Statewide Survey of Laboratories Performing *Mycobacterium tuberculosis* Testing in Minnesota

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SYNOPSIS

RAPID AND ACCURATE laboratory detection and identification of *Mycobacterium tuberculosis*, particularly multidrug-resistant strains, is critical to both public health control measures and patient management. The authors surveyed microbiology laboratories to evaluate whether their methods met national guidelines. As needed, laboratories received individualized recommendations for improvement. The laboratories were resurveyed a year later to assess changes in methods.

Current guidelines recommend fluorochrome acid-fast smears, broth cultures, identification by nucleic acid probe or BACTEC-NAP, and BACTEC primary susceptibility panels, which should include pyrazinamide. Of 27 laboratories performing acid-fast smears, 15 used fluorochrome methods. Six of 16 laboratories performing mycobacterial cultures used broth media. Of six laboratories performing species identification, five used nucleic acid probes or BACTEC-NAP. Of five laboratories evaluating drug sensitivity, two used BACTEC and two included pyrazinamide in their protocols. Overall, 24 (89%) laboratories needed improvements; a year later, 16 (67%) of those had altered their methods or made definite plans to do so.

Survey results suggest that health departments can facilitate improvements in laboratory testing for pathogens of public health importance.

Recently, tuberculosis (TB) epidemiology has demonstrated the emergence of multidrug-resistant strains of TB in the United States, particularly in New York City.[1] A number of large outbreaks of multidrug-resistant TB in institutional settings such as hospitals and correctional facilities have been reported.[2,3] Delays in both diagnosis and initiation of effective therapy regimens due to slow completion and reporting of acid-fast smears, mycobacterial cultures, and drug susceptibility results have contributed to these extensive outbreaks.[3] Prompt identification and drug susceptibility testing of *Mycobacterium tuberculosis* isolates are essential to devising appropriate therapeutic regimens for TB patients.[4–6] The United States Public Health Service's "National Action Plan to Combat Multidrug-Resistant Tuberculosis"[4] includes strategies to maximize laboratory capacity for *M. tuberculosis* testing and to increase awareness and knowledge of this pathogen among public health and microbiology laboratory personnel.

Many areas of the United States have experienced increases in TB case numbers as well as the emergence of multidrug-resistant disease.[7–11] Successful public health control measures for TB require systematic and thorough public health surveillance for disease caused by *M. tuberculosis*.[12,13] Accurate and timely surveillance data are essential to identify cases and assure adequate therapy, to monitor trends in TB disease over time in specific population groups or geographic areas, and to conduct contact investigations and other efforts to break the chain of transmission in the community.[5,13] While TB surveillance is usually conducted through clinicians' reports of TB cases, laboratory-based surveillance can be used to assure complete reporting in a given state or local area. Therefore, the clinical laboratory can play a primary role in public health efforts to curtail transmission of the disease.

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Current laboratory guidelines for microbiologic testing for *M. tuberculosis* were recently outlined by Tenover et al.[5] To achieve these goals in our state, the Minnesota Department of Health (MDH) surveyed all clinical microbiology laboratories performing *M. tuberculosis* testing in the state. The purpose of the surveys was to evaluate whether the microbiologic methods used at testing facilities met national laboratory guidelines, to make recommendations for improvement, and to establish a laboratory-based surveillance network. Results of the surveys are described here.

Methods

In September 1992, we surveyed all major clinical laboratories in Minnesota (N=125) to determine which laboratories performed diagnostic tests for specific communicable diseases, including TB. Of these 125 laboratories, 31 indicated that they tested for *M. tuberculosis*. We resurveyed these 31 laboratories in June 1993 to evaluate specific laboratory practices for identification of *M. tuberculosis*.

The June 1993 survey assessed each laboratory's use of microbiologic tests for *M. tuberculosis* (acid-fast smears, mycobacterial cultures, species identification, and drug susceptibility testing) and determined the volume of tests processed at each facility during the previous calendar year (1992). The survey also evaluated laboratory practices for reporting to MDH isolates of *M. tuberculosis* identified on site. To obtain complete information, we made telephone calls to laboratories that did not return or fully complete the survey within three weeks.

National Guidelines[1] and Minnesota Reporting Requirements[2] for Microbiologic Isolation and Testing of *M. tuberculosis*

Test	*Recommendation*
Acid-fast smear	Fluorochrome staining (concentrated specimens)
Mycobacterial culture	Broth culture methods (e.g., BACTEC or BBL Septi-Chek AFB[3])
Species identification	Nucleic acid probes or BACTEC-NAP
Drug susceptibility	BACTEC
	All *M. tuberculosis* isolates (unless isolate tested within past 3 months)
	INH, RIF, SM, EMB, PZA
Reporting to state health department	Medical laboratories are required to report identification of *M. tuberculosis* to MDH within one working day of completion of test

[1] Tenover, et al. The resurgence of tuberculosis: is your laboratory ready? J Clin Microbiol 1993;31: 767–770.
[2] Minnesota Rule 4605.7040.
[3] Formerly Roche Septi-Chek.

We compared each laboratory's reported microbiologic methods to current laboratory guidelines outlined by Tenover et al.[5] (See Table.) In addition, we compared laboratory practices for reporting *M. tuberculosis* isolates to MDH to procedures required by the Minnesota Rules Governing Communicable Diseases (Minnesota Rules 4605.7000-4605.7800). Based on these comparisons, we developed recommendations for improvement specific to each facility. We then provided participating laboratories with summarized survey results and, as needed, individualized recommendations. The summarized information did not identify any of the laboratories.

Twenty-four laboratories received recommendations for improvement. We sent a follow-up survey to these 24 laboratories in June 1994 (one year later) to determine changes made since the initial survey in microbiologic methods and reporting practices for *M. tuberculosis*. The follow-up survey consisted of a subset of questions from the initial survey that elicited information on laboratory methods and reporting

practices. Laboratories that did not return the follow-up survey by mail within three weeks were contacted by telephone.

Results

Of the 31 clinical laboratories surveyed in June 1993, 27 indicated that they routinely performed microbiologic tests for *M. tuberculosis* (acid-fast smears, mycobacterial cultures, species identification, or drug susceptibility testing) on site. The four remaining laboratories were not testing for *M. tuberculosis*; they either had discontinued testing for *M. tuberculosis* since the 1992 survey or had misidentified themselves in the earlier survey. We evaluated compliance with national laboratory guidelines for these 27 laboratories at the times of the initial and follow-up surveys. Results (see Table) were examined by number of laboratories and by number of specimens processed in 1992 to reflect the size of laboratories involved.

Those laboratories that received recommendations for improvement accounted for a minority of specimens processed in 1992, suggesting that laboratories needing improvements are most often those processing fewer specimens.

Current guidelines recommend use of fluorochrome methods for acid-fast smears.[5,14] All 27 laboratories performed acid-fast smears in 1993. Only 15 (56%), representing 90% of acid-fast smears processed overall in 1992 by the laboratories surveyed, had used fluorochrome methods in June 1993. The remaining 12 laboratories had received recommendations from MDH for improvement. Seven of these either adopted fluorochrome methods by the time of the follow-up sur-

Table. Compliance with National Guidelines for Microbiologic Tests for *M. tuberculosis* by laboratories performing tests and by number of specimens processed, Minnesota, 1992

Test (Recommendation)[1]	Labs performing tests	Total Specimens 1992	Compliant at Initial Survey Number (Percent)		Compliant at Follow-up Survey Number (Percent)		Compliant by July 1995 Number (Percent)		Not Compliant by July 1995 Number (Percent)	
			Labs	Specimens	Labs	Specimens	Labs	Specimens	Labs	Specimens
Acid–fast smear (fluorochrome)	27	38,372	15 (56)	34,504 (90)	3 (11)	826 (2)	4 (15)	2,817 (7)	5 (19)	225 (1)
Mycobacterial culture (broth culture)	16	38,235	6 (38)	31,156 (81)	6 (38)	4,101 (11)	3 (19)	2,878 (8)	1 (6)	100 (<1)
Species identification (nucleic acid probe or BACTEC–NAP)	6	3,843	5 (83)	3,803 (99)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	40 (1)
Reporting to state health department (report to MDH within one working day of completing test)	6	718	2 (33)	160 (22)	1 (17)	400 (56)	0 (0)	0 (0)	3 (50)	158 (22)
Drug susceptibility: method (BACTEC)	5	581	2 (40)	138 (24)	1 (20)	0 (0)	1 (20)	423 (73)	1 (20)	20 (3)
Drug susceptibility: panel (INH, RIF, SM, EMB, PZA)	5	581	2 (40)	423 (73)	1 (20)	65 (11)	1 (20)	20 (3)	1 (20)	73 (13)
Drug susceptibility: frequency (all isolates unless tested in past 3 mos.)	5	581	1(20)	73 (13)	2 (40)	488 (84)	1 (20)	20 (3)	1 (20)	0 (0)

[1] Tenover FC, et al. The resurgence of tuberculosis: is your laboratory ready? J Clin Microbiol 1993;31:767–770.

vey or had made definite plans to do so by July 1995.

National guidelines recommend use of broth culture methods such as BACTEC (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD), BBL Septi-Chek AFB (Becton Dickinson Microbiology Systems, Cockeysville, MD), or MGIT (Becton Dickinson Microbiology Systems, Cockeysville, MD) for mycobacterial cultures.[3,5,14] Sixteen laboratories performed mycobacterial cultures in 1993. Of these, six (38%), representing 81% of mycobacterial cultures processed in 1992 by the laboratories surveyed, used broth culture methods at that time. Of the ten laboratories that received recommendations from MDH for improvement, nine had either adopted broth culture methods by the time of the follow-up survey or made definite plans to do so by July 1995.

Current guidelines recommend use of nucleic acid probes or BACTEC-NAP (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) for species identification of *M. tuberculosis*.[3,5,14] Of six laboratories performing *M. tuberculosis* species identification on-site in 1993, five (83%), which identified 99% of the mycobacterial isolates overall in 1992 by the laboratories surveyed, had used a recommended method for these tests in 1992. The one remaining laboratory had received recommendations for improvement. This laboratory planned to adopt polymerase chain reaction (PCR) techniques by July 1995 to confirm the identification of *M. tuberculosis*, although the use of PCR to identify *M. tuberculosis* from laboratory media has not yet been recommended by national guidelines.

These surveys suggest that state or local health departments can positively influence statewide laboratory practices for identification of infectious agents such as *M. tuberculosis* by working closely with laboratories to enhance surveillance and information sharing.

Any medical laboratory that isolates *M. tuberculosis* is required to report to MDH within one working day of completion of the species identification test (Minnesota Rule 4605.7040). Of the six laboratories performing species identification of *M. tuberculosis*, two (33%), which together identified 22% of *M. tuberculosis* isolates identified overall in 1992 by the laboratories surveyed, reported the identification of these isolates to MDH in a manner consistent with Minnesota Rules at the time of the initial survey in June 1993. Four laboratories received recommendations from MDH regarding reporting of *M. tuberculosis*, one of which altered its practices to conform with the communicable disease reporting rule prior to the follow-up survey. MDH subsequently implemented active laboratory-based surveillance for *M. tuberculosis* in August 1995 to assure reporting from all sites. Isolates from all laboratories statewide are now received at MDH and forwarded to the regional reference laboratory, the Molecular Epidemiology Unit of the Michigan Department of Public Health, for restriction fragment-length polymorphism (RFLP) testing.

National guidelines recommend the BACTEC method for drug susceptibility testing of *M. tuberculosis* isolates.[3,5,14] Of five laboratories testing *M. tuberculosis* isolates for drug sensitivity in 1993, two (40%), representing 24% of such isolates tested overall in 1992 by the laboratories surveyed, used the BACTEC method. The three remaining laboratories received recommendations for improvement; two of these either adopted the BACTEC method by the time of the follow-up survey or made definite plans to do so by July 1995.

According to current guidelines, the primary drug susceptibility panel for *M. tuberculosis* isolates should include isoniazid (INH), rifampin (RIF), ethambutol (EMB), streptomycin (SM), and pyrazinamide (PZA).[5,14] In 1993, drug susceptibility panels at each of the five laboratories performing this test included INH, RIF, EMB, and SM; two laboratories (40%), representing 73% of *M. tuberculosis* isolates tested for drug susceptibility in 1992 by the laboratories surveyed, also included PZA. Of the three laboratories that received recommendations from MDH to incorporate PZA testing, two had either added PZA by the time of the follow-up survey or made definite plans to do so by July 1995.

Current guidelines recommend that drug susceptibility testing should be performed for all initial isolates of *M. tuberculosis* and for patients who have received at least three months of therapy.[5] Of five laboratories performing drug susceptibility testing in 1993, only one (20%), which performed 13% of *M. tuberculosis* drug susceptibility tests performed overall in 1992 by the laboratories surveyed, tested *M. tuberculosis* isolates for susceptibility at least as frequently as recommended. Of four laboratories that received recommendations for change, three either adopted these recommendations prior to the follow-up survey or made plans to do so by July 1995.

Discussion

The surveys revealed several areas in which ongoing improvements are needed in *M. tuberculosis* testing practices in Minnesota. In 1992, most *M. tuberculosis* isolates were identified at laboratories where methods and frequency of drug susceptibility testing did not meet current guidelines. Although substantial improvements were made in the methods used by the laboratories and the frequency of drug susceptibility testing, addition of PZA to the primary drug susceptibility panel is still needed at one of the five laboratories performing these tests. This laboratory represents 13% of isolates processed overall in 1992 by the laboratories surveyed. The surveys also highlighted the need to improve laboratory-based surveillance for *M. tuberculosis*. Active laboratory-based surveillance was subsequently initiated statewide by August 1995.

The results of these surveys of microbiology laboratories in Minnesota are comparable to findings of a national survey conducted by the Centers for Disease Control in collaboration with the Association of State and Territorial Public Health Laboratory Directors. In late 1991, 56 state and territorial public health laboratories were surveyed to assess whether the most rapid methods for *M. tuberculosis* testing were being used. The investigators concluded that many laboratories were performing the most rapid techniques available for microscopy and species identification, although most laboratories were not using rapid radiometric methods for mycobacterial cultures and drug susceptibility testing.[15]

Overall, 16 (67%) of the 24 laboratories that received recommendations from MDH either altered their methods for *M. tuberculosis* testing by the time of the follow-up survey or made definite plans to do so by July 1995. Twelve of these 16 laboratories instituted at least one change prior to the follow-up survey. Those laboratories that received recommendations for improvement accounted for a minority of specimens processed in 1992, suggesting that laboratories needing improvements are most often those processing fewer specimens. While we cannot assess the direct impact of our efforts to improve laboratory practices, these results clearly suggest that the initial laboratory survey and subsequent recommendations enhanced laboratory capability for identification and testing of *M. tuberculosis* statewide. By July 1995, the majority of laboratories in Minnesota, accounting for nearly all specimens processed for *M. tuberculosis* testing, met the guidelines outlined by Tenover et al.[5]

The findings of these surveys suggest that state or local health departments can positively influence statewide laboratory practices for identification of infectious agents such as *M. tuberculosis* by working closely with laboratories to enhance surveillance and information sharing. Collaboration between epidemiologists and laboratorians within MDH facilitated these efforts to improve laboratory methods and surveillance statewide.

Creating networks between health departments and clinical laboratories to improve laboratory practices and disease surveillance is also critical for the identification and control of other infectious diseases of public health importance. This is particularly important for recently emerging pathogens such as *Escherichia coli* O157:H7, antibiotic-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *enterococci*.[16]

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