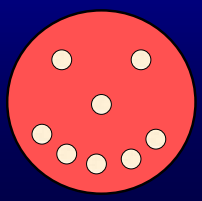
Antimicrobial Susceptibility Testing (AST): Current Issues & Implications for Public Health

Janet Fick Hindler, MCLS MT(ASCP)
jhindler@ucla.edu
UCLA Medical Center
Los Angeles, CA



At the conclusion of this talk, you will be able to……

- Discuss current issues in antimicrobial susceptibility testing and antimicrobial resistance.
- List current sources for information on antimicrobial susceptibility testing.
- Describe the effects of these issues on public health.

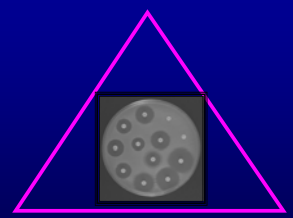
Current Issues in AST

- Identify methods to detect and report "emerging resistance"
  - Reliable
  - Practical for clinical laboratories
- Communicate AST and reporting issues to clinical labs
- Assess performance of clinical labs in detecting "emerging resistance"

Communicate AST & Reporting Issues to Clinical Labs

- Primary resources for continuing education
  - Workshops / seminars
  - Internet
  - Publications
- CDC / NLTN efforts
  - MASTER website (www.phppo.cdc.gov/dls/master/default.asp)
  - AST CD ROM (available late 2002)
  - NLTN workshops, teleconferences, other

Detect / Report Emerging Resistance



Clinical Lab

Public Health Lab
(Define role)

Public Health Lab as A Resource for Clinical Labs in AST & Reporting

- Performs AST (confirms unusual results)
- Answers questions
  - Identifies other resources, when needed
- Provides news "bulletins"
- Provides CE

Much Variability Among PH Labs!

Clinical Lab Need

Knowledge of "resources" for AST & reporting in their community ….particularly when a "real problem" arises.

Assess Performance of Clinical Labs in Detecting Emerging Resistance

- Inspections
- Proficiency surveys
- Post CE monitoring
- (Competency assessment)

Contemporary Resistance Concerns

- *Staphylococcus aureus*
  - MRSA – new tests
  - VISA
  - VRSA
- *Streptococcus pneumoniae*
  - Reporting using new breakpoints

Contemporary Resistance Concerns (con't)

- *Streptococcus* spp.
  - Beta *Streptococcus* Group B (in pregnancy) and clindamycin / erythromycin
- *Enterobacteriaceae*
  - Extended-spectrum beta-lactamases (ESBLs)

NCCLS Standards - 2002

- M2-A7 Disk Diffusion
- M7-A5 MIC
- M100-S12 Tables*

*Updated annually
www.nccls.org (610) 688-0100

Staphylococcus spp.

Organism	1st Choice Drugs	Alternative Drugs
MSS	P'ase stable penicillin (e.g., oxacillin)	a cephalosporin, vancomycin, ß-lac/ß-lac inhibitor combo, imipenem or meropenem, clindamycin, a fluoroquinolone
MRS	vancomycin +/- gentamicin +/- rifampin	linezolid, quin-dalfo, a fluoroquinolone, a tetracycline, trim-sulfa

The Medical Letter; 2001; 43:69.

MRSA (ORSA)

- Genetic determinant – *mecA*
- *MecA* codes for PBP2a

MRSA Test Methods

- NCCLS disk diffusion or MIC
- Commercial method
- Agar screen
- *mecA* assay
- PBP2a assay

MRSA

"Isolates of staphylococci that are shown to carry the *mecA* gene, or that produce PBP2a, the gene product, should be reported as oxacillin resistant."

NCCLS M100-S12

PBP2a

Can *mecA* or PBP2a Assays be Used Alone?

- MRSA?
- MSSA?
- Are these tests consistently reliable "on the bench"?
- Are results for other drugs routinely needed?

Staphylococcus - ß-Lactams

Pen	Ox	Comments
S	S	S to penicillins, cephems, carbapenems
R	S	R to ß-lactamase labile pens; S to ß-lactamase stable pens; S to ß-lac / ß-lac inhibitor combos, cephems, carbapenems
R	R	R to all ß-lactams

NCCLS M100-S12

Staphylococcus aureus

clindamycin	S
erythromycin	S
oxacillin	S
penicillin	R
vancomycin	S

"Cefazolin and other beta-lactams (except amoxicillin, ampicillin, and penicillins) are active against oxacillin-S and penicillin-R staphylococci."

VISA* (GISA**)

- Mostly MRSA
- Vanco MIC=8 μg/ml
- Japan, USA (MI, NY, NJ, CA), Europe
- Patients previously Rx with vanco

* vancomycin-intermediate *S. aureus*
**glycopeptide-intermediate *S. aureus*
(vancomycin is a glycopeptide)

Detection of VISA

	MIC (μg/ml)
MicroScan ON	8
Etest	6-8
Sensititre	4,8
Vitek	4
BHI-Van (6 μg/ml)	growth
MicroScan rapid	≤2, ≥ 16
Disk diffusion	inadequate

Tenover, et al. 1998. JCM. 36:1020

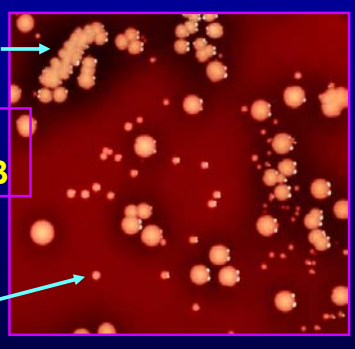
Case Study
VISA - Pt. JB (UCLA)

- 27 y.o. referral patient
- liver transplant candidate
- Bile drainage (liver abscesses)
- 2 strains VISA

Marlowe et al. 2001. JCM. 39:2637

Case Study - VISA Pt. JB

Strain	*mecA*	MIC (μg/ml) OX	Van
1	-	0.5	8
2	+	>16	8



Confirmation of VISA

- If VISA suspected:
  1. Repeat ID and susceptibility tests
  2. Contact your institution's infection control department
  3. Contact CDC at SEARCH@cdc.gov
  4. Contact your local health department
  5. Save isolate

VRSA – 1st Case

- Michigan
- 40 y.o., diabetic on dialysis
- Previous catheter site with MRSA
- Previous vancomycin Rx (6.5 wks)

MMWR 51:565-567, July 5, 2002

VRSA – 1st Case (con't)

- VRSA with *mecA* and *vanA* genes
- Patient also had VRE (*vanA*)
- Vancomycin MIC =1024 µg/ml
- S to chloramphenicol, linezolid, minocycline, quin-dalfo, tetracycline, TMP-SMZ

MMWR 51:565-567, July 5, 2002

VRSA – 2nd Case

- Pennsylvania
- Chronic foot ulcer
- VRSA with *mecA* and *vanA* genes
- Vancomycin MIC = 32 μg/ml
- DD zone = 12 mm
- Van (6 μg/ml) BHI screen = growth
- S to chloramphenicol, linezolid, minocycline, quin-dalfo, rifampin, TMP-SMZ

MMWR 51:902. Oct. 11, 2002

Streptococcus pneumoniae

If pen ≤ 0.06 μg/ml (S)...

1st Choice Drugs	Alternative Drugs
penicillin G or V; amoxicillin	a cephalosporin; erythromycin; clarithromycin; azithromycin; levofloxacin, gatifloxacin, or moxifloxacin; meropenem; imipenem; trim-sulfa; clindamycin; a tetracycline

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae

If pen 0.12 - 1 μg/ml (I)...

1st Choice Drugs	Alternative Drugs
penicillin G IV or ceftriaxone or cefotaxime	levofloxacin, gatifloxacin, or moxifloxacin; vancomycin clindamycin

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae

If pen ≥ 2 µg/ml (R)...

1st Choice Drugs	Alternative Drugs
Meningitis:	
vancomycin + cefotaxime or ceftriaxone +/- rifampin	meropenem; imipenem
Other:	
as above or levofloxacin, gatifloxacin, or moxifloxacin	quinupristin/dalfopristin linezolid

The Medical Letter; 2001; 43:69.

Streptococcus pneumoniae Breakpoints

- **Ceftriaxone and Cefotaxime**
  - Originally based on treatment of meningitis
  - NCCLS saw need for breakpoints for treatment of respiratory infections
  - New non-meningitis breakpoints
- **Penicillin**
  - Originally based on treatment of meningitis
  - No change in breakpoints

Streptococcus pneumoniae

	MIC (µg/ml) S	Int	R
Ceftriaxone or cefotaxime (meningitis)	≤ 0.5	1	≥ 2
Ceftriaxone or cefotaxime (non-meningitis)	≤ 1	2	≥ 4
Penicillin	≤ 0.06	0.12-1	≥ 2

NCCLS M100-S12

Streptococcus pneumoniae Penicillin

"High doses of IV penicillin (e.g. at least 2 million units every 4 hours in adults with normal renal function) or similarly ampicillin (e.g. 2 g at every 6 hours) are effective in treating pneumococcal pneumonia due to strains in the intermediate category."

NCCLS M100-S12

S. pneumoniae (CSF)

	MIC (µg/ml)
ceftriaxone (meningitis)	≤0.25 S
penicillin	≤0.03 S
vancomycin	0.5 S

Patients with meningitis require therapy with maximum doses of ceftriaxone.

S. pneumoniae (blood)

	MIC (µg/ml)
ceftriaxone (meningitis)	1 I
ceftriaxone (non-meningitis)	1 S
erythro, trim-sulfa	R
levofloxacin	0.5 S
penicillin	1.0 I
vancomycin	0.5 S

Pts. with meningitis require therapy with max doses of ceftriax; High dose IV pens (e.g. at least 2 mil U every 4 h in adults with normal renal function) or amp (e.g. 2 g at every 6 h) are effective in treating pneumococcal pneumonia due to strains in the penicillin "int" category.

Beta *Streptococcus* spp.

	1st Choice Drugs	Alternative Drugs
Beta Group A, C, G	penicillin G or V	clindamycin; erythromycin; a cephalosporin; vancomycin; clarithromycin; azithromycin
Group B	penicillin G or ampicillin	a cephalosporin; vancomycin; erythromycin

The Medical Letter; 2001; 43:69.

Throat Culture

Many Group A Streptococcus

"Group A Streptococcus remains universally susceptible to penicillin"

Beta *Streptococcus* spp. Erythromycin/Clindamycin

- Erythromycin and/or clindamycin are alternatives in penicillin-allergic patients
- Group A - 5-10% erythromycin-R
- Group B - up to 25% erythromycin-R and 15% clindamycin-R (prophylaxis in pregnant women issue)

1Schrag et al. 2002. MMWR Recomm Rep. 51:1-22.

Extended-Spectrum ß-lactamases (ESBLs)

- Result from mutation of common ß-lactamase genes (e.g., bla_{TEM-1}, bla_{SHV-1})
- Inactivate extended-spectrum ß-lactam agents
- Genes located on plasmids (often with other R genes)
- Approx. 100 TEM and 30 SHV types

Bradford, P. 2001. CMR. 14:933.

Significance of ESBLs

- Cause nosocomial infections
- Serious, infections. Treatment failures (esp. bacteremia) when patient treated with 3rd gen. cephalosporins.

ESBLs – Testing

- Organisms:
  E. coli, Klebsiella spp.
- Screen test:
  Decreased susceptibility to extended-spectrum ß-lactams
- Phenotypic confirmatory test:
  ß-lactam activity restored by ß-lactamase inhibitor (e.g. clavulanic acid)
- Other clues:
  "S" to cephamycins; multi-R

NCCLS M100-S12

Klebsiella pneumoniae ?ESBL

PRELIM:

	MIC (µg/ml)
cefoxitin	1 S
ciprofloxacin	0.5 S
imipenem	≤ 0.25 S
pip-tazo	8 S
am, cfaz, gm, T-S	R

***hold cefepime, cefotaxime, if "S"

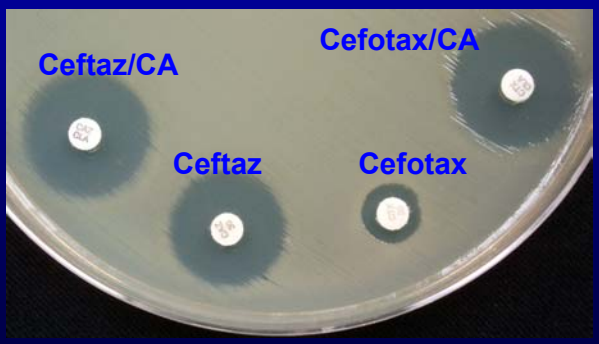
This *K. pneumoniae* is suspicious for extended-spectrum beta-lactamase (ESBL) production; confirmatory tests pending.

ESBL
Phenotypic Confirmatory Test

- Test:
  - cefotaxime
  - cefotaxime/clavulanic acid
  - ceftazidime
  - ceftazidime/clavulanic acid
- Results:
  - clavulanic acid restores activity of cefotaxime or ceftazidime or both

NCCLS M100-S12

ESBL Confirmatory Test



ESBL Reporting

- **Confirmed ESBLs** - report "R" for:
  - cephalosporins (not cephamycins)
  - penicillins (not ß-lac inhibitor combos)
  - aztreonam

NCCLS M100-S12

Klebsiella pneumoniae ESBL

FINAL:	MIC (µg/ml)
cefepime	2 S R
cefoxitin	1 S
cefotaxime	1 S R
ciprofloxacin	0.5 S
imipenem	0.25 S
pip-tazo	8 S
am, cefaz, gent, T-S	R

Confirmatory tests for this *K. pneumoniae* indicate unusual resistance [extended-spectrum beta-lactamase (ESBL) production]; ID consult suggested.

Issues - ESBLs

- Reporting ESBLs from urine isolates (e.g. acute cystitis)
- Changing results for other β-lactams (e.g. pip-tazobactam)
- Detection of…..
  - all types of ESBLs (≅100 TEM and 30 SHV)
  - ESBLs present with other "R" mechanisms
  - ESBLs beyond *E. coli* and *Klebsiella* spp.
  - other β-lactamases (e.g. plasmid-mediated ampC)

NCCLS M39-A Guideline

"Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data"

Focus - clinical laboratories

Guide MDs on empiric therapy

% Susceptible 2001 – Exmp.

	n	Am	Cf	Ctx	Cip	Gm	Pp	T-S
E. coli	*729*	66	81	98	97	97	66	76
E. cloacae	*144*	-	-	66	95	88	65	88
P. aerug	*221*	-	-	10	79	81	76	-

CAP Checklist MIC.21950 (2001)

- Does the procedure manual address unusual or inconsistent antimicrobial susceptibility testing results?
  - Testing QC strains doesn't guarantee accurate patient results
  - Unusual or inconsistent results should be investigated

Verification Rules - Exmps.

Biologically implausible or infrequent

- Repeat test
  - amik-R + gent-R + tob-R Enterobacteriaceae
  - amp-R *E. faecalis*
  - imipenem-R Enterobacteriaceae
- Reexamine or repeat test
  - amp-S *Klebsiella* spp.

NCCLS M39-A

Enterobacter aerogenes

ampicillin	R
cefazolin	R
ceftizoxime	S
gentamicin	S
imipenem	R*
trimeth-sulfa	S

* "R" likely due to drug deterioration

Conclusions

- Clinical laboratory detection of emerging resistance
  - Labs must have access to current testing / reporting guidelines
  - Lab personnel must be competent in testing
  - Resources for "problems" in emerging resistance must be available to labs
- *Staphylococcus aureus*
  - *mecA* or PBP2a tests can be used to identify MRSA
  - VISA are uncommon and may be difficult to detect
  - VRSA are rare and can be detected readily; however the resistance may be unstable

Conclusions (con't)

- *Streptococcus pneumoniae*
  - The new NCCLS breakpoints for ceftriaxone and cefotaxime should be reported
  - MICs should be performed on CSF isolates ASAP
  - A comment should be added to explain the meaning of a penicillin "Int" result on non-CSF isolates
- *Streptococcus* spp.
  - Group B streptococci from penicillin-allergic pregnant women should be tested with clindamycin and/or erythromycin

Conclusions (con't)

- *Enterobacteriaceae*
  - *E. coli* and *Klebsiella* spp. should be tested for ESBL production
  - Urine isolates associated with acute uncomplicated cystitis may not require ESBL testing
  - There are currently no standard NCCLS methods for detecting ESBLs in isolates other than *E. coli* and *Klebsiella* spp.
- Local resistance patterns
  - When possible, NCCLS M39-A should be followed for preparing a cumulative antibiogram report

Conclusions (con't)

- Verifying results on patient isolates
  - Each laboratory should develop a written protocol for verification of unusual or atypical patient results prior to reporting them
- Resources
  - a listing of antimicrobial susceptibility testing resources can be found on MASTER website

Additional References

Livermore, D. M., T. G. Winstanley, and K. P. Shannon. 2001. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J. Antimicrob. Chemother. 48:87-102.

Websites:

http://www.cdc.gov/drugresistance/factsheets/index.htm
CDC antimicrobial susceptibility testing fact sheets

http://www.cdc.gov/drugresistance/
CDC drug resistance

http://www.cdc.gov/drugresistance/community/
CDC antibiotic resistance

http://www.asmusa.org/division/c/index.htm
ASM Division C (clinical microbiology) website includes "Askit" feature

Thank you!

www.phppo.cdc.gov/dls/master/default.asp