ALPHABET SOUP OF ANTIMICROBIAL RESISTANCE

LABORATORY MEDICINE COURSE
2004
CLINICAL MICROBIOLOGY SERVICE
Dr. Preeti Pancholi 5-6237

ALPHABET SOUP OF ACRONYMS
• MRSA- METHICILLIN-RESISTANT S. aureus ✓ 46% AT CUMC
• VISA- VANCOMYCIN (GLYCOPEPTIDE)-INTERMEDIATE S. aureus
• VRSA- VANCOMYCIN-RESISTANT S. aureus
• VRE- VANCOMYCIN R Enterococcus faecium ✓ 80% AT CUMC
• ESBLs - Extended-spectrum β-lactamases ✓ GRAM-NEGATIVE RODS ✓ 18% AT CUMC

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, β-lactams)

ANTIBIOTIC SUSCEPTIBILITY TESTS Role of Clinical Microbiology
• FOLLOW CURRENT NATIONAL COMMITTEE CLINICAL LAB STANDARDS (NCCLS)
• USE OPTIMAL SUSCEPTIBILITY METHODS & QUALITY CONTROL MEASURES
• PROVIDE MIC & INTERPRETATIONS ✓ e.g. SUSCEPTIBLE, INTERMEDIATE, RESISTANT
• WHAT DRUGS SHOULD BE TESTED & REPORTED?
 ✓ APPROPRIATE DRUG/BUG COMBINATIONS ✓ ID, PHARMD & CLINICAL MICRO TEAM
• ANNUAL ANTIBIOTIC REPORTS

NCCLS GUIDELINES
• SELECTIVELY TEST ONLY DRUG/BUG COMBINATIONS WITH IN VIVO/IN VITRO CORRELATION ✓ Campylobacter, Bacillus, Corynebacterium • NO ESTABLISHED CRITERIA ✓ Enterococcus • Do not report cephalosporins, aminoglycosides, clinda, T/S ✓ Salmonella, Shigella • Stool: ONLY test ampicillin, quinolone, T/S • Extraintestinal: above + chloramphenicol, 3rd gen cephalosporin ✓ Enterobacter, Serratia • Do not report ampicillin & 1st & 2nd gen cephalo • Routine resistance ✓ Stenotrophomonas • Inherent resistance to nearly all antimicrobics • ONLY Test T/S, Timentin & fluoroquinolone

DEFINING CLASS DRUGS
<table>
<thead>
<tr>
<th>OXACILLIN</th>
<th>Cephalosporins &amp; penicillins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus</td>
<td></td>
</tr>
<tr>
<td>TETRACYCLINE</td>
<td>Doxycline, minocycline, chlorotetracycline</td>
</tr>
<tr>
<td>All except (Staph &amp; Acinetobacter)</td>
<td></td>
</tr>
<tr>
<td>ERYTHROMYCIN</td>
<td>Clarithromycin, azithromycin</td>
</tr>
<tr>
<td>Gram + cocci</td>
<td></td>
</tr>
<tr>
<td>CEPHALOTHINS</td>
<td>Cefazolin, cefaclor, cepalexin</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
</tr>
</tbody>
</table>
WHAT ARE MIC VALUES?

- **MINIMUM INHIBITORY CONCENTRATION (MIC)**
  - LOWEST CONCENTRATION OF ANTIMICROBIC WHICH WILL INHIBIT GROWTH
- **METHODOLOGIES**
  - MICROBROTH DILUTION BY SEMI-AUTOMATED INSTRUMENTS, e.g. MICROSCAN, VITEK
  - 2-FOLD ANTIMICROBIC DILUTIONS
  - E-TEST
  - PLASTIC STRIPS-GRADATED ANTIBIOTIC CONCENTRATIONS
- **MIC BREAKPOINTS SEPARATE SUSCEPTIBLE, INTERMEDIATE & RESISTANT STRAINS**
- **REFLECTS ACHIEVABLE SERUM CONCENTRATIONS OF THE DRUG**

SIR INTERPRETATIONS

- **SUSCEPTIBLE (S)**
  - INFECTION BY THE STRAIN MAY BE APPROPRIATELY TREATED WITH THE DOSE OF ANTIMICROBIC
- **INTERMEDIATE (I)**
  - RESPONSE RATES MAY BE LOWER THAN FOR SUSCEPTIBLE ISOLATES
- **RESISTANT (R)**
  - STRAINS NOT INHIBITED BY THE USUALLY ACHIEVABLE SERUM CONCEN OF THE AGENT WITH NORMAL DOSING

PREDICTABLE SUSCEPTIBILITIES

<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>RESISTANT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumonia</em></td>
<td>Ampicillin</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>Ampicillin, 1st/2nd cephal</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1st/2nd cephal, aminoglycosides</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td></td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>All penems, cephems, pip/tazo, other ß-lactams</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>Vancomycin</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>Cephalosporins</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>Aminoglycosides, Carbapenems, T/S,often Vancomycin <em>(E. faecium)</em></td>
</tr>
</tbody>
</table>

ANTIMICROBIC SUSCEPTIBILITY TESTS (AST)

<table>
<thead>
<tr>
<th>MICROBROTH</th>
<th>DESCRIPTION</th>
<th>DETECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICROSCAN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VITEK</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AST METHODS

<table>
<thead>
<tr>
<th>AGAR</th>
<th>DESCRIPTION</th>
<th>DETECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>E STRIP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIRBY-BAUER</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MRSA PROFILE

- **PENICILLIN INTRODUCED IN 1944**
  - Plasmid-mediated resistance by ß-lactamase that hydrolyzes ß-lactam ring
  - Prevalent in hospitals in 1950s
- **METHICILLIN INTRODUCED IN 1959**
  - MRSA appeared in 1961 & prevalent in 1970s
  - Resistance from 4 Penicillin Binding Proteins (PBP) encoded by *mec* genes (30-50 kb)
  - Chromosomal, not plasmid
  - MRSA acquired the *mec A* gene which codes for the production of unique PBP2a
- **Oxacillin is the indicator drug for testing**
  - *S.aureus* = MIC < 2 ug/ml (S)
  - Coag Neg Staph = MIC < 0.25 ug/ml (S)
MRSA DETECTION

MICROSCAN

OXACILLIN SCREEN

ETEST

PBP2a mecA PCR

STAPHYLOCOCCUS AUREUS
WHAT'S UP DOC?

Clindamycin S

Erythromycin S

Oxacillin R

Penicillin R

Vancomycin I/R

"Tu quoque, fili?" (You, my son, as well?)
Julius Caesar’s outcry when he discovered Brutus,
his adopted son, was ready to stab him.
Analogy: Vancomycin, now, as well?

VANCOMYCIN & STAPH

• Vanco is traditional MRSA treatment
  ✓ 3-4% Hypersensitivity, no p.o.
  ✓ Vanco non-susceptible rare
    ✓ VISA (11) and VRSA (3)
    ✓ Linezolid (CAP, other infections), daptomycin
      (skin & soft tissue) are alternatives
• MIC Breakpoints to VANCOMYCIN
  ✓ SUSCEPTIBLE < 4 ug/mL
  ✓ INTERMEDIATE 8-16 ug/mL
  ✓ RESISTANT ≥ 32 ug/mL
• Retest S. aureus with MIC of ≥ 4 µg/ml & use alternate method
  ✓ Vancomycin agar screen plates (test all MRSA), Etest, reference lab
• Disk test will NOT detect VISA

VISA ISOLATES

VISA colonies may be smaller and slower growing than typical S. aureus as shown on this 48th BIAP

VISA

MRSA (not VISA)


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

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

THE USA VRSA ISOLATE
  ✓ MRSA
  ✓ VANCOMYCIN MIC 1,024 ug/mL
  ✓ CONJUGATIVE TRANSFER
  ✓ VRSA HAD vanA & mecA
  ✓ vanA TRANSPONSON JUMPED FROM VRE PLASMID TO MRSA→ VRSA
**STAPHYLOCOCCUS AUREUS**

**CLINDAMYCIN INDUCED RESISTANCE**

<table>
<thead>
<tr>
<th>MECHANISM</th>
<th>DETERMINANT (GENE)</th>
<th>ERY</th>
<th>CLINDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFLUX</td>
<td>RIBOSOMAL ALTERATION</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REQUIRES INDUCTION TO BE DEMONSTRABLE

**MACROLIDE RESISTANCE**

- **MLSB**
  - MACROLIDE LINCOSAMIDE (e.g. CLINDAMYCIN) STREPTOGRAMIN (type B)
  - R mediated by \( \text{erm} \) gene
  - RIBOSOMAL METHYLATION
  - INDUCIBLE (MLSB\(_\text{i}\))
  - CONSTITUTIVE (MLSB\(_\text{c}\))
- ALSO APPLICABLE FOR GROUP B STREP

**ENTEROCOCCI**

- COMMENSAL ORGANISM
  - INFECTION OR COLONIZATION
- RESISTANCE
  - INTRINSIC R (aminoglycosides & \( \beta \)-lactams)
  - ACQUIRED R (chloramphenicol, tetracycline, macrolides, quinolones)
  - SOURCE OF R GENES
- INFECTIONS
  - CLINICAL
  - NOSOCOMIAL
  - INFECTION CONTROL
    - VRE SCREENING (PERI-RECTAL/ANAL SWABS)
    - MOLECULAR TYPING TO DETERMINE CLONAL SPREAD

**ENTEROCOCCI: LAB TESTING**

- ANTIBIOTICS
  - AMPICILLIN MIC, \( \beta \)-LACTAMASE, VANCO SCREEN, OTHERS (e.g. Linezolid)
- SYNERGY SCREEN
  - BLOOD ISOLATES TEST
    - COMBINATION OF \( \beta \)-LACTAM (e.g. PENICILLIN OR VANC WITH AN AMINOGLYCOSIDE (GENT OR STREP)) BACTERICIDAL
    - HLG (Gentamicin 500 ug/mL); Strep (2000 ug/mL)
### VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE)

- **SPECIATION NECESSARY**
  - Intrinsic resistance (E. gallinarum & E. casseliflavus)
  - Acquired resistance (E. faecium & E. faecalis; also in E. raffinosus, E. avium, E. durans)
- Higher Vanco R in *E. faecium* vs. *E. faecalis*
  - 8% (*E. faecalis*) & 80% (*E. faecium*)

<table>
<thead>
<tr>
<th>GENE</th>
<th>VANCO (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van A</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Van B</td>
<td>16–64</td>
</tr>
<tr>
<td>Van C (Intrinsic)</td>
<td>2–16</td>
</tr>
<tr>
<td>Van D</td>
<td>64–128</td>
</tr>
</tbody>
</table>

### EXTENDED SPECTRUM β-LACTAMASES
- FIRST DESCRIBED IN 1983
- ESBLS ARE β-LACTAMASES THAT MEDIATE R TO
  - 3rd generation cephalosporins, (e.g. cefotaxime, ceftiraxone, ceftazidime) 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), β-lactamase inhibitors
    - Susceptible to carbapenems
  - **CHROMOSOME-MEDIATED**
    - AmpC in SPICE (Serratia, Pseudo, Proteus, Citro, Enterobacter)
      - Also have plasmid-mediated AmpC
    - K1 in K. oxytoca
    - Resistant to cefoxitin (cephamycin) & β-lactamase inhibitors

### CARBAPENEM R
- Carbapenems (imipenem, meropenem)
  - Used as antibiotics of last resort for multidrug-resistant GNR
  - Drug of choice for ESBL producers
- Mechanisms include
  - Altered porins, metallo-β-lactamases or other carbapenemases
  - Etest strips
  - More likely found in *Pseudomonas* or *Acinetobacter*
  - Polymyxin is drug of last resort

### AMINOGLYCOSIDE R
- Aminoglycosides (e.g. gentamicin, tobramycin, amikacin)
  - Used as antibiotics usually in combination with β-lactams
  - Drug of choice for *Enterobacteriaceae* or *P. aeruginosa*
- Mechanisms include
  - Inactivation of drug by aminoglycoside-modifying enzymes (AME’s), ribosomal alterations, efflux, permeability loss
  - AME’s most common. Can be passed via plasmids & transposons

### TOUGH BUGS ON THE BLOCK
- Resistant Staph: MRSA, VISA, VRSA
  - Cost to treat MRSA 3X MSSA
  - 44% MRSA CUMC
- 18% ESBLS CUMC
- 81% VRE (*E. faecium*)
- Metallo-β-lactamases
  - *Acinetobacter baumannii*
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*
- Penicillin R *S. pneumoniae*
  - 48% Susceptible
  - 24% Low Level Resistance
  - 28% High Level Resistance

### FUTURE NIGHTMARES
- Widespread linezolid resistance in VRE and Staph
- *Van A* gene transfer to all *S. aureus* to result in increase in VRSA
- Spread of metallo-β-lactamases in nosocomial GNR carbapenem resistant GNR
- Depletion of antimicrobial agents
  - Few new classes, e.g. ketolides (telithromycin) for RTIs