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Rapid Drug Susceptibility Testing with a Molecular Beacon Assay Is Associated with Earlier Diagnosis and Treatment of Multidrug-Resistant Tuberculosis in California▽

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To assess the clinical impact of a molecular beacon (MB) assay that detects multidrug-resistant tuberculosis (MDR TB), we retrospectively reviewed records of 127 MDR TB patients with and without MB testing between 2004 and 2007. Use of the MB assay reduced the time to detection and treatment of MDR TB.

The emergence and spread of multidrug-resistant tuberculosis (MDR TB) threatens TB control worldwide. MDR TB is resistant to the most effective first-line agents, isoniazid (INH) and rifampin (RIF). The cornerstone of MDR TB diagnosis is culture and drug susceptibility testing (DST) of *Mycobacterium tuberculosis* isolates. Conventional methods for culture and first-line DST require weeks, causing delays in MDR TB diagnosis and treatment, which in turn are associated with advanced disease (6, 8), treatment failure (4), and transmission. Rapid molecular methods have been developed for the detection of mutations that confer drug resistance (3, 5, 7), and several public health organizations have endorsed molecular DST for TB (2, 9).

The California Department of Public Health has been performing a molecular beacon (MB) assay in which real-time PCR is performed on acid-fast bacilli (AFB) smear-positive sputa or *M. tuberculosis* cultures to detect mutations conferring resistance to INH and RIF. TB providers in California are encouraged to submit specimens for MB testing from patients in whom drug-resistant TB is suspected (i.e., patients with a history of prior TB treatment, patients born in nations with high rates of resistant TB, patients failing TB treatment, and those who are known contacts to MDR TB cases). The assay is not FDA approved but is highly sensitive and specific (5), detects silent mutations infrequently, and shows >95% agreement with phenotypic DST results after testing on nearly 200 unique samples (unpublished). We performed a retrospective cohort study among MDR TB patients to assess the clinical impact of the MB assay in a public health setting in California.

This study used deidentified demographic and clinical data that were collected by the California Department of Public Health for TB surveillance. Standard TB treatment was defined as any regimen containing at least 3 drugs, including INH and RIF. MDR treatment was defined as any regimen containing at least 4 drugs and including at least 2 second-line anti-TB medications (e.g., a fluoroquinolone, injectable agent [amikacin, kanamycin, capreomycin], para-aminosalicylic acid, cycloserine, ethionamide, or linezolid) (1, 10). The time to sputum culture conversion was defined as the interval between the collection dates of the first positive sputum culture and the first consistently negative sputum culture. Median times were compared using Wilcoxon's two-sample test. Proportions were tested using chi-square testing or Fisher's exact test. The MB assay was performed as previously described (5).

Of 139 culture-positive MDR TB cases reported in California from 2004 through 2007, 12 cases were excluded because the MDR TB treatment start date could not be determined. Among the remaining 127 MDR TB cases, 27 (21%) had specimens tested by MB with confirmatory phenotypic DST, while 100 (79%) had phenotypic DST alone. In the MB group, 19 (70%) had a concentrated sputum specimen tested, and 8 (30%) had an *M. tuberculosis* isolate tested.

In both groups, the majority of patients were foreign-born and of Asian ethnicity. Patients with MB testing were more likely to be female (70% versus 43%; $P = 0.0116$), reside in rural jurisdictions (51.9% versus 27%; $P = 0.0143$), have a history of previous TB (62% versus 24%; $P = 0.0003$), have a positive sputum smear (82% versus 60%; $P = 0.0404$), and have a cavity on their chest radiograph (48% versus 24%; $P = 0.0166$) compared to patients tested by phenotypic DST only (Table 1). In the MB group, all patients had pulmonary TB, while in the non-MB group, 80% had pulmonary disease and 20% had either extrapulmonary or both pulmonary and extrapulmonary disease.

Compared to patients without MB testing, patients with MB testing spent less time on standard TB therapy and started MDR treatment regimens more promptly after the case report

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TABLE 1. Characteristics of MDR TB patients with and without MB testing

Demographic	No. of patients (%)[a]: With MB testing (n = 27)	Without MB testing (n = 100)	P value
Median age	34	38	0.4775
Age range	21–56	27–49	
Sex			0.0116
Male	8 (29.6)	57 (57.0)	
Female	19 (70.4)	43 (43.0)	
Race/ethnicity			0.6485
White	1 (3.7)	4 (4.0)	
Black	2 (7.4)	4 (4.0)	
Hispanic	4 (14.8)	25 (25.0)	
Asian/Pacific Islander	20 (74.0)	67 (67.0)	
Place of birth			0.3654
United States	3 (11.1)	5 (5.0)	
Outside the United States	24 (88.9)	95 (95.0)	
Previous TB			0.0003
Yes	16 (61.5)	24 (24.2)	
No	10 (38.5)	75 (75.8)	
Homeless			0.6800
Yes	2 (7.4)	6 (6.1)	
No	25 (92.6)	93 (93.9)	
Excess alcohol use			1.0000
Yes	1 (3.9)	5 (5.1)	
No	25 (96.2)	94 (95.0)	
Injection or noninjection drug use			0.5792
Yes	0 (0.0)	4 (4.0)	
No	26 (100.0)	95 (96.0)	
AIDS			1.0000
Yes	0 (0.0)	3 (3.0)	
No	27 (100.0)	97 (97.0)	
Moved during treatment			0.1744
Yes	5 (18.5)	9 (9.0)	
No	22 (81.5)	91 (91.0)	
Cavity noted on chest radiograph			0.0166
Yes	13 (48.2)	22 (24.2)	
No	14 (51.9)	69 (75.8)	
AFB sputum smear			0.0404
Positive	22 (81.5)	59 (60.2)	
Negative	5 (18.5)	39 (39.8)	
Jurisdiction			0.0143
Urban	13 (48.2)	73 (73)	
Rural	14 (51.9)	27 (27)	
Provider type			0.3582
Health department	14 (51.8)	57 (57)	
Private	1 (3.7)	10 (10)	
Both	4 (14.8)	17 (17)	
Unknown	8 (29.6)	16 (16)	

[a] Values for median age and age range are given in years. All other values represent the no. of patients (%).

(Table 2). In addition, among patients with positive smears, the time to the culture conversion was less in those with MB testing than in those with phenotypic DST (median, 63 versus 90 days; $P = 0.1698$), although this difference was not statistically significant. In both groups, approximately two-thirds of patients completed treatment. Twenty percent of patients without MB testing and 30% of patients with MB testing remained on therapy at the time of data collection. Four of 100 (4.0%) patients without MB testing died while on therapy. There were no deaths in the MB group.

Within the MB group, there were no differences in demographic variables, culture conversion times, and lengths of therapy between patients who had MB testing performed on concentrated sputum sediments and those who had MB testing performed on *M. tuberculosis* isolates. The MB assay was not performed directly on smear-negative sediments due to low sensitivity, so patients with MB testing on sediments were more likely to have positive AFB sputum smears (100% versus 37.5%; $P = 0.0006$) than patients with MB testing on isolates. Also, MB testing on sediments had faster turnaround times than testing on isolates. As a result, patients with MB testing on sediments had shorter intervals between sputum collection and MB results (6 days versus 26 days; $P = 0.0002$) and started MDR treatment regimens sooner after the case report (34 days versus 68.5 days; $P = 0.0525$) than patients with MB testing on isolates.

There are several limitations to this study. The group with MB testing was comprised of patients with higher organism burdens and more advanced disease, as indicated by the high proportion of patients with positive sputum smears and with cavities noted on chest radiographs (Table 1). These differences in the two study groups may have led to underestimating the impact of MBs, since patients with more severe disease are likely to require more time for sputum culture conversion. Because the MB assay does not have sufficient sensitivity to use directly on smear-negative sediments, MB testing had less impact on patients with smear-negative disease. In addition, the study was retrospective, and we could not ensure that sputum cultures were obtained at standardized intervals.

Nevertheless, our findings show that the use of the MB assay in California was associated with more timely detection and treatment of MDR TB. This study is among the first to quantify the impact of molecular DSTs in a nation with low TB incidence and suggests such assays may improve MDR TB outcomes and control within the United States.

TABLE 2. Treatment characteristics of MDR TB patients with and without MB testing[a]

MB testing	From case report to MDR treatment initiation		From standard treatment initiation to MDR treatment initiation		To culture conversion		To culture conversion, AFB smear positive only		For treatment among patients that completed treatment	
	No. of patients/ total no. of patients	No. of days (IQ range)	No. of patients	No. of days (IQ range)	No. of patients	No. of days (IQ range)	No. of patients	No. of days (IQ range)	No. of patients	No. of days (IQ range)
Yes	27/27	38 (24–65)	27/27	13 (0–26)	18/27	61.5 (35–105)	15/27	63 (36–143)	17/27	732 (659–787)
No	100/100	79 (53.5–120)	100/100	53 (30–84)	68/100	84 (49–128)	47/100	90 (65–141)	73/100	751 (608–818)
P value	<0.0001		<0.0001		0.2582		0.1698		0.6063	

[a] IQ, interquartile.

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The authors declare no conflicts of interest.

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