ALPHABET SOUP OF ANTIMICROBIAL RESISTANCE

ANTIMICROBIAL RESISTANCE
HOW CAN THE LAB HELP?

Dr. Susan Whittier, X 5-6281

ANTIBIOTIC SUSCEPTIBILITY TESTING ROLE OF THE LAB

- FOLLOW CURRENT CLSI (NCCLS) GUIDELINES
- WHAT DRUGS SHOULD BE TESTED & REPORTED?
  - SELECTIVE DRUG/BUG COMBINATIONS BASED ON IN VIVO & IN VITRO CORRELATION OF DATA
  - ID, PHARMD & CLINICAL MICRO TEAM
- ANNUAL ANTIBIograms
  - HELPS WITH MICROBES WITH PREDICTABLE RESISTANCE PATTERNS
- LAB REPORTING SYSTEMS
  - SIR, YES/NO (DISK DIFFUSION)
  - MIC (DISK GRADIENT &/OR MICRODILUTION)
  - TESTING NEW ANTIMICROBIAL AGENTS
    - JUST SIR BY DISk DIFFUSION
    - MIC BY DISk GRADIENT STRIP

ANTIBIOTIC SUSCEPTIBILITY TESTS

- MIC VALUE
  - LOWEST CONCENTRATION OF ANTIMICROBIAL WHICH WILL INHIBIT GROWTH
  - MICROSCAN or VITEK SEMIAUTOMATED
  - E-STRIPS (DISK GRADIENT)
  - TIME TO RESULTS: 18 - 24 HRS
- YES SIR, NO MIC
  - QUALITATIVE INTERPRETATION
  - DISK DIFFUSION (KIRBY-BAUER)
  - TIME TO RESULTS: 18 - 24 HRS
- QUESTIONS TO ASK......
  - S.aureus IS ERYTHRO RESISTANT
    - IS IT A PREDICTOR OF CLINDA RESISTANCE?
  - LAB REPORTS PENICILLIN RESISTANT GP A STREP
    - IS THIS BELIEVABLE?
  - LAB REPORTS YEAST FROM BLOOD CULTURE
    - WHAT EMPIRIC TREATMENT IS RECOMMENDED?

WHAT AFFECTS CHOICE OF ANTIMICROBIAL AGENTS?

- ANTIMICROBIAL SUSCEPTIBILITY TEST RESULTS
- PHARMACODYNAMICS
  - AUC:MIC90 RATIO
  - HALF LIFE OF DRUG
  - TIME ABOVE THE MIC
  - CONCENTRATION DEPENDENT KILLING
    - Greater cidal activity with higher concen (e.g. aminoglycosides, B-lactams)

NAME CALLING AST JARGON

- MRSA - Methicillin-Resistant S.aureus
  - 44% at CUMC
- VISA- Vanco-intermediate S. aureus
- VRSA- Vanco-resistant S. aureus
- VRE- Vanco R E. faecium
  - 81% in CUMC
- ESBLs in GNR
  - 18% in CUMC
PREDICTABLE RESISTANCE

- **Salmonella, Shigella**
  - Stool: Ampicillin, quinolone, T/S ONLY will be reported
  - Extraintestinal: above + chloramphenicol, 3rd gen cephalosporin
- **Enterobacter, Serratia**
  - Ampicillin & 1st & 2nd generation cephalosporins are NOT reported
  - Routine resistance
- **Stenotrophomonas**
  - Inherent resistance to nearly all antimicrobics
  - ONLY T/S, Timentin & fluoroquinolone are reported

**Enterobacter, Serratia**
- Ampicillin & 1st & 2nd generation cephalosporins are NOT reported
- Routine resistance
- **Stenotrophomonas**
  - Inherent resistance to nearly all antimicrobics
  - ONLY T/S, Timentin & fluoroquinolone are reported

**Campylobacter, Bacillus, Corynebacterium**
- NO ESTABLISHED CRITERIA

**Enterococcus**
- Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported

**Enterococcus**
- Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported
- Chloramphenicol, 3rd gen cephalosporin

**Enterococcus**
- Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported

**Stenotrophomonas**
- Inherent resistance to nearly all antimicrobics
- ONLY T/S, Timentin & fluoroquinolone are reported

**Enterococcus**
- Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported

**Enterococcus**
- Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported

**Stenotrophomonas**
- Inherent resistance to nearly all antimicrobics
- ONLY T/S, Timentin & fluoroquinolone are reported

THE “USED TO BE” PREDICTABLE AST PATTERNS

<table>
<thead>
<tr>
<th>ORGANISMS</th>
<th>PREDICTABLE [Not so much…]</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumo</td>
<td>Susceptible to Imipenem</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>Susceptible to Cipro</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Susceptible to Cipro</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Susceptible to Vanco</td>
</tr>
<tr>
<td>E. faecium</td>
<td>Susceptible to Linezolid</td>
</tr>
<tr>
<td>Any organism</td>
<td>Susceptible to at least one antibiotic</td>
</tr>
</tbody>
</table>

ENDOCARDITIS CASE

- 61 yo male with persistent fevers
- Suspected subacute bacterial endocarditis
- Two sets of blood cultures collected
- Positive the next day for coagulase negative *Staphylococcus*
- AST panels are set up for isolates 1 & 2

ENDOCARDITIS CASE MIC VALUES

- **ISOLATE #1**
  - **OXACILLIN 0.5** Resistant
  - **PENICILLIN 1.0** Resistant
  - **VANCO** Susceptible
  - **CLINDA** Susceptible
  - **ERYTHRO < 0.25** Susceptible
- **ISOLATE #2**
  - **OXACILLIN 1.0** Resistant
  - **PENICILLIN 0.5** Resistant
  - **VANCO** Susceptible
  - **CLINDA** Susceptible
  - **ERYTHRO < 0.25** Susceptible

ARE THESE THE SAME ISOLATE? MICS WITHIN 1 2-FOLD DILUTION OF EACH OTHER ARE CONSIDERED THE SAME

ENDOCARDITIS CASE POINTS TO PONDER

- ARE THE ISOLATES REALLY RESISTANT?
  - MICs ARE VERY LOW [0.5 AND 1.0]
  - **S. AUREUS** OXACILLIN RESISTANCE > 4
  - BREAKPOINTS FOR CNS & OXACILLIN WERE REVISED
  - MANY CNS STRAINS CONTAINED MECA BUT HAD OXACILLIN MICs BELOW THE 4 UG/ML BREAKPOINT
  - NOW THERE ARE TWO SETS OF OXACILLIN BREAKPOINTS

ENDOCARDITIS CASE ONE MORE WRINKLE!

- ONE SPECIES OF CNS UTILIZES THE S. AUREUS BREAKPOINTS
  - *Staphylococcus lugdenensis*
  - Ubiquitous to skin & mucous membranes
  - Portal of entry often unidentified
  - Chronic renal failure
  - Neoplastic disease
  - Post-pneumonia
  - High mortality associated with aggressive destruction of native valve
Staphylococcus lugdenensis
- Able to bind vitronectin & fibrinogen to extracellular matrix proteins
- Produces a delta-like toxin similar to that of S. aureus
- Demonstrate nucleic acid sequences related to SA accessory gene regulator (agr), a determinant of virulence
- Frequently emboligenic
- All traits are more typical of S. aureus

Beta-hemolytic Streptococci
Erythromycin/Clindamycin

<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>DETERMINANT</th>
<th>ERY</th>
<th>CLIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFLUX</td>
<td>MEF</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>RIBOSOME MODIFICATION</td>
<td>ERM</td>
<td>R</td>
<td>S**</td>
</tr>
<tr>
<td>RIBOSOME MODIFICATION</td>
<td>ERM</td>
<td>R</td>
<td>R CONSTITUTIVE</td>
</tr>
</tbody>
</table>

* Groups A, B, C, G
**Requires induction to show resistance

NEONATAL SEPSIS
- Female full-term neonate developed fever of 103 at 2 days of age
- Irritable & not feeding well
- Mom’s pre-natal screen at 36 wks gestation was positive for Grp B strep
  - MOM WAS PEN ALLERGIC SO RECEIVED IV CLINDAMYCIN DURING DELIVERY
  - PREGNANCY UNEVENTFUL OTHER THAN PROM @ 20H PRIOR TO DELIVERY
- Blood cultures collected from neonate & prophylactic ceftriaxone was initiated
- Signs of improvement w/in 6 hrs

NEONATAL SEPSIS
- NEXT DAY, BLOOD CULTURES WERE POSITIVE FOR:
  - GPC chains & pairs
- DAY 2
  - Catalase negative
  - Beta hemolytic
  - Grp B strep latex positive

AST Results
- Ampicillin <0.25 Susceptible
- Ceftriaxone <0.12 Susceptible
- Clinda <0.25 Susceptible
- Erythro >1 Resistant
- Penicillin ≤0.12 Susceptible
- Vanco ≤0.5 Susceptible

WHY WAS CLINDA NOT EFFECTIVE IN PREVENTING THIS INFECTION?

BETA-HEMOLYTIC STREPTOCOCCUS RESISTANCE RATES (USA)*
- Beta-hemolytic Streptococcus spp.
  - AMPCILLIN / PENICILLIN / VANCOMYCIN: 0%
  - Group A
    - ERYTHROMYCIN: UP TO 10%
    - CLINDAMYCIN: UP TO 7%
  - Group B
    - ERYTHROMYCIN: UP TO 25%
    - CLINDAMYCIN: UP TO 15%

*commonly quoted rates; select studies may have reported higher rates

When the pieces of the puzzle don’t quite fit....
- URINE CULTURE OBTAINED FROM LONG-TERM-CARE FACILITY PT
  - Patient hx significant for diabetes, peripheral vascular disease & chronic renal failure
- CULTURE RESULTS:
  - >100,000 CFU/ml Staphylococcus aureus

<table>
<thead>
<tr>
<th>DRUG</th>
<th>RESISTANT</th>
<th>SUSCEPTIBLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXACILLIN</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>CHLORAMPHENICOL</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>LINEZOLID</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>RIFAMPIN</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TRIMETH/SULFA</td>
<td>2/38</td>
<td></td>
</tr>
<tr>
<td>VANCOMYCIN</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Patient was started on vancomycin
Urinary cultures remained positive for *S. aureus*
Further testing by lab
  - E test MIC = >256 RESISTANT!!
Isolate was positive for
  - *mecA* OXACILLIN RESISTANCE
  - *vanA* VANCOMYCIN RESISTANCE
MECHANISM FROM VRE
WHAT HAPPENED???????
Automated systems are unable to detect VRSA
CDC recommends utilization of vancomycin screen agar plate

---

**VRSA JUNE 2002**

1st case in 40 yr old diabetic woman from Michigan
VRSA from dialysis cath tip
Recurrent foot ulcer infected with VRE & MRSA

---

**VRSA**
(3 isolates encountered to date)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Vanco MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,024</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
</tr>
</tbody>
</table>

1 Reference broth microdilution MIC
2 Missed or inconsistent results (some ≤ 2 µg/ml) with automated methods

4/04 CDC RECOMMENDATION:
ADD VANCOMYCIN AGAR SCREEN WITH AUTOMATED METHOD

---

**NEW TESTS**

- **LATEX AGGLUTINATION ASSAY**
  - PBP2a low-affinity penicillin binding protein
  - Latex beads sensitized with monoclonal Ab vs PBP2a
  - PURE CULTURE ONLY (NOT SPECIMEN)
  - Need 10⁶ cells
  - 1 HR TEST
- **PCR – GOLD STANDARD**
  - *mecA* & *nuc* genes – COAMPLIFICATION
  - BLOOD CULTURE BOTTLES or PURE CULTURE
  - LYSE CELLS
  - SMART CYCLER (amplification & detection)
  - UNSTANDARDIZED
  - EXPENSIVE, TECHNICALLY CHALLENGING
  - 4 HR TEST

---

**VISA**

- VISA – INTERMEDIATE TO VANCO
  - 1st ISOLATED IN 1996 IN JAPAN
  - 8 PTS TO DATE IN USA
  - MECHANISM OF RESISTANCE: THICKENED CELL WALL AND/OR AN EXTRACELLULAR MATRIX ???
  - PATIENTS HAD PRIOR EXPOSURE TO LONG TERM VANCOMYCIN THERAPY
- 2 VISA ISOLATES FOUND SUSCEPTIBLE TO OXACILLIN
  - ONE WAS *mecA* POS & ONE NEG
  - OXACILLIN RESISTANCE IS NOT NECESSARY FOR VISA PHENOTYPE
- NO CLONAL SPREAD OF SINGLE STRAIN

---

**PUZZLE PIECES**

- E. faecalis
- *S. aureus*
- VanA
- *S. aureus*
- VanA
- *S. aureus*
- VanA
- VanA
- Resident plasmid
- VanA
- *E. faecalis*
ICU SEPSIS

- 64 yo male patient, cardiac ICU post-CABG
- Becomes febrile and hemodynamically unstable
- Blood cultures x 2 are collected
- Culture Results:
  - Klebsiella pneumoniae
    - Amikacin 8 S
    - Cefoxitin 4 S
    - Ceftazidime ≥32 R
    - Ceftriaxone 8 S
    - Imipenem 4 S
- Based on AST, patient treated w/ ceftriaxone
- Remains febrile
- Blood cultures collected
- Positive for K. pneumoniae
- What’s going on?

EXTENDED SPECTRUM ß-LACTAMASES

- FIRST DESCRIBED IN 1983
- ESBLS ARE ß-LACTAMASES THAT MEDIATE R TO
  - 3rd GEN CEPHALOSPORINS BUT THESE CAN APPEAR SUSCEPTIBLE WHEN TESTED IN LAB
  - MONOBACTAMS (E.G. AZTREONAM)
  - EXTENDED SPECTRUM PENICILLINS (E.G. PIPERACILLIN)
- STRUCTURAL GENES
  - PLASMID- MEDIATED
    - Altered configuration of TEM-1 & 2, SHV-1 near active sites to increase hydrolytic ability for cephalosporins
    - Susceptible to cefoxitin (cephamycin), ß-lactamase inhibitors (but enzyme hyperproduction might overwhelm inhibitors)
    - Susceptible to carbapenems
  - CHROMOSOME-MEDIATED AMP C
    - AmpC in SPICE (Serratia, Pseudo, Proteus, Citro, Enterobacter)
  - PLASMID-MEDIATED AMP C
    - K1 in K. oxytoca
    - Resistant to cefoxitin (cephamycin) & ß-lactamase inhibitors

ESBL PHENOTYPIC CONFIRMATORY TESTS

- To confirm screening results, compare the MIC values of:
  - Ceftazidime to ceftazidime+clavulanate
  - Cefotaxime to cefotaxime+clavulanate
- ESBL = ≥3 DOUBLING DILUTION DECREASE FOR EITHER DRUG IN THE PRESENCE OF CLAVULANATE

You “Pneumo” Than You Thought

- A 35 year old obese female was admitted for elective knee replacement surgery following an automobile accident
- Post-surgery she developed ARDS and was placed on a ventilator
- The patient’s condition continued to deteriorate and she developed a nosocomial pneumonia

KLEBSIELLA PNEUMONIAE TYPICAL ESBL AST PATTERN

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≥32</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥8</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>8/2</td>
</tr>
<tr>
<td>Aztreonam (monobactam)</td>
<td>≥32</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>8/152</td>
</tr>
</tbody>
</table>
You “Pneumo” Than You Thought

- What gram-negative was recovered from BAL, an empyema collection, urine, and blood?
  - *Klebsiella pneumoniae*

You “Pneumo” Than You Thought

- At least three mechanisms described that result in imipenem resistance among strains of *K. pneumoniae* among isolates recovered from patients in New York City
  - ampC hyperproduction with concomitant loss of outer membrane porins
    - KPC-2
    - KPC-3

The Case of the Flavorful Bacterium

- A 30 day old male was seen by his pediatrician as an outpatient for routine circumcision
- The following day the mother noted a fever and brought the child to the emergency department of a rural hospital
- The child was admitted with a temperature of 103°F and started empirically on ampicillin and cefotaxime after collection of blood cultures and performance of a spinal tap due to “meningeal signs”
- Bladder catheterization was attempted but the tubing crimped and could not be properly placed or removed

You “Pneumo” Than You Thought

- What additional antibiotics might be tested?
  - Polymyxin B: disk diffusion
    - Zone size = 12 mm
    - Interpretation?

A Bladder Case I have Never Seen

- The child was transferred emergently to CUMC for catheter removal and treatment of infection
- Blood cultures became positive at the outlying hospital with a gram-negative rod
- Upon admission to CUMC blood cultures were again collected, a 2nd spinal tap was performed, and cotrimoxazole was added to the antibiotic regimen
- Colonies had a very faint yellowish pigment; identified at the outside hospital using API NF-ID as a *Chryseobacterium* sp.
**Chryseobacterium meningosepticum**
- Isolates recovered both from CSF and blood at CUMC were identified as *C. meningosepticum*
- Natural habitats: soil, plants, foodstuffs, and water sources (including hospital)
- Oxidase and indole positive; nonmotile
- The patient was not responding optimally to therapy
- Pending results of antimicrobial susceptibility testing what changes were made to the antibiotic regimen?

**Water Case This Was!**
- Blood cultures were collected and the patient was admitted to the PICU
- He was started empirically on cefepime plus vancomycin
- The following day one of the two blood cultures became positive (aerobic bottle only) with a "diphtheroid" which was deemed a contaminant
- The young man responding to has antibiotics defervesced and his cellulitis felt less warm to the touch

**Chryseobacterium meningosepticum**
- *Chryseobacterium* spp. are inherently resistant to many antibiotics commonly used to treat infections caused by gram-negative bacteria (aminoglycosides, β-lactams, tetracyclines, and chloramphenicol)
- Susceptible to agents generally used for treating infections caused by gram-positive bacteria (rifampin, clindamycin, erythromycin, levofloxacin, trimethoprim-sulfamethoxazole, and vancomycin)
- Di Pentima et al (1998; CID; 26:1169-1176) provided evidence that IV vancomycin plus rifampin are appropriate empiric therapy for *C. meningosepticum* meningitis in newborns

**Water Case This Was!**
- The following morning the second blood culture became positive with the same "diphtheroid"
- Colonies developed a yellow pigment over a 3 day period of time
- The pediatric ID physician requested that the isolate be further identified and that antimicrobial susceptibility testing be performed
- Any thoughts as to the identity of the isolate?

**“Water” Case This Was!**
- A fifteen year old male just completing a course of methotrexate therapy for osteogenic sarcoma visited Myrtle Beach with his family
- While walking on the shore he stepped on a razor clam and sustained a cut to the bottom of his foot
- The following morning he noticed redness around the cut and treated it with triple ointment
- Upon returning home he presented to the ED with a cellulitis and low grade fever

**Leifsonia aquatica**
- Previously named *Corynebacterium aquaticum*
- Rarely encountered in clinical specimens
- Identity confirmed by NYC DOH
- Always motile; very strong DNAse activity
- Yellow pigment of colonies develops slowly over three to four days
- Vancomycin MICs for some strains are elevated (8 μg/mL)
How to Perform Susceptibility Tests?

- No NCCLS recommended methods for testing of coryneform bacteria (orphan organisms)
- No FDA or NCCLS breakpoints for interpreting results of MIC testing
- No disk diffusion interpretive criteria
- Three options
  - Do not test
  - Test and use breakpoints from other gram-positives
  - Test and report MIC results with no interpretations using PK to judge whether achievable levels can be reached at the site of infection

How “Sporing” Can a Case Get?

- Susceptibility testing is requested
- Which of the following approaches should be taken?
  - Set up disk diffusion tests?
  - Set up E-tests on Blood M-H?
  - Set up broth macrodilution testing in Mueller-Hinton (M-H) broth?
  - Go to literature and/or textbooks to assess published results?
  - Use NCCLS guidelines for testing of B. anthracis?

You’ll Take a “Lichen” to This Case

- A 5 year old boy with ALL with an indwelling intravascular line for administration of chemotherapeutic agents becomes febrile
- Redness and purulent discharge are noted at the line insertion site
- Blood cultures yield Bacillus licheniformis
- The child is treated empirically with ceftriaxone, defervesces, and clears his blood cultures

Bacillus spp.

- No correct/incorrect answers
- In Table 2K of M100-S14 it states: “Criteria for B. anthracis do not apply to other Bacillus spp.”
- Suggest using inoculum and incubation conditions listed in Table 2K and reporting MIC values without interpretations (unless result is > highest value tested; report as R)

I’m Not Giving You a ‘Line”

- Because of poor access, the vascular line is not removed
- 48 hours after cessation of therapy the child again shows signs of sepsis and additional blood cultures are collected
- Cultures again yield B. licheniformis
- Possible explanations?

Bacillus spp.

- Usually resistant to β-lactams
- Vancomycin and clindamycin recommended pending availability of susceptibility testing results
- Cephalosporins contraindicated
- Ciprofloxacin has been successfully used
TO THE RESCUE?

- NEW ANTIBIOTICS
  - LINEZOLID
  - SYNERCID
  - DAPTOMYCIN
  - ERTAPENEM
  - TIGECYCLINE
- BACK FOR A 2ND CHANCE!
  - COLISTIN
  - POLYMIXIN B

SYNERGY TESTING
A NEW PLAN OF ATTACK!

- CHOOSE TWO ANTIBIOTICS WITH DIFFERENT MECHANISMS OF ACTION
- COMBINE THEM TO SEE WHETHER THEY ARE MORE EFFECTIVE IN COMBINATION THAN EITHER IS INDIVIDUALLY
- HISTORICALLY EFFECTIVE
  - I.E. PENICILLIN & GENTAMYCIN FOR ENTEROCOCCI
- CLINICAL OUTCOME DATA SUPPORTS SYNERGY TESTING FOR:
  - GRAM NEGATIVE INFECTIONS IN NEUTROPENICS
  - CYSTIC FIBROSIS ISOLATES
  - PAN-RESISTANT GRAM NEGATIVES
- DETERMINE FIC (FRACTIONARY INHIBITORY CONCENTRATION)

\[ \text{\checkmark SYNERGISTIC} \quad \checkmark \text{ADDITIVE} \quad \checkmark \text{ANTAGONISTIC} \]