The Molecular 'MDR Screen' is an Important Tool in the Diagnosis and Initiation of Appropriate Therapy in TB Patients in the State of Florida

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Abstract

Objective: The Florida Department of Health Bureau of Public Health Laboratories (BPHL) algorithm for the diagnosis and anti-mycobacterial susceptibility testing (AST) of tuberculosis (TB) includes a molecular 'MDR (multi-drug resistance) screen' for the rapid and accurate detection of drug resistance in TB. This study reviews the performance of the MDR screen and initiation of appropriate TB patient therapy.

Study Design: Analysis of all AST data over a 2-year period was performed, followed by review of patient treatment records for MDR cases. The following data was analysed: date of MDR screen result by Hain Genotype® MTBDR*plus* assay, version 2 (Hain LifeScience); date of phenotypic AST result by Sensititre MIC assay (TREK Diagnostic Systems, Thermo Fisher); and date of initiation of appropriate MDR treatment regimen.

Results: From January 1, 2014 to December 31, 2015, MDR screen was performed on n=603 primary clinical specimens. Of 603 primary specimens a result could not be determined for n=81 (indeterminate or positive control band for TB missing). Of the 522 primary specimens for which a result was known, n=56 showed resistance to one or both rifampin (RIF) and isoniazid (INH): 13 were MDR (resistant to both RIF and INH), 5 were RIF mono-resistant, and 38 were INH resistant. In-depth review of the MDR cases showed that 9 were Florida patients, for which treatment data was available. Of these patients, the average number of days from date of MDR screen result to change to appropriate therapy was n=6.9 days (range 1-16 days). The number of days from change in appropriate therapy to when the phenotypic AST was available was n=30.2 days (range 11-77 days).

Conclusions: From 2014-2015, 9.3% of primary clinical specimens tested by the MDR screen showed resistance to one or both RIF and INH, MDR-TB was detected in 2.15%. The MDR screen provided results for change to appropriate therapy for MDR patients that would not have been available for several days, and in most cases weeks, if clinicians had waited for phenotypic AST results. Ultimately placing patients on appropriate therapy in a timely manner has a positive impact on patient and public health outcomes

Background

- Over the last several years there have been tremendous advances in understanding the mechanisms of antimicrobial resistance, with the implementation of molecular techniques. Mutations within *Mycobacterium tuberculosis* complex (MTBC) chromosome associated with resistance are well-characterized for several drugs including rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), ethambutol, fluoroquinolones and aminoglycosides (1).

- Multi-drug resistant TB (MDR-TB) is defined as resistance to RIF and INH and molecular detection of MDR-TB is a rapid and accurate method for screening for resistance.
  - Resistance to RIF is most often associated with mutations in the Rifampin Resistance Determining Region (RRDR) of the *rpoB* gene (2). These mutations cause phenotypic resistance in 97% of the cases (3).
  - Resistance to INH, in 80-90% of cases, is caused by mutations in either the *katG* gene or the *inhA* gene promoter region (3).

- Effective treatment regimens must be started as soon as possible to ensure better patient outcomes and prevent transmission of disease (4). Therefore detecting patients with drug resistant strains as quickly as possible is critical. One method for detection of mutations associated with resistance to RIF and INH is the line probe assay, MTBDR*plus* from Hain LifeScience (5).

- Florida Bureau of Public Health Laboratories (FBPHL) utilizes the MTBDR*plus* assay as its molecular "MDR Screen". The test can be performed directly on the specimen with a turnaround time (TAT) of approximately 8 hours. The instrumentation is inexpensive, results are easily visualized and interpreted.

- This study analyzed the performance of the Hain MTBDR*plus* assay in our TB testing algorithm over a two-year period to determine the impact of test results on patient therapy.

Methods

MDR Screen: The Hain GenoType MTBDR*plus* Assay (Hain Test, Hain Lifescience GmbH) was performed according to manufacturer's instructions. In brief, DNA was extracted from clinical specimens (or isolates), amplified in a multiplex PCR with specific biotinylated primers for *rpoB*, *katG* and *mabA-inhA*, followed by reverse hybridization with *wild type* or *mutant* probes and detection of hybridization by streptavidin-conjugated alkaline phosphatase. The test was batched and performed twice/week on all first-time positive TB samples (whether a first-time real-time PCR positive specimens or a first-time positive MTBC isolate, see Figure 1).

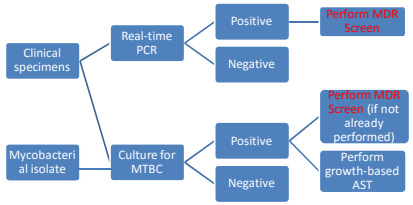

Figure 1. Algorithm for eligibility of samples for MDR screen

DNA sequencing: Sanger DNA sequencing was performed for confirmation of resistance mutations on a Genetic Analyzer ABI Prism 3100 (Life Technologies). Sequences were analyzed using BioNumerics software (Applied Maths) and compared to *wild type* sequence.

Growth-based Antimicrobial Susceptibility Testing (AST): The TREK Sensititre® plate method (Thermo Scientific) was performed with pure MTBC isolates cultured on solid media. In brief, 0.5 McFarland dilutions of isolates were diluted 100x and incubated in the presence of increasing concentrations of drug, including INH (0.06-4mcg/mL) and RIF (0.12-16 mcg/mL). The first drug level without visible growth was determined to be the minimal inhibitory concentration (MIC) and interpreted as susceptible, INH <0.25 mcg/mL, RIF ≤1 mcg/mL

Results

MDR Screen results were analyzed for 603 specimens over a 2-year period

- 522 clinical specimens had results that could be analyzed. Relevant mutations were detected in n=56 specimens (10.72%), see Table 1.

MDR screen result	Specimens tested (%)
No mutations	466 (89.27)
Isoniazid resistant (*katG/inhA*)	38 (7.28)
Rifampin resistance (*rpoB*)	5 (0.96)
MDR (*rpoB* + *katG/inhA*)	13 (2.49)

Table 1. Mutations associated with resistance in clinical specimens (2014-2015)

The average TAT for the MDR Screen was 9.44 days, the mode was 2.5 days

- 82.9% of all specimens tested by MDR Screen were resulted within 10 days or less from the receipt of the specimen in our laboratory and more than 55% within 5 days, the mode was 2.5 days (Table 2).

TAT for MDR Screen	< 5 days (avg. 3.57)	6-10 days (avg. 7.48)	11-15 days (avg. 13.73)	>16 days (avg. 35.95)
Number of specimens (%)	334 (55.38)	166 (27.52)	20 (3.31)	83 (13.75)

Table 2. TAT for MDR screen on clinical specimens (2014-2015)

Results cont.

MDR Screen results were available, on average, 34.26 days earlier than growth-based AST results

- An assessment of n=384 clinical specimens tested by both GenoType MTBDR*plus* Assay and Sensititre MIC revealed an average TAT for Sensititre MIC of 43.67 days (from date of receipt to growth-based AST result) compared to 9.41 days for the molecular MDR Screen.
- If an isolate was submitted, n=127, TAT was 35.6 days and if an MTBC-positive isolate was submitted, n=243, TAT was reduced to 17.8 days.

MDR Screen results correlated with changes in patient therapy

Isoniazid mono-resistant cases, n=33

- n=33 Florida patients with INH resistance were detected by MDR Screen.
- Modifications to the drug regimen were documented in 19 patients (n=9: increased dosage, n=10: INH discontinuation).

Multi drug resistance, n=9

- n=9 Florida patients with RIF and INH resistance were detected by MDR Screen.
- Modifications to the drug regimen were documented in all 9 cases
- The average number of days from date of MDR Screen result to change to appropriate therapy was n=6.9 days (range 1-16 days).
- The average number of days from change in appropriate therapy to when the phenotypic AST was available was n=30.2 days (range 11-77 days).
- Of the 9 patients, 7 completed the treatment and were considered cured (average of 19.2 months or 581 days). One patient left the state/country having completed 8 months of treatment, and one is still under treatment (started on 5/28/2015).

Conclusions

The MDR Screen is a reliable tool in early detection of resistance to first line drugs

- The average TAT for MDR Screen results is considerably less than for growth-based AST. 83% of the tested specimens could be resulted within 10 days and results were available in most cases on average 34 days earlier than the growth-based AST results.
- The MDR Screen detected mutations in 9.3% of patients tested at FBPHL over a 2-year period, which is a significant number for which valuable information on susceptibility could be determined early on.

The MDR Screen has an impact on drug regimen modifications

- Examination of drug regimen data of 33 Florida patients infected with INH-mono-resistant strains revealed that modification of regimen occurred in 19 patients upon receipt of MDR screen laboratory results.
- Nine MDR-TB patients were identified by the MDR Screen and were switched to appropriate therapy 23 days sooner on average.

References

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