Detecting Antimicrobial Resistance
A Partnership of PHL and CDC

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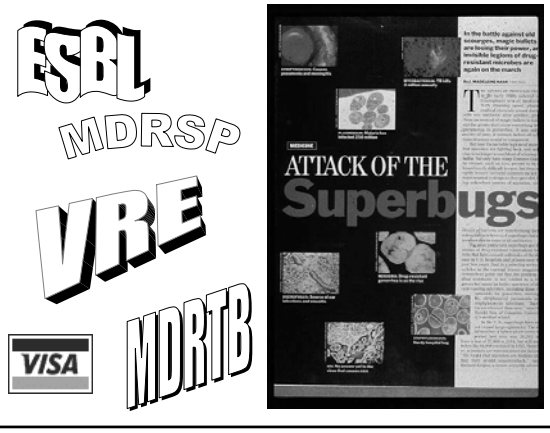
Objectives

1. Understand the clinical and public health importance of resistant microorganisms
2. Learn what organisms are the most important to monitor
3. Recognize the pitfalls in susceptibility testing for detecting resistant bacteria
4. Know the requirements for sending organisms to CDC
5. Understand how PHL and CDC can work together to facilitate rapid reporting of results



Why are we seeing more resistance?

- Overuse of antibiotics for viral illness
- Under-treating infections
  - Stop taking medication before finished
  - Use less potent antibiotics in underdeveloped countries
- Millions of dollars of antibiotics incorporated into livestock feed to bring a better price in the market

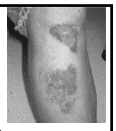
How does this affect public health?

- Resistant bacteria transmitted person-to-person perpetuate disease
  - Nosocomial- hospital
  - Community- daycare, nursing homes
- No antibiotics left to use!!
  - Only 8 new agents approved since 1998
- Deadly combination of virulence and resistance
- Resistant organisms in one part of the world only a plane ride away from your world

MRSA

- Methicillin resistant *Staphylococcus aureus* resistant to all penicillinase resistant antibiotics (nafcillin, oxacillin, dicloxacillin)
- Use oxacillin to predict resistance since it is more stable
  - If ox R, then cephalosporin R, amp/sulbactam R
- Population of *S. aureus* is heterogeneous which makes detection of resistance difficult
  - Tests to detect *mecA* gene or its product altered PBP2a more accurate

MRSA



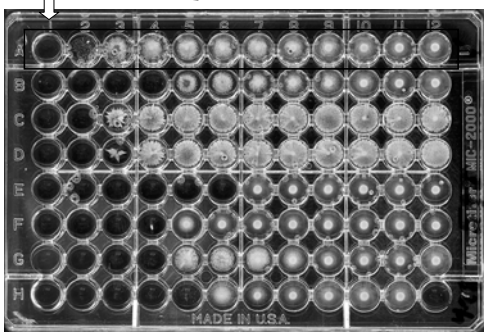
- Common in healthcare setting, now in outpatient population with skin/soft tissue infections, necrotizing pneumonia, septicemia
  - Daycare centers, football and wrestling teams, jails/prisons, msm
  - Unique PFGE type USA300
  - Different *mecA* gene (*mec* type IV)
  - Carries the gene for Panton -Valentine leukocidin (PVL)
  - Clindamycin for therapy requires additional testing to check for inducible resistance (D zone test)
- Nasal colonization facilitates spread of *S. aureus*

VRSA / VISA

- Vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus*
- Public health issue since vancomycin is routinely used to treat MRSA
- 3 strains of VRSA: MI, PA, NY
  - *S.aureus* acquired the *vanA* gene from enterococci
  - Surveillance revealed no transmission
- All potential VRSA and VISA (vanc > 4 µg/ml, growth on vancomcyin screening agar) should be sent to CDC for confirmation ASAP

Vancomycin MIC =128 µg/ml

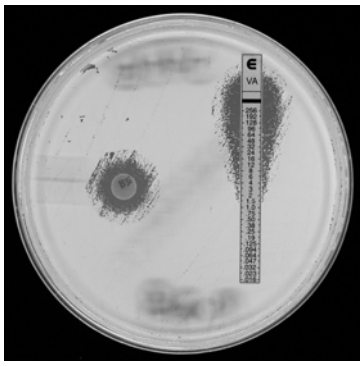
Increasing concentrations of vancomycin

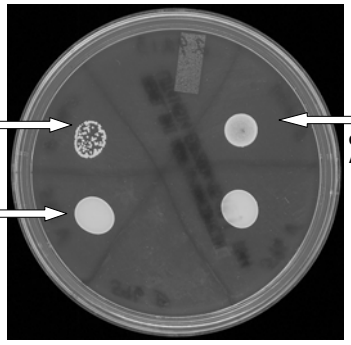


VA

***S. aureus* #595 tested in reference BMD panel**

***S. aureus* #595 tested after overnight growth on BAP**





Brain Heart Infusion agar containing 6 µg/ml of vancomycin (BHI-V6)

Algorithm for Testing *S. aureus* with Vancomycin (VA)

April 2004

Acceptable Primary Test Methods Include:

- **MIC method[1] plus VA screen plate** (BHIA with 6 µg/ml of VA)
  - **VA MIC ≤2 µg/ml And NO growth on VA screen plate** → **Report as VSSA[3]**
  - **VA MIC ≤2 µg/ml AND GROWTH on VA screen plate (rare)** → **Possible VISA/VRSA**
  - **VA MIC ≥4 µg/ml AND GROWTH on VA screen plate** → **Possible VISA/VRSA**
- **Disk diffusion[2] plus VA screen plate** (BHIA with 6 µg/ml of VA)
  - **VA zone ≤14 mm AND GROWTH on VA screen plate** → **Possible VISA/VRSA**
  - **VA zone >14 mm AND GROWTH on VA screen plate** → **Possible VISA/VRSA**
  - **VA zone >14 mm and NO growth on VA screen plate** → **Report as VSSA[3]**

CHECK purity

CONFIRM isolate ID

RETEST using non-automated MIC method[4]

SAVE ISOLATE

NOTIFY infection control, physician, local health department and CDC[5] of "possible VISA/VRSA"

SEND to reference laboratory for confirmation

Important Footnotes

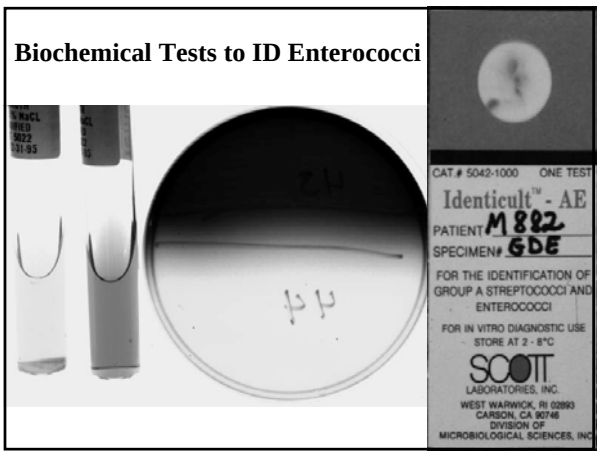
[1]Laboratories using automated susceptibility test methods should add a commercial vancomycin agar screen plate.
[2]Disk diffusion alone is not sufficient to detect VISA.
[3]If a laboratory is concerned about a result based on a patient's history, MIC testing can be performed at CDC.
[4]Non-automated methods: reference broth microdilution, agar dilution, agar gradient diffusion (Etest; use a 0.5 McFarland inoculum and Mueller-Hinton agar).
[5]Report to CDC by email: SEARCH@cdc.gov

More VISA/VRSA info: www.cdc.gov/ncidod/hip/vanco/vanco.htm

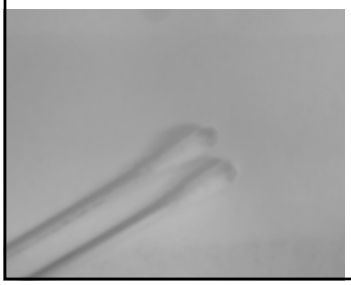
VRE

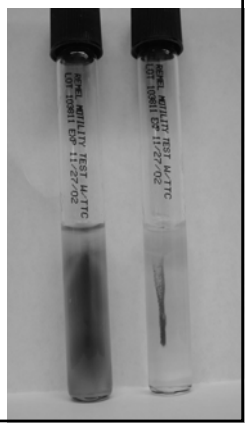
- Vancomycin-resistant enterococci (*E. faecium* and *E. faecalis*)
- Dangerous mix of already resistant bugs now resistant to the most widely prescribed antibiotic used to treat Gram positive infections
- Enterococci important cause of nosocomial bacteremia, surgical site infection and UTI
- Spread in healthcare environment on hands of personnel or contact with contaminated objects
- Treatment with new antibiotics (linezolid, quinopristin/dalfopristin)

Biochemical Tests to ID Enterococci



Additional Tests to Detect Species with Intrinsic Vancomycin Resistance





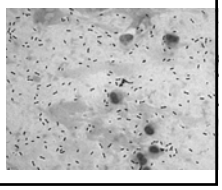
Pneumococci

⇩

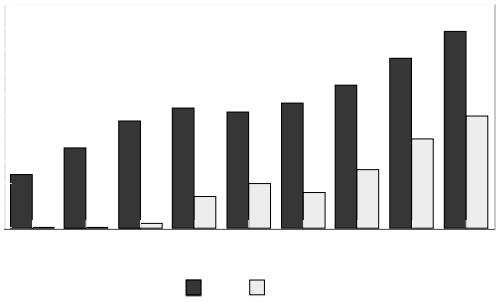
Pen R pneumococci

⇩

MDRSP



Trends in S. pneumoniae at LUMC



MDRSP

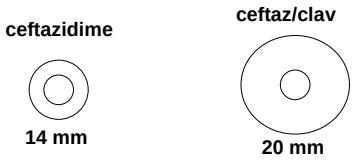
- Multidrug resistant Streptococcus pneumoniae
  - Resistant to pen, SXT, erythromycin, tetracycline, third generation cephalosporins
  - Resistant organisms easily spread in daycare settings
- New polysaccharide conjugate vaccine with 7 most common serotypes of pneumococci seen in children protects vaccinated and those around them (herd effect)

ESBL

- Extended spectrum beta lactamases destroy activity of all penicillins, cephalosporins, aztreonam
- Clinical importance
  - High failure rates with ESBLs treated with cephalosporins
  - ESBL producing organisms significant infection control problems
  - *E. coli, Klebsiella* spp*., Proteus mirabilis, Salmonella*
  - Carbapenems (imipenem) drug of choice

ESBL

- Screen for resistance based on MIC ≥2 µg/ml or disk diffusion zone sizes
- Must perform confirmatory tests using clavulanic acid to reverse activity ≥3 fold decrease in MIC or 5mm increase
- If positive, change S/I to R for all cephalosporins



AmpC Beta lactamases

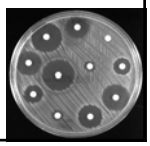
- Chromosomal mediated in *Enterobacter, Serratia, Citrobaccter, Morganella, Providencia*
- Plasmid-mediated in *E. coli* and *Klebsiella*
- Enzymes are resistant to the effects of beta-lactamase inhibitors
- Enzymes hydrolyze cephalosporins and cephamycins (cefoxitin and cefotetan)
- No standardized method of detection in the lab

MDR GNR

- *Acinetobacter baumannii* resistant to all routinely used antibiotics
- *Klebsiella* resistant to imipenem and cephalosporins
- Resistance to > 3 classes of antibiotics in *Ps. aeruginosa* increased from 4% in 1993 to 14% in 2002
- Inappropriate empiric treatment group had 38.4% mortality rate compared 27.4% for patients given at least one active antibiotic

How do clinical labs detect resistance?

- Large labs with high volume testing use automated instruments for ID/susceptibility
  - Rapid results, labor saving
  - May sacrifice accuracy, limited flexibility of antibiotics tested
- Disk diffusion
  - Technically simple
  - More cumbersome, slower to get a result
- E test
  - Combines MIC and diffusion method
  - Labor intensive and costly



Pitfalls in Current Susceptibility Testing

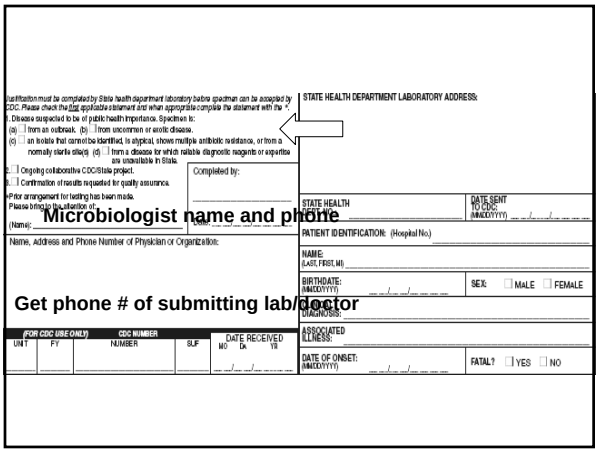
- Automation may overcall MRSA
- Automation may not detect VRSA
- Automation does not detect inducible resistance in clindamycin
- Disk diffusion may not detect VRE and VISA
- ESBL detection requires confirmatory testing
- Interpretation of pneumococcal antibiotics depends on meningitis or non-meningitis

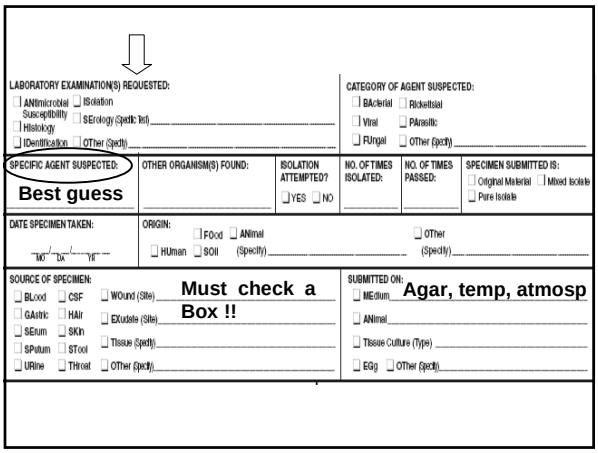
How can PHL help?

- First line resource to the smaller clinical labs
- Disseminate accurate advice
- Perform reference testing to confirm unusual results
- Gather data to track area-wide resistance
- Compile state-wide antibiogram

How can PHL be a good partner with CDC?

- Provide required information about the isolate
  - Patient demographics
  - Specimen source
  - Growth requirements of the organism (temperature, atmosphere, media)
  - Biochemical reactions of your testing
  - Give your “best guess” so it goes to the right lab
- Your name and phone number at PHL





LABORATORY EXAMINATION(S) REQUESTED:
☐ ANtimicrobial Susceptibility ☐ ISolation ☐ Histology ☐ SErology (Specify Test) ☐ IDentification ☐ OTher (Specify)

CATEGORY OF AGENT SUSPECTED:
☐ BActerial ☐ Rickettsial ☐ Viral ☐ PArasitic ☐ FUngal ☐ OTher (Specify)

SPECIFIC AGENT SUSPECTED:	OTHER ORGANISM(S) FOUND:	ISOLATION ATTEMPTED? ☐ YES ☐ NO	NO. OF TIMES ISOLATED:	NO. OF TIMES PASSED:	SPECIMEN SUBMITTED IS: ☐ Original Material ☐ Mixed Isolate ☐ Pure Isolate
Best guess					

DATE SPECIMEN TAKEN: ___/___/___ MO DA YR

ORIGIN: ☐ FOod ☐ ANimal ☐ HUman ☐ SOil (Specify) ☐ OTher (Specify)

SOURCE OF SPECIMEN:
☐ BLood ☐ CSF ☐ WOund (Site) ☐ GAstric ☐ HAir ☐ EXudate (Site) ☐ SErum ☐ SKin ☐ SPutum ☐ STool ☐ TIssue (Specify) ☐ URine ☐ THroat ☐ OTher (Specify)

Must check a Box !!

SUBMITTED ON: Agar, temp, atmosp
☐ MEdium ☐ ANimal ☐ TIssue Culture (Type) ☐ EGg ☐ OTher (Specify)

PREVIOUS LABORATORY RESULTS/OTHER CLINICAL INFORMATION: (Information supplied should be related to this case and/or specimen(s) and relative to the test(s) requested.

Copy of your biochemical reactions or the submitting laboratory. Tell us anything and everything !!

How can PHL be a good partner with CDC?

- Check isolate for **purity** before it's sent !!!!
  - Use selective agars to find contamination
  - Chocolate, MacConkey, CNA, bile esculin
- If you are unsure of the importance or urgency of a request, call CDC
  - Staph lab 404-639-3570
  - Strep lab 404-639-1237
  - Enterics 404-639-2316
  - Anaerobes 404-639-3654
  - Special bacti 404-639-5458
  - E.coli/Shigella 404-639-4372