Drug Susceptibility Testing of *Mycobacterium tuberculosis* Complex

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Topics

- Drug susceptibility testing
  - Methods & interpretation rules
  - Key points of DST
- Discordant results
  - Possible causes
  - Examples
  - Suggestions to resolve and interpret

Drug Susceptibility Testing Methods

- Agar proportion (conventional)—21 d
  - Observation of growth—colony counts
- Broth systems
  - BACTEC 460 (radiometric)—4-12 d
    - $^{14}CO_2$ production—GI
  - MGIT 960 (non-radiometric)—4-13 d
    - O_2 consumption—GU
  - VersaTrek (ESP) (non-radiometric)—3-13 d
    - O_2 consumption
    - Headspace pressure changes

Interpretation for Resistance by AP

- ***An isolate is considered resistant to a drug if the bacterial growth in the presence of the drug at the critical concentration is > 1 % of the growth in the control (without the drug).***

Reading A Drug Plate

- INH & RIF
  - **150** colonies on the control quadrant
  - **30** colonies on the INH quadrant
  - 0 colonies on the RIF quadrant
- Calculation:
  - For INH:

$$\frac{30}{150} \times 100\% = 20\ \%$$

- Interpretation:
  - INH: Resistant (20%)
  - RIF: Susceptible (0%)

Interpretation of Broth DST

- MGIT 960:
  - GU of GC has to reach 400 within 4-13 days
  - GU of a MGIT with drug >100----R
  - GU of a MGIT with drug ≤ 100----S
- VersaTrek (ESP II)
  - Detection time of : >3 and <10 days
  - Detection time of GC GC +3 days = X days
  - Detection time of a vial with drug is >X----S
  - Detection time of a vial with drug is ≤ X----R

Key Points of DST

- Must test on PURE cultures
  - Does not guarantee no contamination during DST set-up.
  - Resistance must be verified before reporting.
    - Must rule out presence of contaminants or NTM.
- Put a good lab system in practice
  - Sterile technique
  - Adding all ingredients properly
  - Good training & good QA
- Test at the "right" critical concentration for the method used.
  - No consensus on CC for some 2nd-line drugs. Be cautious in interpreting inter-lab results. (main cause for discrepancy)

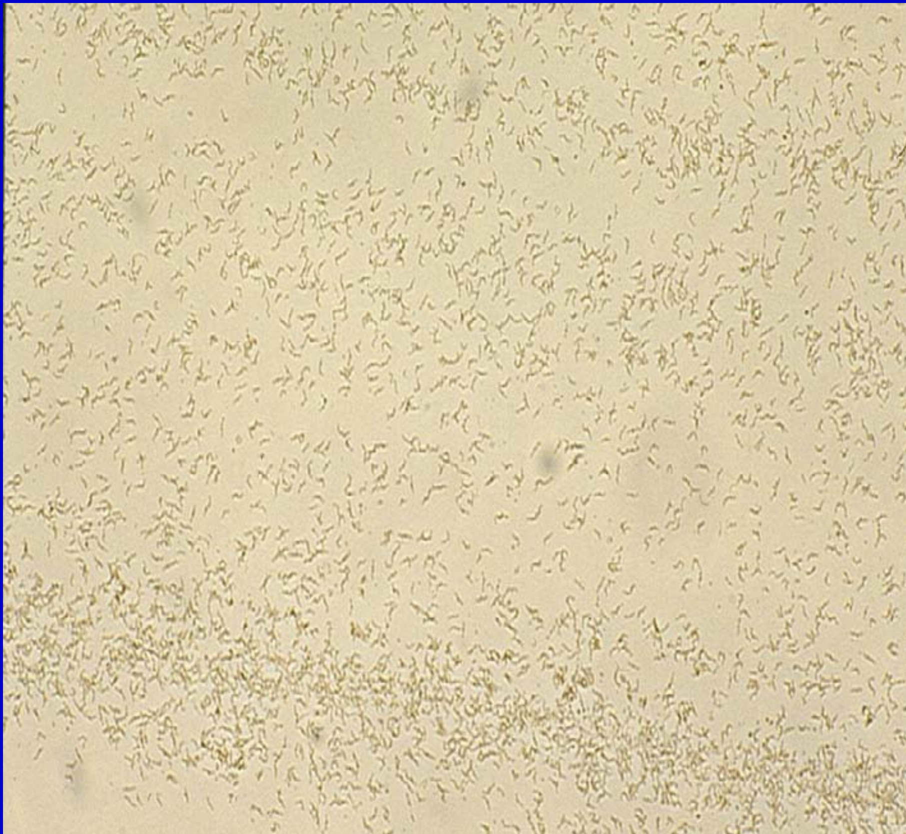
After Obtaining MGIT DST Printout

- Check the tube order and match drug order on the printout.
- Check growth in the tube
  - If a strain does not use O_2 much, you may see substantial growth without increased GUs. It will be incorrectly interpreted as "S".
  - Check for drug resistance mutations, or retest with other methods.
- Verify resistance
  - Must rule out presence of contaminants or NTM.
  - Advisable to re-test mono-R to RIF, EMB or PZA.

Verification of Resistance

- AP—colony morphology compatible with MTBC.
- Broth methods
  - Visual check—if turbid, contaminants/NTM may be present.
  - Cell morphology—make a smear
    - Presence of AFB, cell morphology is compatible with MTBC.
    - Absence of contaminants
    - Absence of NTM
  - If in doubt, make a subculture on 7H10.
    - Check microscopic morphology in 1-2 days for NTM.

MAC on 7H10 after 1-2 days incubation



If MTBC, you'll not see these "squiggles" after 1-2 days incubation.

If rapid growers, more growth than this.

If other bacteria (non-AFB), you may see colonies with bare eyes.

Test Concentrations for Primary Drugs

	AP-7H10	BT 460	MG 960	VersaT
RIF	1	2	1	1
INH	0.2, 1	0.1, 0.4	0.1, 0.4	0.1, 0.4
PZA	---	100	100	300
EMB	5, (10)	2.5, (7.5)	5	5, (8)

When 2 concentrations are listed,

the lower concentration is the critical concentration.

Test Concentrations (1)
Second-line Drugs (limited data)

	BAC 460	MGIT 960	AP-7H10
AMK	1	1, (1.5 MDL)	4
CAP	1.25	2.5 (3 MDL)	10
ETH	2.5	5	5, (10 CDC)
KNA	2.5	2.5	6
PAS	4	---	2
RFB	0.5	0.5	0.5

Test Concentrations (2) Second-line Drugs (limited data)

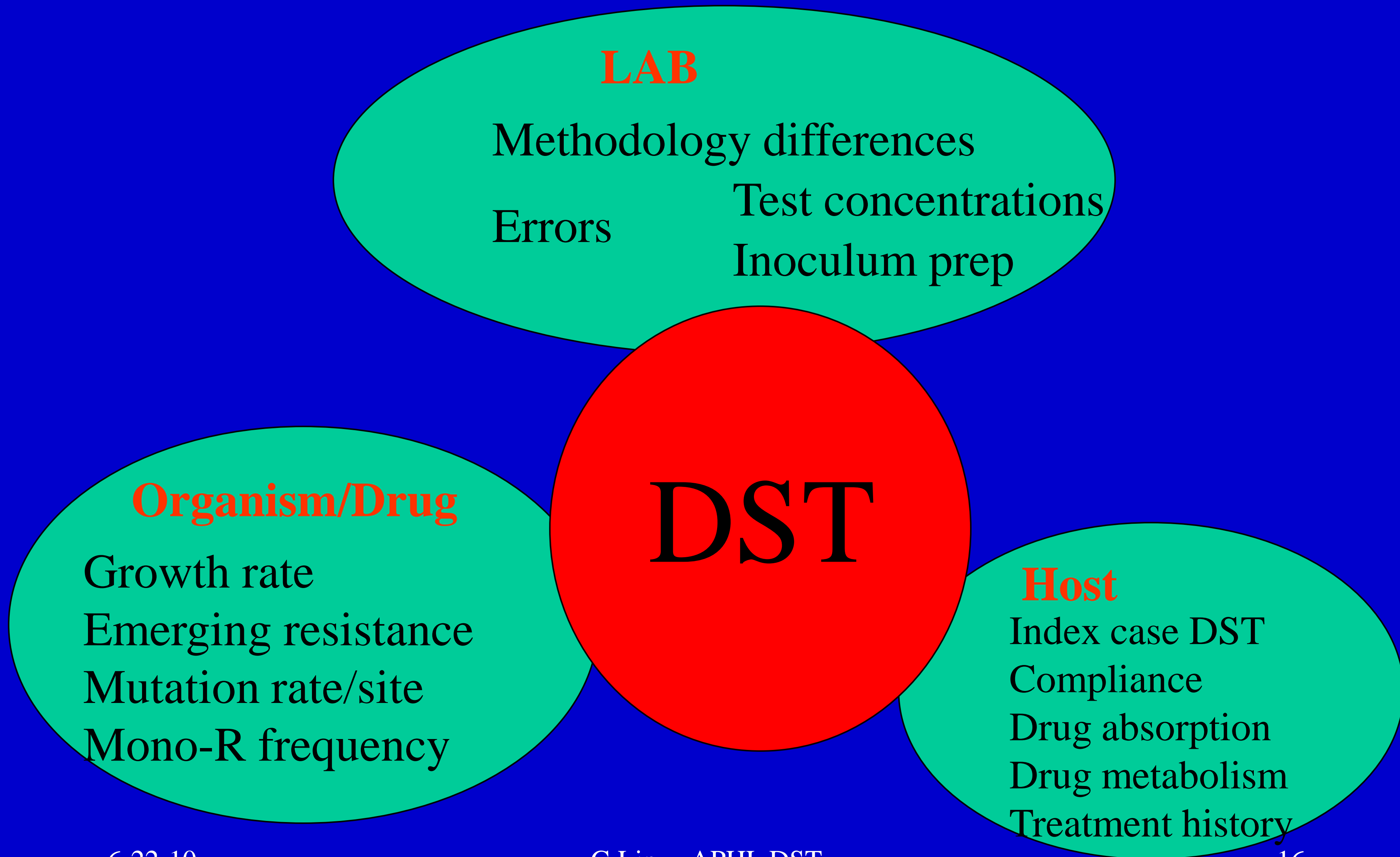
	BACTEC 460	MGIT 960	AP-7H10
Linezolid	1	1	--
LEV	2	1.5	1
MOX	0.5	0.25*	0.5
OFX	2	2	2
STR	2, (6)	1, (4)	2, 10

DST of Quinolones

- No need to test all 3 fQs listed
  - OFX is the representative of the class.
- MOX has higher pharmacokinetic activities and intracellular accumulation.
  - It may have clinical efficacy when a strain is resistant to CIP/OFX/LEV.
- Testing MOX at 0.25 µg/ml by MGIT 960
  - If S at 0.25, predicts susceptibility to LEV (ref: MDL study)
  - If R at 0.25, test at 0.5, 1, 2, 4 µg/ml.
  - According to MOX MIC to modify MOX dosage to treat.
    - Clinical studies are warranted to investigate clinical efficacy of MOX for strains with MIC between 0.5-4.

Discrepancy

Components affecting Drug Susceptibility



Causes for discrepancy: not due to errors (1)

- Methodology differences
  - Differences in medium components (affect org's metabolism), detection mechanisms, instrumentation, drug concentrations, etc.
  - Different stains may be affected differently on their metabolism by drugs & media.
  - Different interpretation of "microcolonies".
  - It is unlikely to get 100% correlation among different methods.
    - ETH--<90% correlation between AP & MGIT.
- Variability of strains & strain' status→ Poor reproducibility
  - MIC is near the test concentration (not emerging R)
  - Emerging resistance (mixed populations)

Causes for discrepancy: not due to errors (2)

- Genetic & phenotypic discrepancy
  - Not all mutations confer resistance
    - Silent mutations
    - Mutations cause slight increase of MIC but lower than the test concentrations
    - Mutations cause hypersusceptibility (surprise!)
      - Dual mutations in gyrA (T80A+A90G) cause fQ hypersensitivity—MIC 14x lower. (T80A alone also does not confer resistance.)

Causes for discrepancy due to errors

- Wrong specimens tested
  - Rare, but it happens due to mislabeling at collection or at lab, swapping specimens, inoculating wrong media….
- Testing errors
  - Forget to add drugs or supplement
  - Cell suspension too light or too heavy or clumpy
- Fail to rule out:
  - Mixed infection (MTBC + NTM)
  - Contaminations

Examples

Discrepant EMB Results

	Method	EMB conc	Results
Lab 1	MGIT 960	5	S
Lab 2	MGIT 960	5	S
Lab 3	Bactec 460	2.5	R
Lab 4	Bactec 460	7.5	S
Lab 5	Agar Proportion	5	R (5%)

The patient had not been treated before. Other drugs were S.

What to Consider to Resolve a Discrepant EMB Result

- EMB: the critical concentration is near the wild-type MIC.
- Mono-EMB resistance is very rare. If the pt has not been treated before. It is less likely to be R.
- Test concentrations:
  - 2.5 ug/ml by BACTEC 460 is slightly low; tend to have false R.
  - 7.5 ug/ml by BACTEC 460 is high, tend to have false S.
  - AP-5% R: may not be very reproducible.
- Pt status:
  - If MDR, emerging EMB-R may be considered.
  - Treatment history. Past regimen coverage—if covered by 3 or 4 effective drugs, on DOTS, R is less likely to develop.
  - What is the index case EMB result?

Discrepant ETH Results

	Method	ETH conc	Results
Lab 1	BAC 460	2.5	Low R
Lab 1	MGIT 960	5	R, GU 165
Lab 2	AP	5	S
Lab3	AP	10	S

What to Consider to Resolve a Discrepant ETH Result?

- The MICs for S & R strains are not well separated.
- If inhA mutation is detected, it is more likely to be R.
- Methodology differences
  - Some strains may have different metabolic pathways in different media under the influence of ETH.
  - The test concentration:
    - 5 ug/ml by MGIT; 89% correlation with AP; may be falsely R especially when GU is just slightly >100.
    - 2.5 ug/ml by BACTEC 460; correlated well with MGIT 5 ug/ml.
    - 5 ug/ml (7H10) or 10 ug/ml (7H11) by AP. (10 ug/ml with 7H10 may be high—it is possible to generate false S.)

Capreomycin

- Grandma—Pan-S
- Grandma developed MDR & died.
- Grandson was infected.
  - CAP: Bactec 460 showed slightly increase in GI, not high enough to call R.
  - CAP: AP showed inconsistent results: R at 12.5%; 3 months later tested S.
- Both patients were not treated with CAP.
- Not an emerging R.
- This strain's MIC is near the test concentration.

PZA

- False R seems to be more common with MGIT 960 than BACTEC 460
  - Coarse clumps in inoculum may be the cause.
- If PZA-R,
  - Is it a pure culture?
  - Is it M. bovis?
    - Check Morphology on LJ & do spoligo typing.
  - Mono-PZA-R (not M. bovis) is very rare.
    - Re-testing to confirm is recommended.
  - When re-test, pay attention to inoculum preparation.
    - Use homogenous cell suspension & standardize to McF 0.5 if use MGIT 960.

Quinolone

	CIP 1	CIP 2, MG	CIP 2, AP	OFX 1	OFX 2, MG	OFX 2, AP	SQ gyrA
Lab1	---	S	---	---	S	---	---
Lab2	R	---	---	R	---	---	---
Lab3	---	---	S	---	---	R, 12.5%	NO MUT

Quinolone Discrepancies

- Are there discrepancies in those results?
  - Maybe not (?)
- Critical concentration is 2 ug/ml.
  - R at 1 ug/ml should not be consider as R.
- No mutations in *gyrA*.
  - CDC found a novel mutation in *gyrB*; association with fQ resistance to be confirmed.
- R to OFX, but S to CIP at 2 ug/ml by AP.
  - Suggest retesting OFX and CIP.

Vertical Discrepancy

- 1st isolate: S to SIREP
- 3 months later: R to SIREP
- Acquired Resistance?
- All 5 drugs? in 3 months?—very unlikely!
- A key question was asked: Was SM included in the regimen? **No!** How could SM become R?
- Errors?! Lab investigation found:
  - Specimen switching occurred during processing for the first isolate—confirmed by genotyping. The patient had MDR TB initially.

Molecular & Phenotypic Discrepancies

- Limited genes are targeted.
  - If no mutations detected, but DST shows R, make sure DST is done properly and contamination/NTM is ruled out.
- Not all mutations confer resistance
- RIF
  - Silent mutations
    - 514 (TTT) & 527 (AAA)
  - Mutations causing slight increase in MIC
    - L533P, L511P, etc….
  - Mutations not causing increase in MIC
    - 526 (H to N), others…
  - Mutations not causing cross-R between RIF & RFB
    - D516V, H526L, H526G, K527R, etc.
- fQ
  - Not conferring R—(T80A), (T80A+A90G); (S95T)

Some Tips to Sort Out Discrepancies

- When reviewing DST results, one must know:
  - What method? What test concentration?
- Communicate with microbiologists.
  - Get lab's input. Lab reports do not contain all details. Lab may tell you some observations that were not reported. (such as GU, TIP, etc.)
  - The validation results.
  - How resistance is confirmed? R/O contamination, mixed culture?
- Is it due to methodology difference or lab errors?
  - If in doubt, ask lab to investigate.
  - Repeat testing may be the 1st step of investigation.
    - may not always resolve discrepancy.
    - Retest another isolate.
- Use molecular methods: may be helpful but not always.

Questions?
Comments?