ALPHABET SOUP OF ANTIMICROBIAL RESISTANCE

ANTIMICROBIAL RESISTANCE
HOW CAN THE LAB HELP?

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ANTIBIOGRAM

- **Antimicrobial susceptibility profile of pathogen**
  - Guides empiric therapy based on intrinsic resistance patterns & predictable drug bug combinations
  - CAN YOU PROVIDE SOME EXAMPLES?
- **Fickle pathogens**
  - *S. maltophilia* & Trimeth/sulfa
  - *P. aeruginosa* & cipro
  - *K. pneumo* & imipenem
- **Antibiogram NOW ON LINE!!**
  - "Real-time" analysis
  - Make formulary decisions
  - Establish guidelines for antibiotic management

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:MIC_{90} 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
- ***Campylobacter, Bacillus, Corynebacterium***
  - NO ESTABLISHED CRITERIA
- ***Enterococcus***
  - Cephalosporins, aminoglycosides, clinda, T/S will NOT be reported

ENDOCARDITIS CASE
MIC VALUES

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

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

THE "USED TO BE" PREDICTABLE AST PATTERNS

ORGANISMS	PREDICTABLE [Not so much…]
K. pneumo	Susceptible to Imipenem
P. aeruginosa	Susceptible to Cipro
Salmonella	Susceptible to Cipro
S. aureus	Susceptible to Vanco
E. faecium	Susceptible to Linezolid
Any organism	Susceptible to at least one antibiotic

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

	SUS	RES
SA	≤ 2	≥4
CNS	≤ 0.25	≥0.5

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
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

MECHANISM	DETERMINANT	ERY	CLIN
EFFLUX	*MEF*	R	S
RIBOSOME MODIFICATION	*ERM*	R	S**
RIBOSOME MODIFICATION	*ERM*	R	R CONSTITUTIVE

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

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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

BETA-HEMOLYTIC *STREPTOCOCCUS* RESISTANCE RATES (USA)*

- Beta-hemolytic *Streptococcus* spp.
  - AMPICILLIN / 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

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NEONATAL SEPSIS

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

WHY WAS CLINDA NOT EFFECTIVE IN PREVENTING THIS INFECTION?

- 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

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*

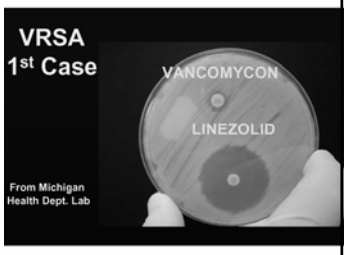
OXACILLIN	4	RESISTANT
CHLORAMPHENICOL	4	SUSCEPTIBLE
LINEZOLID	2	SUSCEPTIBLE
RIFAMPIN	1	SUSCEPTIBLE
TRIMETH/SULFA	2/38	SUSCEPTIBLE
VANCOMYCIN	4	SUSCEPTIBLE

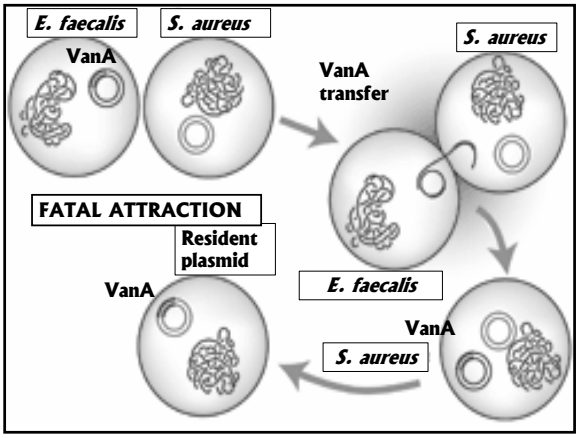
PUZZLE PIECES

- Patient was started on vancomycin
- Urine 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)

Isolate	Vanco MIC[1] (µg/ml)
1	1,024
2	32 [2]
3	64 [2]

[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

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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 PHENOTOYPE
- NO CLONAL SPREAD OF SINGLE STRAIN

NEW TESTS

- LATEX AGGLUTINATION ASSAY
  - ✓ *PBP2*a low-affinity penicillin binding protein
  - ✓ Latex beads sensitized with monoclonal Ab vs PBP2a
  - ✓ PURE CULTURE ONLY (NOT SPECIMEN)
    - Need 10^9 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

ICU SEPSIS

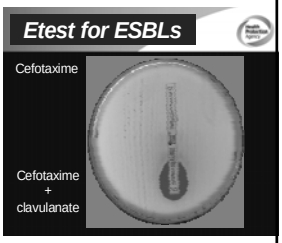
- 64 yo male patient, cardiac ICU post-CABG
- Becomes febrile and hemodynamically unstable
- Blood cultures x 2 are collected
- Culture Resuts:
  - *Klebsiella pneumoniae*

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?

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



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

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

Amikacin	8	S
Ampicillin	≥32	R
Cefoxitin	4	S
Cefazolin	≥32	R
Ceftazidime	≥32	R
Ciprofloxacin	≤1	S
Gentamicin	≥8	R
Imipenem	≤4	S
Piperacillin/Tazobactam	8/2	S
Aztreonam (monobactam)	≥32	R
Trimethoprim/Sulfamethoxazole	8/152	R

You "Pneumo" Than You Thought

- The antimicrobial susceptibility pattern of the isolate was as follows:
- Resistant to: ampicillin, piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, aztreonam, cefazolin, cefuroxime, cefotetan, ceftriaxone, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, levofloxacin, ciprofloxacin, and trimethoprim-sulfamethoxazole
- Intermediate susceptibility to: amikacin
- Susceptible to: tetracycline

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

You "Pneumo" Than You Thought

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

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 103^0F 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

- Clinical isolates should not be tested for susceptibility to polymyxin B by the disk diffusion technique (Susceptible > 11 mm)
  - Large molecule; diffuses slowly
  - Poor correlation with MICs
  - QC ranges too large to be meaningful
  - MIC testing by E-test
  - The susceptibility testing breakpoints for polymyxin B are:
    - Susceptible ≤ 2 µg/mL
    - Resistant ≥ 4 µg/mL

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
  - POLYMYXIN 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)

√ SYNERGISTIC	√ ADDITIVE	√ ANTAGONISTIC