive value	100 (1,496/1,496)	99.8–100
PZA monoresistance		
Sensitivity	81.8 (9/11)	48.2–97.7
Specificity	99.4 (1,506/1,515)	98.9–99.7
Positive predictive value	50.0 (9/18)	26.0–74.0
Negative predictive value	99.9 (1,506/1,508)	99.5–100

[a] CI, confidence interval.
[b] Values are percentages. Values in parentheses were used to calculate the percentages and were obtained as follows: sensitivity, $a/(a + c)$; specificity, $d/(b + d)$; positive predictive value, $a/(a + b)$; and negative predictive value, $d/(c + d)$. In these calculations, a is the number of *M. bovis* isolates that were PZA (mono)resistant, b is the number of *M. tuberculosis* isolates that were PZA (mono)resistant, c is the number of *M. bovis* isolates that were PZA susceptible, and d is the number of *M. tuberculosis* isolates that were PZA susceptible.

Among the nine *M. bovis* isolates with IS*6110* and PGRS restriction fragment length polymorphism data available, eight had a single IS*6110* band. There were two clusters corresponding to two persons with identical genotyping patterns, but there were no epidemiological links between them.

Our results suggest that definitive testing to distinguish *M. bovis* from *M. tuberculosis* should include all PZA-resistant isolates, not only those with PZA monoresistance.

The prevalence of *M. bovis* in San Francisco was 0.6%, similar to the prevalence in other countries with good bovine tuberculosis control programs but lower than the reported 6.6% prevalence in San Diego (7). The association between birth in Mexico and *M. bovis* infection was similar to that found in the study in San Diego. However, the lower prevalence of *M. bovis* in San Francisco likely reflects the greater distance of San Francisco from the Mexican border.

The lack of DNA for 5 of the 30 isolates classified by biochemical test results might have introduced misclassification of *M. bovis* and *M. tuberculosis* isolates. However, the biochemical test results correlated well with the results of spoligotyping and genomic deletion analysis. Although 28% of the isolates were not tested for PZA resistance, PZA testing was random and should not bias our estimates of the sensitivity and specificity. Given the small number of cases of *M. bovis* infection, our estimates of sensitivity and positive predictive value have wide confidence intervals. The positive predictive value of PZA resistance for the identification of *M. bovis* would likely be higher in areas where *M. bovis* is endemic in cattle. Since the techniques we used are not universally available, optimal testing strategies will vary depending on local resources.

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