ANTIMICROBIAL RESISTANCE
HOW CAN THE LAB HELP?

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LEARNING OBJECTIVES

- Describe difference between qualitative & quantitative antimicrobial susceptibility testing [AST] and the clinical value of each
- Discuss resistance mechanisms utilized by gram negative bacteria
- Recognize unusual/improbable AST result

ANTIBIOTIC SUSCEPTIBILITY TESTING ROLE OF THE LAB

- IMPLEMENT CURRENT CLSI (CLINICAL LAB STANDARDS INSTITUTE) GUIDELINES
- TEST & REPORT DRUG SUSCEPTIBILITIES BASED ON PATHOGEN & SOURCE OF INFECTION
  - E.G. URINE, BLOODS
  - IN VIVO & IN VITRO CORRELATION
  - DRUG RESISTANCE MECHANISMS OF ACTION
- ANNUAL ANTIBIOGRAMS
  - UNIT SPECIFIC, e.g. MICU, SICU, PICU
  - CHOOSE APPROPRIATE EMPIRIC THERAPY BASED ON PREDICTABLE RESISTANCE PATTERNS
- LAB REPORTING SYSTEMS
  - SIR vs. MIC
- TESTING NEW ANTIMICROBIAL AGENTS
  - PROVIDE INTERPRETIVE CONSULTATION

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)

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 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
- SIR, NO MIC
  - QUALITATIVE INTERPRETATION
  - DISK DIFFUSION (KIRBY- BAUER)
  - TIME TO RESULTS: 18 - 24 HRS
  - NOT SUFFICIENT FOR STERILE FLUIDS
- 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?

SUSCEPTIBILITY TESTING Assumptions

- BLOODSTREAM INFECTION
- HOMOGENEOUS BACTERIAL POPULATION
- SUCCESS = ERADICATION OF ORGANISM

SUSCEPTIBILITY TESTING Reality

- SITE OF INFECTION:
  - ✓ INFLAMED LUNG W/ PURULENT SECRETIONS
  - ✓ ABSCESS
  - ✓ CSF
- HETEROGENEOUS BACTERIAL POPULATION
  - ✓ MULTIPLE SPECIES
  - ✓ MULTIPLE MORPHOTYPES
- BIOFILM

Implications of Multi-Resistant Organisms

- EPIDEMIOLOGY
- TREATMENT
- INFECTION CONTROL
- TRANSPLANT ELIGIBILITY

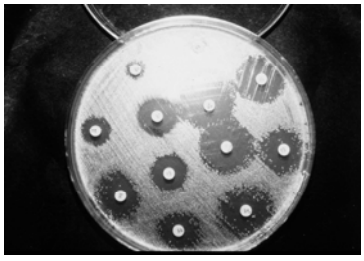
ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODOLOGIES

- DISK DIFFUSION
  - Kirby-Bauer
- BROTH DILUTION
  - Microbroth
  - Microscan
  - Vitek
- E TEST

DISK DIFFUSION

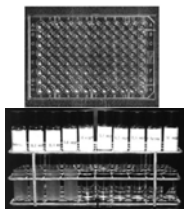
- PROS
  - ✓ EASY TO PERFORM
  - ✓ FLEXIBILITY
  - ✓ NO EQUIPMENT NEEDED
- CONS
  - ✓ NON QUANTITATIVE
  - ✓ NON AUTOMATED
  - ✓ INACCURATE FOR POORLY DIFFUSING DRUGS
  - ✓ DATA LACKING FOR SLOW GROWING ORGANISMS

DISK DIFFUSION



BROTH DILUTION

- PROS
  - QUANTITATIVE
  - WIDE RANGE OF ORGANISMS
  - COMMERICALLY PREPARED PANELS
  - AUTOMATION
- CONS
  - PRE-DETERMINED FORMAT
  - LESS RELIABLE FOR CERTAIN ORGANISMS



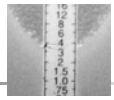
E- STRIP MIC TEST

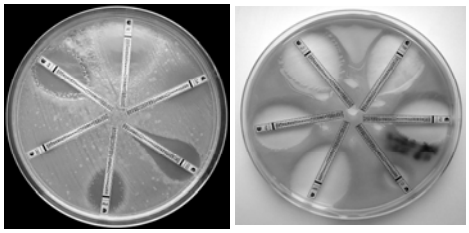
- Gradient agar diffusion preformed antimicrobial gradient
- Plastic coated strip
- MIC read at point of elliptical growth inhibition
- Can use on fastidious organisms
- Confirmation of unusual resistance
- Expensive

E TEST

- PROS
  - SIMPLE TO PERFORM
  - FLEXIBILITY
- CONS
  - EXPENSIVE

E TEST





EXAMPLES OF FLAGGED RESULTS

- *E. coli* : Imipenem Resistant
- *S. pneumoniae*: Vancomycin Resistant
- *Kleb pneumo*: Amikacin Resistant, Gent/Tobra Susceptible
- *S. aureus*: Penicillin Susceptible
- *E. cloacae*: Ampicillin Susceptible
- *A. baumanii*: Aztreonam Susceptible

SUSCEPTIBILITY TESTING CURRENT CHALLENGES

- Focus time, effort and finances on critical care patients
- Does every patient isolate need an MIC?
- How good are we at detecting resistance?

WHEN IS MIC TESTING NECESSARY?

- Life threatening infections
  - ✓ Endocarditis
  - ✓ Meningitis
  - ✓ Osteomyelitis
- Immunocompromised patients
- Critically ill patients

HOW TO USE MIC DATA

- For individual patient therapy
  - ✓ Selection of antibiotic
  - ✓ Dosage
    - Efficacy
    - Efficiency
    - Toxicity
  - ✓ Combination therapy
- Investigation of unusual AST results
- Detection of specific resistance mechanisms

USING MICs TO OPTIMIZE THERAPY

- More institutions are utilizing MIC data to manage critical patients
- Pharmacokinetics
  - Drug levels in blood, CSF, tissue, infection site vs the MIC
- Pharmacodynamics
  - Drug properties that affect bacterial eradication rate vs the MIC

PHARMACOKINETICS

- Antibiotic:
  - ✓ Route of administration
  - ✓ Dose
  - ✓ Metabolism
  - ✓ Elimination
- Drug levels in blood and infected tissues
- PK is what the body does to the drug

PHARMACODYNAMICS

- Antibiotic penetration
- Receptor binding affinity
- Resistance mechanism
- Host immunity
- Virulence
- PD is what the drug does in the body

PK/PD REQUIRE PRECISE MICs

- Aminoglycosides
  - ✓ C max
- Fluoroquinolones
  - ✓ AUC
- Beta lactams
  - ✓ Time over the MIC

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

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

TOUGH BUGS ON THE BLOCK

- MRSA & VRE
  - COST TO TREAT MRSA 3X MSSA
- ESBLs
- Carbapenam- resistant GNR
  - *Klebsiella pneumoniae*
  - *Acinetobacter baumannii*
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*
- Metallo- ß –Lactamases

AST NOT AS EASY AS IT SEEMS !

- > 1 method sometimes needed
  - MRSA
  - VRE
  - ESBL
- Review results for unusual antibiogram patterns
- Update new interpretive guidelines
- Some microbes lack CLSI guidelines

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 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?

ENDOCARDITIS CASE
MIC VALUES

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- 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

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 *MEC*A 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

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

- Beta-hemolytic *Streptococcus* spp.
  - AMPICILLIN/PENICILLIN/VANCO: 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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Clindamycin Disk Diffusion Induction Of Resistance

- WHEN?
  - Erythromycin resistant/Clinda susceptible isolates
- WHY?
  - 2 mechanisms of macrolide resistance
    - Efflux [msrA]
    - Ribosome alteration [erm]
      - Inducible resistance requires ery-produced methylase

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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*

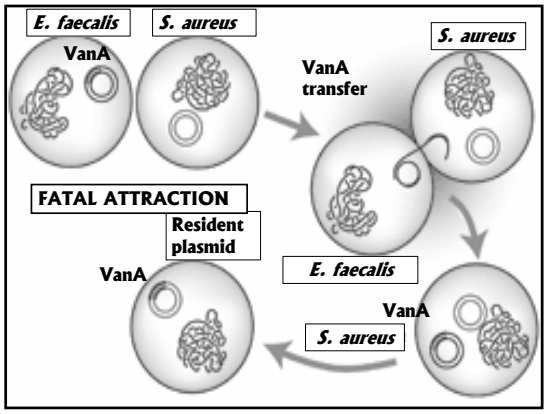
OXACILLIN	4	RESISTANT
CHLORAMPHENICOL	4	SUSCEPTIBLE
LINEZOLID	2	SUSCEPTIBLE
RIFAMPIN	1	SUSCEPTIBLE
TRIMETH/SULFA	2/38	SUSCEPTIBLE
VANCOMYCIN	4	SUSCEPTIBLE

PUZZLE PIECES

- Patient was started on vancomycin
- Urine cultures remained positive for *S. aureus*
- WHAT'S GOING ON?

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

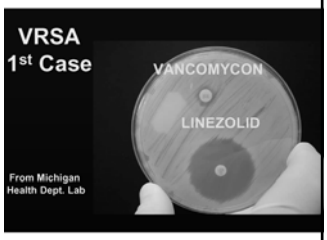


VISA

- 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

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



MICHIGAN VRSA CASE

- THE USA VRSA ISOLATE
  - MRSA – METHICILLIN MIC >16 ug/mL
  - VANCOMYCIN MIC 1,024 ug/mL
  - SUSCEPTIBLE TO LINEZOLID, MINOCYCLINE, QUIN/DALFO, CHLORO and SXT
- CONJUGATIVE TRANSFER
  - VRSA ISOLATE HAD *vanA* RESISTANCE GENE & *mecA*
  - *vanA* TRANSPOSON JUMPED FROM VRE CONJUGAL PLASMID TO A RESIDENT PLASMID IN MRSA STRAIN TO BECOME VRSA

CLSI Interpretive Criteria Vancomycin *Staphylococcus* spp.

		VISA	VRSA
METHOD	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
MIC (µg/ml)	≤4	8-16	≥32
Disk (30 µg) (mm)	≥15	-	-

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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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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?

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

IMPACT OF DRUG RESISTANT GNRs IN HOSPITALS

- NOTORIETY: OUTBREAKS & DRUG RESISTANCE
  - NOSOCOMIAL NEMESIS
- Drug resistance makes Gram-negatives more difficult to treat
- Real-time molecular detection methods for GNR lag behind its Gram-positive relatives
- Nosocomial infections are associated with poor patient outcomes
  - 5%–35% admitted to ICUs
  - Nosocomial pneumonia in the ICU is a leading cause of mortality

BIRD'S EYE VIEW RESISTANT GNRs

- SUPER RESISTANT GNR PATHOGENS
  - *ACINETOBACTER*
  - *KLEBSIELLA*
  - *PSEUDOMONAS*
- ANTIMICROBIAL RESISTANCE ARSENAL
- AST CHALLENGES
- FUTURE PROSPECTS

DEFINING DRUG RESISTANCE

- MULTIDRUG-RESISTANCE (MDR)
  - >1 of 5 drug classes
- PANRESISTANCE
  - Complete/nearly complete lack of treatment options
    - Due to multiple resistance mechanisms
  - Resistance to all antibiotics recommended for treatment
    - Colistin/polymyxin B

WHAT DRIVES MDR INFECTIONS?

- ANTIBIOTIC SELECTIVE PRESSURE
- LONG TERM CARE FACILITIES & PRIOR HOSP
  - Colonization pressure & transmission
- RISE IN IMMUNOCOMPROMISED & ICU PATIENTS
  - Require ventilation, catheters, etc
- THE POWER OF THE MICROBE!!!

A.baumanni
Variety Pack of Resistance Factors

- Metallo-β-Lactamases
  - OXA, IMP, VIM on Integrons, i.e., Genetic elements with
    - Gene cassettes with antibiotic resistance genes
    - Integration sites for the cassettes
    - Some are located within transposons
  - Carbapenemase-hydrolyzing Class D
- AmpC
- Outer membrane protein (OMP)
  - Porin loss- Less antibiotic entering periplasmic space
- Efflux Pumps
  - Natural role is to remove chemicals that could disorganize cytoplasmic membrane
  - Resistance: expels β-lactams & other antibiotics by pump out of porin channels to outer membrane
  - Aminoglycoside- modifying enzymes (Target modification)

β -LACTAMASES: CLASS A

- KPC
  - The most common Carbapenemase in the USA
  - Enzyme may be hard to detect in vitro
  - Plasmid-mediated
    - *K. pneumoniae*
    - *Enterobacter*
  - CUMC *K. pneumoniae*
    - 13 strains KPC-2
    - 2 strains KPC-3

CARBAPENEMASE DETECTION

- AUTOMATED INSTRUMENTS
  - ACCURACY OF CARBAPENEM NONSUSCEPTIBLE RESULTS IS QUESTIONABLE
  - IF CARBAPENEM INTERMEDIATE/RESISTANCE CONFIRM WITH OTHER TEST METHOD
- E-TEST CONFIRMATION
- SUBSTRATES FOR ENZYME HYDROLYSIS
  - ERTAPENEM, IMIPENEM, MEROPENEM
    - ERTAPENEM IS THE BEST IN VITRO INDICATOR FOR CARBAPENEM RESISTANCE

EXTENDED SPECTRUM ß-LACTAMASES

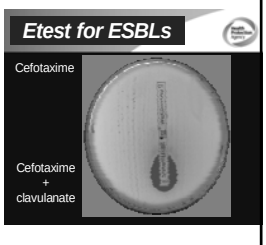
- FIRST DESCRIBED IN 1983
- ESBLS ARE ß-LACTAMASES THAT MEDIATE R TO
  - 3RD GEN CEPHALOSPORINS BUT THESE CAN APPEAR SUSCEPTIBLE WHEN TESTED IN LAB
  - MONOBACTAMS (E.G. AZTREONAM)
  - EXTENDED SPECTRUM PENICILLINS (E.G. PIPERACILLIN)
- STRUCTURAL GENES
  - PLASMID- MEDIATED
    - Altered configuration 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 LAB CONFIRMATION

- COMPARE SYNERGISTIC ACTIVITY OF CEFTAZIDIME & CEFOTAXIME WITH/ WITHOUT CA
  - E-strip shows MIC decrease of ≥3 doubling dilutions for either drug in presence of CA
- SUSCEPTIBLE TO 2ND GEN CEPHALOSPORINS
- RESISTANT TO AZTREONAM
- LAB DETECTION: *K.pneumoniae, K. oxytoca, E.coli* & most recently *Proteus mirabilis*
  - ESBLS exist in many *Enterobacteriaceae*
    - Detection masked by other resistance factors
- LAB REPORTS FOR ESBLs
  - Resistant to all ß-lactams, penicillins, & ß-lactam combination drugs (due to hyperproduction of enzyme that might overwhelm inhibitors)

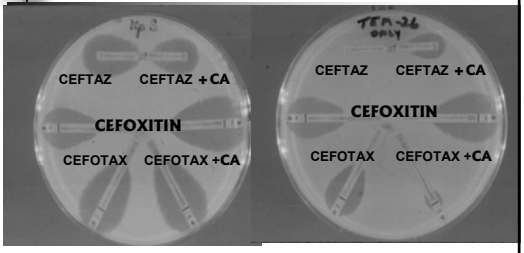
ESBL PHENOTYPIC CONFIRMATORY TESTS

- 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



E-STRIP *K. PNEUMONIAE*

NON ESBL PRODUCER ESBL PRODUCER



MORE KLEBSIELLA RESISTANCE

- 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

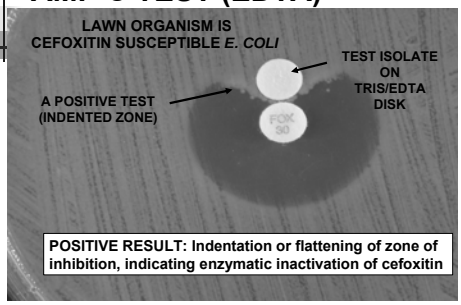
AMP C β-Lactamases

- Class C β-Lactamase
- Plasmid-mediated or Chromosomal
- Hydrolyzes 2nd & 3rd generation cephalosporins
- Not inhibited by ß-lactamase inhibitors

Test Amp C (plasmid-mediated)

- Disk impregnated with EDTA
- Place on lawn of cefoxitin-susceptible *E.coli*
- Disk inoculated with clinical strain, almost touching EDTA disk
- Incubate overnight
- Amp C Positive = cefoxitin resistance

AMP C TEST (EDTA)



JCM 2005; 43:3110-3113

PLASMID-MEDIATED Metallo-β-Lactamases

- Resistance to all β -lactams except Aztreonam
- Zinc containing β -lactamases
  - Inhibited by EDTA
- Not inhibited by CA, tazobactam or sulbactam
- IMPs 1-8: *K. pneumoniae, P. aeruginosa, A.baumannii*
  - Imipenem Resistant
- VIMs 1-4: *P. aeruginosa*
  - Imipenem Resistant

TREATMENT OF RESISTANT GNRs

- Bad bugs, No drugs !!
  - Limited new antimicrobials on the horizon
  - Optimize control measures & antimicrobial restriction policies
- Monotherapy
  - *A. baumannii*
    - Carbapenems, polymyxins
  - *Stenotrophomonas*
    - Bactrim
    - Timentin, Levoquin
- Combination Therapy? Still controversial
  - Many *in vitro* and *in vivo* studies are associated with improved outcomes

TO THE RESCUE?

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

NEW DRUG

- **TIGECYCLINE (TYGACIL)**
  - Active against *A. baumannii, K. pneumoniae, S. maltophilia*
  - Not affected by any β -lactamases, including ESBLs
  - Do NOT test *P. aeruginosa*
    - Not indicated
  - Indicated for intra-abdominal infections
  - No CLSI breakpoints currently exist

NEW APPLICATIONS OF OLD DRUG

- **Colistin (polymyxin E) or Polymyxin B**
  - IV: *P. aeruginosa* or *A. baumannii*
  - Inhalation treatment for ventilator-associated pneumonia
  - Nephrotoxicity 8-36%
  - Neurotoxicity rare

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
- CLINICAL OUTCOME DATA NEEDED TO SUPPORT SYNERGY TESTING FOR:
  - CYSTIC FIBROSIS ISOLATES (*Pseudomonas*)
  - PANRESISTANT GRAM- NEGATIVES
    - Polymyxin + Rifampin, Imipenem or Azithro
    - Rifampin + Sulbactam, Imipenem or Azithro
- DETERMINE FIC (FRACTIONARY INHIBITORY CONCENTRATION)

√ SYNERGISTIC	√ ADDITIVE	√ ANTAGONISTIC

CONTROLLING RESISTANCE

STRATEGIES

- ANTIBIOTIC AGENTS RESTRICTED TO ID CONSULTATION
- COMPUTERIZED GUIDE TO DRUG SELECTION
- SELECTIVE SUSCEPTIBILITY REPORTING
- EDUCATION
- ACTIVE FORMULARY COMMITTEE

CONTROVERSIAL

- COMBINATION THERAPY
  - THEORETICALLY ATTRACTIVE
  - USEFUL FOR TB
  - EFFICACY NOT ADEQUATELY TESTED
  - CAN IT REDUCE OVERALL RESISTANCE?
  - INCREASED COSTS
- ANTIBIOTIC ROTATION OR CYCLING