Drug susceptibility testing to 1st & 2nd line drugs in the diagnosis of MDR & XDR TB

Dr Camilla Rodrigues MD
Consultant Microbiologist
TB - constantly on the back burner

Tuberculosis –
a disease which medicine never cured, wealth warded, or poverty could boast exemption from; which sometimes moves in giant strides & sometimes at a tardy pace
but
slow or quick is ever sure & certain

Nicholas Nickleby – Charles Dickens
### Drug Resistant TB – “Ebola with wings”

<table>
<thead>
<tr>
<th>Type of Resistance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Resistance</td>
<td>no past history of TB drugs</td>
</tr>
<tr>
<td>Initial Resistance</td>
<td>no reliable record of TB drugs</td>
</tr>
<tr>
<td>Sec / Acq Resistance</td>
<td>past h/o TB drugs</td>
</tr>
<tr>
<td>MDR</td>
<td>Resistant to INH + RIF</td>
</tr>
<tr>
<td>XDR</td>
<td>MDR plus</td>
</tr>
<tr>
<td></td>
<td>R to one of three injectables Ak Km Cs &amp; R to any fluoroquinolones</td>
</tr>
</tbody>
</table>
The resumption of consumption ..... 

Patients with resistant TB are

- difficult to treat
- remain infectious for longer
- pose a public health hazard - transmit to 12 persons / year
- more likely to die
- HIV further compounds the problem
TB - Diagnostic Test Coverage

Symptoms  X-ray  Smear  Culture  Solid  Culture  Liquid  DST  Molecular Probes

Developed Countries

Developing Countries

Lack of proper support
? a matter of affordability

Growing shift
• Many national TB programs do not have the resources to screen all TB cases for resistance

• Underestimation of the extent of the progressing epidemic of primary resistance

• Mono resistance to INH or R contributes to MDR in the continuation phase
DST in *M. tuberculosis* - a neglected problem

- Culture
- Confirmation of species
- DST a **must** for
  - Relapse or re treatment
  - Change of regimen (pts continuing to be +)
  - Suspected primary resistance

**may be used** as
- A guide in initial treatment
- To obtain baseline epidemiological data
### Priority rank list for DST

<table>
<thead>
<tr>
<th>Priority Rank</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st line</td>
<td>Rifampicin, Isoniazid, Ethambutol, Pyrazinamide, Streptomycin</td>
</tr>
<tr>
<td>2nd line</td>
<td>Aminoglycoside (kanamycin or amikacin), Polypeptide (capreomycin), Fluoroquinolone (ciprofloxacin or ofloxacin), Thioamide (ethionamide or prothionamide), PAS, Serine analogue (cycloserine or terizidone)</td>
</tr>
</tbody>
</table>
Newer second line drugs

- Moxifloxacin
- Rifabutin
- Linezolid
- Clarithromycin
- Clofazimine
Drug Susceptibility Testing (DST) - \textit{M. tuberculosis}

1. Growth observation
   - Macroscopic observation
     - Proportion method
     - Absolute Conc
     - Resistance Ratio
     - E test
     - Nitrate Reduction Assay
   - Microscopic observation
     - MODS

2. Detection of Metabolic activity or products
   - BACTEC / MGIT / MB/Bact /ESP Myco
   - MABA - microplate alamar blue assay / REMA / MTT

3. Newer methods as Phage based technologies

4. Molecular methods: detection of genetic mutations - Genotypic
Requirements for Drug Susceptibility Tests

- High intra & inter lab reproducibility
- Shortest TAT
- Distinguish between high & low levels of R
- Practical lab application
- Minimal investment & consummable costs
- Minimal labor time
- Applicability to 1st & 2nd line drugs
Problems in DST for *M tuberculosis* still a bottleneck

- Inoculum standardisation (number / dispersion / viability)
- Stability of drugs
- Alteration of drugs in different media (inactivation / protein binding / deterioration / inspissation / pH / antagonistic substances / incomplete dissolution in solvents / inaccurate dilution)
- Incubation temp / time
- Criteria of resistance
- Type of test performed
# Problems in Drug Susceptibility Testing

<table>
<thead>
<tr>
<th>False Resistance</th>
<th>False Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>High inoculum</td>
<td>Low inoculum / low viability</td>
</tr>
<tr>
<td>Contamination mixed</td>
<td>suboptimal growth</td>
</tr>
<tr>
<td>not homogenised</td>
<td>inoculum dilution</td>
</tr>
<tr>
<td>low drug conc</td>
<td>incorrect drug conc</td>
</tr>
<tr>
<td>Low level resistance</td>
<td>- inconsistent results when tested repeatedly</td>
</tr>
<tr>
<td>- inconsistent results when tested repeatedly</td>
<td>- discordant when compared to other systems</td>
</tr>
<tr>
<td>- discordant when compared to other systems</td>
<td></td>
</tr>
</tbody>
</table>
DST – *M tuberculosis*

1. Growth observation

- Macroscopic observation
  - Proportion Method
  - Absolute Concentration Method
  - Resistance Ratio
  - E test
  - Nitrate Reduction Assay
- Microscopic observation
  - MODS

Indirect
1. **Proportion method**
   - Ratio of colonies on the drug containing medium to those on drug-free medium (set at 1%)
   - If >1% is R, the drug will not be useful in therapy
   - Qualitative as well as precise estimate

2. **Absolute concentration method or MIC**
   - Growth is taken as the end point ( >20 colonies)
   - Affected by inoculum size & viability

3. **Resistance ratio method**
   - Determines the resistance ratio between the MIC of the test strain and MIC of reference strain ( RR >8)
   - Affected by variations in the std strains
DST- Growth observation

• Macroscopic
  Absolute conc ( >20)
  Resistance Ratio (RR > 8)
  Proportion Method (set at 1%)
  E - test
  Nitrate Reductase Assay

• Microscopic
  MODS
1. Growth observation: Direct Assays

Nitrate Reduction Assay

Control  INH  RF  ST  ETH
1:10     0.2  40  4.0  2.0

Fully Susceptible

R to all 4 drugs
Color change > Control

J Clin Microbiol 2002;40:553
1. Growth observation
Direct assays for resource limited settings

Microscopic Observation Drug Susceptibility Assay – MODS
accurate detection & simultaneous identification of MDR

Characteristic tangles of *M* *tb* seen under inverted light microscope

Drug Susceptibility Testing (DST): *M. tuberculosis*

1. Growth observation
   - Proportion method
   - Absolute Conc
   - Resistance Ratio
   - E test
   - Nitrate Reduction Assay
   - MODS

2. Detection of Metabolic activity or products
   - BACTEC / MGIT / MB Bact / ESP Myco
   - MABA / REMA / MTT

3. Phage based technologies

4. Molecular methods: detection of genetic mutations
BACTEC 460 TB

- Modified proportion method
- 7H12 does not absorb / inactivate drugs
- Growth monitored radiometrically
- R is determined by comparing rate of growth in control & in drug containing vial
- Critical prop for R is taken as 1% for all drugs
Shorter TAT for $1^0$ isolation
Allows for completion in 1 week
DST on solid media (3 - 4 wks) vs liquid (6 - 12 days)
Approved for indirect DST for $1^{st}$ line drugs

BACTEC 460 TB System

- (14C palmitic acid)
- Detection (GI > 40)
- $^{14}CO_2$
Culture Inoculum – Procedural Flow Chart 460 TB

Test Culture

Subculture on 12B

Incubate at 37 degrees

When GI >500 in 12B

0.1 ml in each drug vial

0.1 ml + 9.9 ml Diluting Fluid

Mix 0.1 ml in Control (w/o Drug)

GI 30 in the control, Interpret results

If growth in drug cont vial progresses to a higher level than the 1:100 diluted control, then >1% R
Critical concentrations

CDC & CLSI recommend testing critical conc. Other conc optional.

- Lowest conc of drugs that inhibits “wild” strains (S) while not inhibiting strains isolated from pts not responding to treatment (R).

- For H & R, the gap bet highest MIC for susceptible & lowest MIC for resistant is substantial. Problem in interpreting cut off values close to critical conc.

- R is growth of >1% of an inoculum in presence of the critical conc of the drug.
# Critical concentrations for 1\textsuperscript{st} line drugs (µg/ml)

<table>
<thead>
<tr>
<th>Drug</th>
<th>LJ</th>
<th>7H10</th>
<th>460 TB</th>
<th>MGIT 960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
<td>0.2/1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>40</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>2</td>
<td>5.0</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>4</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

CLSI & CDC recommend testing critical concentrations
Discrepancies with Emb /Sm
Re testing

- Indeterminate results
- Mono resistance esp rifam, pyrazina, emb
- New case that shows resistance
- Quality Control
Pyrazinamide susceptibility

Issues: low pH inhibits growth

- Enzymatic PZAse (Wayne)
- 460 TB / MGIT 960 TB system
- *pncA* gene sequencing
Increasing demand for 2nd line drugs
- when to test & how?

- MDR (in the absence of drug R, 1st line drugs are highly effective)
- Failure to respond despite long history of treatment
- Follow up in the DOTS Plus - without bacteriological confirmation, utilisation of 2nd line not justified
- Variation in testing systems & methods
## Recommended critical concentrations
### 2nd line drugs - LJ

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Drug conc µg/ml</th>
<th>1% critical prop</th>
<th>10% critical prop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Capreomycin</td>
<td>40</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>PAS</td>
<td>0.5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Thiacetazone</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Cycloserine</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

WHO /CDS/TB 2001 ;288
Critical Concentrations of 2\textsuperscript{nd} line drugs

liquid vs solid

<table>
<thead>
<tr>
<th>Drug</th>
<th>460 TB</th>
<th>7H10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capreomycin</td>
<td>1.25</td>
<td>10.0</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>1.25</td>
<td>5.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>inconsistent &amp; unreliable (difficulty in CC in 7H12)</td>
<td></td>
</tr>
</tbody>
</table>

*J Clin Microbiol 1999;37:3179*
DST - M. tuberculosis

- Treatment of MDR should be based on DST
- Considerable difference in critical concentrations with lack of correlation for 2nd line drugs
- DST for 1st & 2nd line drugs established on 460 TB
- Increasing concern about use of radio active methods
- Inadequate worldwide studies for 2nd line on MGIT 960
MGIT 960 TB

Detects $O_2$ consumption in the presence or absence of drug
Validation of MGIT 960 for DST to 2nd line drugs

J Clin Microbiol 2006;44:668
J Clin Microbiol 2006;44:811
Susceptibility testing of 2nd line drugs on MGIT 960

To establish BACTEC MGIT 960 test procedures & concentrations for 2nd line drugs used to treat R pts

Amikacin
Kanamycin
Capreomycin
Ofloxacin
Moxifloxacin
Ethionamide
PAS
DST calibration – study phases

• Phase 1  :  Susceptible culture isolates from never treated pts – Including a standard strain, H37Rv (11) - Probably S

• Phase 2  :  Resistant culture isolates from patients failing 2\textsuperscript{nd} line treatment (20)– Probably R

• Phase 3  :  Fresh & stock clinical resistant isolates (73)
Study design

Phase 1: Establish a basic test procedure & determine range of test conc

Phase 2: Test conc adjusted based on results of Phase 1 (working critical conc in MGIT 960 that would yield results equivalent to 460 TB)

Phase 3: Final testing of optimal drug conc
   “work in the field”

Critical conc considered the conc with the least amt of discrepant results between the 2 systems
Methods

• All 7 antimicrobial drugs were tested by BACTEC 460 TB & MGIT 960 in parallel
• All drugs obtained in a chemically pure form
• Three concentrations were tested for each drug by both methods
• Stock solutions of drugs aliquoted & stored at -70°C
• M. tb H37Rv ATCC 27294 used for QC
<table>
<thead>
<tr>
<th></th>
<th>MGIT 960</th>
<th>460 TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>0.5, 1.0, 2.0</td>
<td>0.5, 1.0, 2.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>1.25, 2.5, 5.0</td>
<td>1.25, 2.5, 5.0</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>1.25, 2.5, 5</td>
<td>1.25, 2.5, 5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1.0, 2.0, 4.0</td>
<td>1.0, 2.0, 4.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>1.0, 2.0, 4.0</td>
<td>1.0, 2.0, 4.0</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>1.25, 2.5, 5</td>
<td>1.25, 2.5, 5</td>
</tr>
<tr>
<td>PAS</td>
<td>2.0, 4.0, 8.0</td>
<td>2.0, 4.0, 8.0</td>
</tr>
</tbody>
</table>
Discrepant results based on critical concentrations established in Phase 3

<table>
<thead>
<tr>
<th></th>
<th>False Susceptible by MGIT</th>
<th>False Resistant by MGIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 73 isolates</td>
<td>0.78%</td>
<td>0.39%</td>
</tr>
<tr>
<td>Percent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

False Susceptible regarded as a **Very Major Error**
False Resistant      regarded as a **Major Error**
# MGIT critical concentrations - 2nd line drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>MGIT 960</th>
<th>460 TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Capreomycin</strong></td>
<td><strong>2.5</strong></td>
<td><strong>1.25</strong></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Ethionamide</strong></td>
<td><strong>5.0</strong></td>
<td><strong>2.5</strong></td>
</tr>
<tr>
<td>PAS</td>
<td>4.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Drug Susceptibility Testing (DST) : *M.tuberculosis*

1. Growth observation
   - Proportion method / Absolute Conc / Resistance Ratio
   - Nitrate Reduction Assay
   - MODS

2. Detection of Metabolic activity or products
   - BACTEC / MGIT / MB/Bact / ESP Myco
   - MABA - Microplate Alamar Blue Assay
   - REMA - RESazurin Microtitre Assay
   - MTT - Methyl Thiazol diphenyl Tetrazolium bromide

3. Phage based technologies

4. Molecular methods : detection of genetic mutations

   Rapid, low cost show good agreement for H & R
   ? Biosafety & contam
Drug Susceptibility Tests (DST) – *M. tuberculosis*

1. Growth observation
   - Macroscopic observation in drug free & drug containing media
     - Proportion method
     - Absolute Conc
     - Resistance Ratio
     - E-test
     - Nitrate Reductase Assay
   - Microscopic observation
     - MODS

2. Detection of Metabolic activity or products
   - BACTEC / MGIT / MB/Bact
   - MABA - microplate alamar blue assay / REMA / MTT

3. Phage based technology: LRP, PhaB, Fast Plaque

4. Molecular methods: detection of genetic mutations
Lysis with mycobacteriophages

Infection

Phage start to replicate in cells

Overnight incubation

Resistant
Sensitive

Test sample

Treatment with virucidal solution

Phage

Sensor cell

Rifampicin

TB bacilli

24 hrs

10 mins
Lysis with mycobacteriophages

Rifampicin Sensitive

Plate without Rifampicin
Plate with Rifampicin

Rifampicin Resistant

Plate with Rifampicin
Plate without Rifampicin
# Phage assay

<table>
<thead>
<tr>
<th>Test</th>
<th>Ref. Std</th>
<th>Specimen</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF Resistance</td>
<td>BACTEC 460</td>
<td>Culture Isolates</td>
<td>Sensitivity 96%</td>
</tr>
<tr>
<td></td>
<td>L.J Proportion mtd</td>
<td></td>
<td>Specificity 100%</td>
</tr>
<tr>
<td>INH Resistance</td>
<td>BACTEC 460</td>
<td>Culture Isolates</td>
<td>Sensitivity 97%</td>
</tr>
<tr>
<td></td>
<td>L.J Proportion mtd</td>
<td></td>
<td>Specificity 100%</td>
</tr>
<tr>
<td>RIF Resistant</td>
<td>BACTEC 460</td>
<td>Smear + Resp. Spec</td>
<td>Sensitivity 93%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specificity 87%</td>
</tr>
</tbody>
</table>

**Limitations**

- Analytical sensitivity: 100-300 bacilli/ml
- Not useful in smear negative, paucibacillary specimens
- Pts receiving anti-TB treatment

*Ajay K et al. IJMM 2002;20:211-14*
Drug Susceptibility Testing (DST): *M. tuberculosis*

1. Growth observation
   - Macroscopic observation in drug free & drug containing media
     - Proportion Method
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     - MODS

2. Detection of Metabolic activity or products
   - BACTEC / MGIT / MB/Bact / ESP Myco
   - MABA / REMA / MTT

3. Phage based technology
   - Bioluminescence / Flow cytometry

4. Molecular methods: detection of genetic mutations
   - advances in genotypic hardware
DST – Genotypic molecular methods
“Leap frogging technology”

- DNA sequencing
- PCR – SSCP
- PCR – HDX
- RFLP
- Line probe assays
- Molecular beacons
- **Micro Arrays / microfluidic tech**
SSCP analysis was done on 12% poly AA gel with Gels are run overnight at Room temp. and 4°C Stained with EtBr and observed under UV light
## Drugs & genes involved in their resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>RNA polymerase subunit B ( (rpo\beta) )</td>
<td>96%</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Enoyl acp reductase ( (inhA) )</td>
<td>10-20%</td>
</tr>
<tr>
<td></td>
<td>Catalase–peroxidase ( (katG) )</td>
<td>30-60%</td>
</tr>
<tr>
<td></td>
<td>Alkyl hydroxyperoxideoxy reductase ( (ahpC) )</td>
<td>2-8%</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Arabinosyl transferase ( (embC,A,B) )</td>
<td>80%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Ribosomal protein subunit 12 ( (rpsL) )</td>
<td>52-59%</td>
</tr>
<tr>
<td></td>
<td>16 S ribosomal RNA ( (rrs) )</td>
<td>8-21%</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Pyrazinamidase-nicotinamidase ( (pncA) )</td>
<td>72-97%</td>
</tr>
<tr>
<td>Quinolones</td>
<td>DNA gyrase subunit A ( (gyr A) )</td>
<td>75-94%</td>
</tr>
</tbody>
</table>
Probes used in Line probe assays

Rifampicin resistance determining region (RRDR) -81 base-pair region encoding 27 amino acid and involving codon 507-533
Line Probe Assays: Inno Lipa Rif TB

Strip hybridization
• PCR-based strip assay
• Detects *M. tuberculosis* complex 3S-rRna/16S-RNA
• Detection of both H (*catG*) and R (*rpoB*) resistance
Molecular beacons for established mutations

Based on stem & loop structure with the probe in the loop
Fluorescence detected in Real time within 4 hrs w/o post PCR manipulation

*J Clin Microbiol* 2004;42 :4204
MTB Drug Resistance Testing

Probes
1. MYC
2. MTB
3. Rif wt 1
4. Rif wt 2
5. Rif wt 3
6. Rif wt 4
7. Rif wt 5
8. Rif 533 mt
9. Rif 531 TTG
10. Rif 531 TGG
11. Rif 526 TAC
12. Rif 526 GAC
13. Rif 526 CGC
14. Rif 526 TGC
15. Rif 526 AAC
16. Rif 522 TTG
17. Rif 522 TGG
18. Rif 516 TAC
19. Rif 516 GTC
20. Rif 516 GTG
21. Rif 516 GGC
22. Rif 513 CCG
23. Rif 511 CCA
24. Inh A wt
25. Inh A mt
26. KG 315 wt
27. KG 315 mt
28. KG 463 wt
29. KG 463 P
30. RpsL 43 wt
31. RpsL 43 mt
32. RpsL 88 wt
33. RpsL 88 mt
34. RRS 491 wt
35. RRS 491 mt
36. RRS 513 wt
37. RRS 513 mt
38. RRS 516 mt

Wild – Susceptible, Mutant – Resistant
<table>
<thead>
<tr>
<th>Method</th>
<th>Identification Rate</th>
<th>Recovery Time</th>
<th>Susceptibility Testing Efficiency</th>
<th>Susceptibility Testing Time</th>
<th>Total Time for Identification &amp; Susceptibility testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.J</td>
<td>30-50%</td>
<td>2-6 wks</td>
<td>80-99%</td>
<td>2-4 wks</td>
<td>4-12 wks</td>
</tr>
<tr>
<td>BACTEC</td>
<td>50-75%</td>
<td>2-3 wks</td>
<td>92-100%</td>
<td>8-12 days</td>
<td>3-5 wks</td>
</tr>
<tr>
<td>MGIT</td>
<td>50-75%</td>
<td>1-2 wks</td>
<td>98.6-100%</td>
<td>6-10 days</td>
<td>2-4 wks</td>
</tr>
<tr>
<td>RLBH</td>
<td>95-98%</td>
<td>4 days</td>
<td>98-100%</td>
<td>4 days</td>
<td>4 days</td>
</tr>
</tbody>
</table>
MDR TB: in urban & rural settings

A study done at our centre comparing sputum samples (pulmonary TB) from urban & rural areas

<table>
<thead>
<tr>
<th></th>
<th>HNH</th>
<th>Rural</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR-TB</td>
<td>51%</td>
<td>2%</td>
</tr>
<tr>
<td>S, H, E, R</td>
<td>25%</td>
<td>0.6%</td>
</tr>
<tr>
<td>S,H,R</td>
<td>15%</td>
<td>Nil</td>
</tr>
</tbody>
</table>

- Tertiary care centre with a referral bias towards non responding cases

Clin Infect Dis 2003:36:152-4
• At HNH, Mumbai, Beijing genotype most predominant (35%) among MDR isolates

Second largest prevalent group (31%) found to belong to Delhi genotype

RFLP showed Beijing genotypes to be closely related

In the near future..... Lab on a chip

- Several thousand probes on a chip
- Automated data management systems with paperless reporting

....and these tests will provide

- Identification of pathogens
- Drug susceptibilities
- Nature of host response
The men who gave us the cure for tuberculosis

...... losing curable status?
Ending neglect....

Central role of the laboratory in the early diagnosis & management of TB

Knowing is not enough ....we must apply
Willing is not enough ....... we must do

Goethe
Thank you
Media Inoculation

1 ml of standard suspension (1 mg/ml) + 9 ml of sterile distilled water

For each isolate

Control tubes  Drug Conc.  Control tubes  Drug Conc.

2 bacterial dilutions
DST: Reliability of results

Therapeutic index (diff bet in vitro MIC & serum drug levels)
High for INH & Rif
Low for 2nd line drugs as cycloserine / ETA / PAS

In general, Susceptibility results are predictable
If Resistance prevalence is <10%, resistance results show a low PV

*In vitro* criteria should be performed with strains from pts. that
1. Never been treated
2. Failed treatment
3. DST then standardised to obtain reproducible results
Wayne’s Pyrazinamidase Assay

Detectors active Pyrazinamidase enzyme by hydrolysis of PZA to Pyrazinoic acid as evidence by a colour change.

Inoculation of actively growing culture into 2 Dubos Agar butts containing 100mg/Litre of PZA

- Incubation: 37°C, 4 days
- Add 1 ml freshly prepared 1% ferrous ammonium sulphate
- RT, 30 mins
- Examine for a pink band in the agar medium

If 4 day tube is negative or doubtful, repeat the test at 7th day using the second tube

- Positive: Pink band (Susceptible)
- Negative: No pink band (Resistant)
MGIT-PZA

0.8 ml PZA + 1:10 dil
Growth suspension

0.1 ml PZA + 0.8 ml PZA Growth suppl + 0.5 ml Growth suspension

Incubate in MGIT 960 TB system

Results will be flagged by the Machine

Cut off value is 100
# Multidrug resistance in new TB cases in India

<table>
<thead>
<tr>
<th>Place</th>
<th>Study period</th>
<th>Sample size</th>
<th>MDR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujarat</td>
<td>1983-89</td>
<td>570</td>
<td>0</td>
</tr>
<tr>
<td>Pondicherry</td>
<td>1985-91</td>
<td>2127</td>
<td>0.7</td>
</tr>
<tr>
<td>Bangalore</td>
<td>1980-86</td>
<td>1024</td>
<td>1.3</td>
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<tr>
<td>Kolar</td>
<td>1987-89</td>
<td>292</td>
<td>3.4</td>
</tr>
<tr>
<td>Rajasthan</td>
<td>1988-91</td>
<td>1009</td>
<td>0.9</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>1985-2000</td>
<td>6771</td>
<td>2.0</td>
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<tr>
<td>Delhi</td>
<td>1990-91</td>
<td>324</td>
<td>0.6</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>2001-02</td>
<td>273</td>
<td>1.0</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>1982-1989</td>
<td>1108</td>
<td>2.0</td>
</tr>
<tr>
<td>West Bengal</td>
<td>2000-01</td>
<td>350</td>
<td>3.0</td>
</tr>
<tr>
<td>Orissa</td>
<td>2000-02</td>
<td>343</td>
<td>0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Place</th>
<th>Study period</th>
<th>Sample size</th>
<th>MDR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamil Nadu</td>
<td>1986-2000</td>
<td>1408</td>
<td>24.8</td>
</tr>
<tr>
<td>Karnataka</td>
<td>1988-1989</td>
<td>111</td>
<td>17.1</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>1998-99</td>
<td>90</td>
<td>67.0</td>
</tr>
<tr>
<td>Gujarat</td>
<td>1983-2001</td>
<td>2089</td>
<td>30.6</td>
</tr>
<tr>
<td>Delhi</td>
<td>1990-91</td>
<td>81</td>
<td>33.3</td>
</tr>
<tr>
<td>Haryana</td>
<td>1991-95</td>
<td>196</td>
<td>49.0</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>1998-99</td>
<td>1426</td>
<td>8.1</td>
</tr>
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</table>