Drug Resistant TB – In Georgia?

Susan M. Ray, MD
Professor of Medicine (ID), EUSOM
Hospital Epidemiologist, Grady Health System
Medical Consultant, GA State TB Program

March 2014

WHO-Endorsed Molecular Tests for TB

- **Molecular Line Probe Assay (LPA)**
  - Regional or national-level laboratory
  - Smear-positive sputum or MTB cultures

- **Cepheid Xpert® MTB/RIF test**
  - Sub-district or district hospital level laboratory
  - Smear-positive or negative pulmonary and extra-pulmonary specimens from adults and children

MTB: Mycobacterium tuberculosis

Xpert® MTB/RIF Improves TB Testing

A Single Xpert® MTB/RIF Test

- Is about as sensitive and specific as one culture on solid media
- Can increase TB case detection by 40% over direct smear microscopy alone
- Takes only 2 hours to complete, compared to weeks for culture
- Uses simple sputum processing steps
- Detects presence of MTB and rifampicin resistance simultaneously
- Does not require sophisticated BSL-3 facilities or specialized expertise

Sample → **Cartridge** → **Module** → **4 Module Instrument**

MTB: Mycobacterium tuberculosis
BSL-3: Biosafety level 3

Performance of Xpert® MTB/RIF for Rifampicin Resistance and MDR TB

- **Rifampicin resistance (RIF-R) is a marker for MDR TB**
  - >85% of RIF-R strains are MDR strains in most countries
  - WHO recommended treatment of RIF-R TB is similar to MDR TB

- **Strong recommendation by WHO to use Xpert® MTB/RIF as the initial diagnostic test in individuals suspected of having MDR TB**
  - Excellent sensitivity (95%) and specificity (98%) for detecting rifampicin resistance
  - Implementing Xpert® MTB/RIF will cost less than conventional culture and DST to meet diagnostic targets for MDR TB

DST: drug susceptibility testing

World Health Organization. 2013. *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update.* WHO/HTM/TB/2013.14.

GA TB Program – Drug Resistance Testing

- GAPHL –
  - AFB smear and culture (broth/liquid)
  - Identification of mycobacteria by HPLC
  - Xpert MTB/Rif for:
    - Smear positive sample
    - Sample from TB “suspect” identified by the submitter
  - 1st line DST
  - Referral to CDC
    - MDDR service: samples with RifR signal by Xpert
    - 2nd line DST: isolates with any resistance to 1st line agents

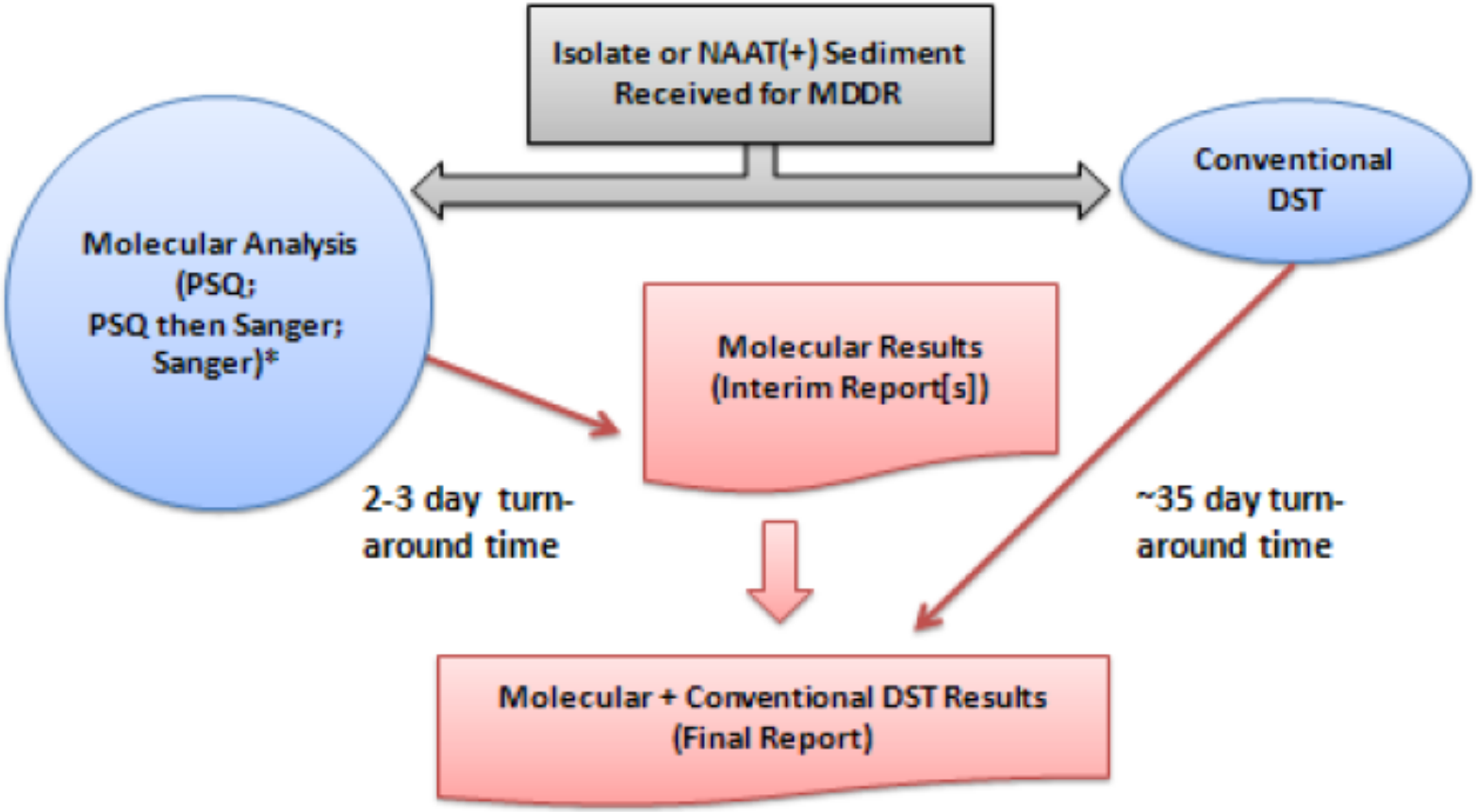
Reference Laboratory
Division of TB Elimination

Laboratory User Guide for U.S. Public Health Laboratories: Molecular Detection of Drug Resistance (MDDR) in *Mycobacterium tuberculosis* Complex by DNA Sequencing (Version 2.0)

June 2012

Centers for Disease Control and Prevention
Division of Tuberculosis Elimination Laboratory Branch
1600 Clifton Road, NE, F08
Atlanta, Georgia 30333
e-mail: TBLab@cdc.gov
Telephone: 404-639-2455
Fax: 404-639-5491

MDDR V2.0 Algorithm



*based on information supplied on request form

Performance characteristics of MDDR by Drug			
Drug	**Locus or loci examined**	**Sensitivity (%)**	**Specificity (%)**
RMP	*rpoB*	97.1	97.4
INH	*inhA* + *katG*	86.0	99.1
FQ	*gyrA*	79.0	99.6
KAN	*rrs* + *eis*	86.7	99.6
AMK	*rrs*	90.9	98.4
CAP	*rrs* + *tlyA*	55.2	91.0
EMB	*embB*	78.8	94.3
PZA	*pncA*	86.0	95.9

GA TB Program – Drug Resistance Testing

- GAPHL –
  - AFB smear and culture (broth/liquid)
  - Identification of mycobacteria by HPLC
  - Xpert MTB/Rif for:
    - Smear positive sample
    - Sample from TB “suspect” identified by the submitter
  - 1st line DST
  - Referral to CDC
    - MDDR service: samples with RifR signal by Xpert
    - 2nd line DST: isolates with any resistance to 1st line agents

Source: TB Specimen / SPUTUM

TEST REQUESTED	RESULTS
MYCOBACTERIOLOGY: CULTURE FOR CLINICAL SPECIMEN	PENDING
MICROSCOPIC TB EXAM: FLUOROCHROME Tested: 01/25/2013 Reported: 01/25/2013	1+ (4-36 AFB/100 fields)
MYCOBACTERIUM TUBERCULOSIS COMPLEX PCR Tested: 01/25/2013 Reported: 01/25/2013	POSITIVE FOR MYCOBACTERIUM TUBERCULOSIS COMPLEX ADDITIONAL INFORMATION: AFB culture pending. Specimen may contain both Mycobacterium tuberculosis and non-tuberculous mycobacteria, or it may contain only Mycobacteria tuberculosis, This test should not be the sole basis for diagnosing tuberculosis. Real-time PCR is not FDA cleared as a diagnostic test for Mycobacterium tuberculosis complex.
RIFAMPIN RESISTANCE BY PCR	Rifampin resistance DETECTED. A mutation which causes resistance has been detected in the rpoB gene. Real-time PCR is not FDA cleared as a diagnostic test for rifampin resistance.

Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); Conventional Drug Susceptibility Test in progress.

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 550 clinical isolates)
rpoB (RRDR)**	Silent mutation: TTC>TTT; Phe514Phe	Probably Rifampin susceptible. (97% of RMP-R isolates in our in-house evaluation of 550 clinical isolates have a mutation, other than the one detected, at this locus.) The mutation detected is a synonymous (silent) single-nucleotide polymorphism (SNP) and does not result in an amino acid change and is not considered clinically significant.
inhA (promoter)	No mutation	Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at one or both of these loci.)
katG (ser315 codon)	No mutation	
embB (Met306,Gly406)	No mutation	Incomplete sequence observed. Repeat testing in progress.
pncA (promoter, coding region)	No mutation	Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
gyrA (QRDR)	No mutation	Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: • 91% of AMK-R isolates have a mutation in the rrs locus; • 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	No mutation	

*A negative results (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

**No amplification was detected in the rpoB locus by Sanger sequencing; the result is based on pyrosequencing.

MDDR assays were developed and the performance characteristics determined by the DTBE Reference Laboratory. They have not been cleared or approved by the Food and Drug Administration.

Why was the Xpert test wrong?

- Xpert detects any change in sequence from wild type
- MDDR: determines the exact DNA sequence

- All base-pair changes do not result in a change in the amino acid – “silent” mutations

Predictive value of Xpert RifR

- Depends on the actual prevalence of Rifampin resistance
- Specificity is 98%
- Sensitivity is 95%
- Prevalence of rifampin resistance in GA
  - <1%
- Predictive value of positive test:
  - 14%

Results

Observation Text	Observation Value	Notes
TB-MTB-PCR=MYCOBACTERIUM TUBERCULOSIS COMPLEX PCR	POSITIVE FOR MYCOBACTERIUM TUBERCULOSIS COMPLEX	Comment: TB-PCR-POS ADDITIONAL INFORMATION: AFB culture pending. Specimen may contain both Mycobacterium tuberculosis and non-tuberculous mycobacteria, or it may contain only Mycobacteria tuberculosis, This test should not be the sole basis for diagnosing tuberculosis. Real-time PCR is not FDA cleared as a diagnostic test for Mycobacterium tuberculosis complex.
Date Observed:	01/31/2014	
TB-FL=MICROSCOPIC TB EXAM: FLUOROCHROME	4+ (> 36 AFB / field)	
Date Observed:	01/28/2014	
RIF-PCR=RIFAMPIN RESISTANCE BY PCR	Rifampin resistance DETECTED.	Comment: 3090-02 A mutation which causes resistance has been detected in the rpoB gene. Real-time PCR is not FDA cleared as a diagnostic test for rifampin resistance.

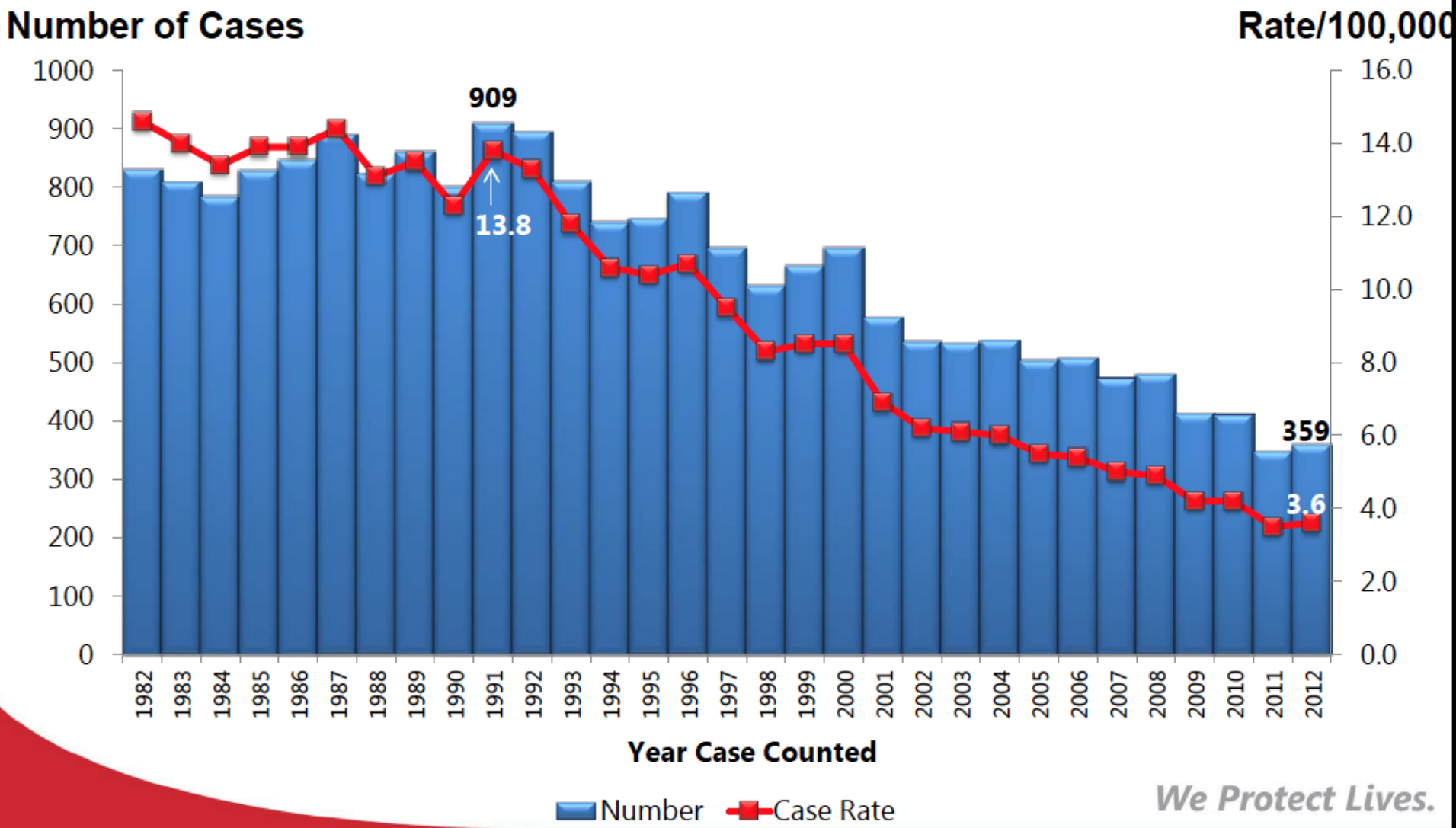
Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); Conventional Drug Susceptibility Test in progress.

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 550 clinical isolates)
rpoB (RRDR)	Mutation: TCG>TTG; Ser531Leu	Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)
inhA (promoter)	No mutation	Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)
katG (ser315 codon)	Mutation: AGC>ACC; Ser315Thr	
embB (Met306,Gly406)	Mutation: GGC>GCC; Gly406Ala	Ethambutol resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are EMB-R).
pncA (promoter, coding region)	Mutation: A-11G	Pyrazinamide resistant (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are PZA-R.)
gyrA (QRDR)	No mutation	Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: • 91% of AMK-R isolates have a mutation in the rrs locus; • 87% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 56% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	No mutation	

*A negative results (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

MDDR assays were developed and the performance characteristics determined by the DTBE Reference Laboratory. They have not been cleared or approved by the Food and Drug Administration.

TB Cases and Case Rates Georgia,1982-2012



We Protect Lives.

South Eastern United States 2012: Culture-confirmed TB Cases and Drug Resistance

2012 Data		Resistance			
	Culture + Cases with DST	INH R		MDR	
		N	(%)	N	(%)
AL	108	5	(4.6)	0	(0.0)
FL	497	40	(8.0)	6	(1.2)
GA	245	25	(10.2)	1	(0.4)
KY	59	6	(10.2)	0	(0.0)
MS	65	7	(10.8)	0	(0.0)
NC	169	16	(9.5)	1	(0.6)
SC	79	7	(8.9)	0	0.0
TN	113	9	(8.0)	0	0.0
VA	172	16	(9.3)	5	(2.9)
WV	6	2	(33.3)	0	(0.0)
PR	54	5	(9.3)	1	(1.9)
SE Region	1567	138	(8.8)	14	(0.9)
US	7250	660	(9.1)	83	(1.1)