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

Calin B. Chiribau,1 M-C. Rowlinson,1 S.Crowe,1 B. Jones,¹ A. Ashkin,¹ H. Perera,¹ D. Ashkin,¹ M. Lauzardo,² D. Urbine²

¹Florida Department of Health, Tallahassee, FL, ²University of Florida, Department of Medicine, Division of Infectious Diseases and Global Medicine, Gainesville, FL
Background

- Multi-drug resistant TB (MDR-TB) is defined as resistance to at least RIF and INH

- Molecular detection of MDR-TB is a rapid and accurate method for resistance screening
  - 97% of the cases of resistance to RIF are associated with mutations in *rpoB* gene RRDR
  - 80-90% of cases of resistance to INH are caused by mutations in either the *katG* gene or *mabA-inhA* gene promoter region

- Florida Bureau of Public Health Laboratories (FL-BPHL) utilizes the MTBDR*plus* assay (HAIN Lifescience) as its molecular “MDR Screen” since July 2009 (assay performed twice/week)
  - Performed directly on clinical specimens
  - Assay turnaround time (TAT) ~ 8 hours
  - Inexpensive instrumentation
  - Results relatively easily visualized and interpreted

- This study analyzed the performance of the Hain MTBDR*plus* assay over two-year period (2014-2015) and the impact of test results on patient therapy
Integration of the MDR screen in the Laboratory Algorithm

- **CLINICAL SPECIMENS**
  - REAL-TIME PCR
    - POSITIVE \(\rightarrow\) PERFORM MDR SCREEN
    - NEGATIVE

- **MYCOBACTERIAL ISOLATE**
  - MTBC+ CULTURE
    - POSITIVE \(\rightarrow\) PERFORM MDR SCREEN*
    - NEGATIVE \(\rightarrow\) PERFORM GROWTH-BASED AST

* IF NOT ALREADY PERFORMED @ SPECIMEN LEVEL
Study design

Analysis of AST data for n=603 clinical specimens over a 2-year period (2014-2015)

- Date of specimen receipt
- Date of MDR screen result by Hain Genotype® MTBDRplus assay, version 2 (Hain LifeScience)
- Date of phenotypic AST result by Sensititre MIC assay (TREK Diagnostic Systems, Thermo-Fisher)
- Date of initiation of appropriate therapy:
  - MDR patients: Addition of fluoroquinolone(s)
  - INH-resistant: Modification of treatment regimen
Laboratory Methods: MDR screen

MDR patient detected by MDR screen (by Hain GenoType MTBDR\textit{plus} Assay)
\textit{rpoB}:Ser531Leu, \textit{katG}:Ser315Thr

CC: conjugate control, AC: amplification control

\textit{rpoB} His526Arg mutation identified by Sanger DNA sequencing
Laboratory Methods: Growth-based AST

RIF  STR  RFB  INH

MIC (µg/ml):  >16  >32  4  2

MDR patient detected by growth based assay (by Sensititre)
Results: MDR screen turnaround time

- The average TAT (date of specimen receipt to result release) for the MDR Screen was 9.44 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

<table>
<thead>
<tr>
<th>TAT for MDR Screen</th>
<th>&lt; 5 days (avg. 3.57)</th>
<th>6-10 days (avg. 7.48)</th>
<th>11-15 days (avg. 13.73)</th>
<th>&gt;16 days (avg. 35.95)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of specimens (%)</td>
<td>334 (55.38)</td>
<td>166 (27.52)</td>
<td>20 (3.31)</td>
<td>83 (13.75)</td>
<td>603</td>
</tr>
</tbody>
</table>
Results: Mutations

- Out of n=603 tested, 81 could not be resulted (missing TUB⁺ bands, indeterminate)
- 522 specimens had valid results that could be analyzed. Relevant mutations were detected in n=56 specimens (10.73%)
Results: Molecular AST vs Growth based AST

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 MTBDRplus 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.
Results: Impact on patient care. MDR patients

- 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, upon receipt of MDR screen results
- 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)
Results: Impact on patient care.
Isoniazid mono-resistance

Isoniazid mono-resistant cases (*katG/ inhA*), n=33

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

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

- The average overall 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 made 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, a significant number for which valuable information on susceptibility could be determined early on.

The MDR Screen has a significant 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 (on average) sooner.