

**LABORATORY MEDICINE COURSE
2006
ROLE OF THE CLINICAL
MICROBIOLOGY LAB IN DX OF
INFECTIOUS DISEASES**



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**COURSE OBJECTIVES
YOU ARE HERE TODAY BECAUSE??**

- UNDERSTAND HOW MICRO TESTS IMPACT ON YOUR DX
- REVIEW DIFFERENTIAL DIAGNOSES IN CASE PRESENTATION FORMAT
- PRE-ANALYTICAL PARAMETERS
 - ✓ WHICH DIAGNOSTIC TESTS TO ORDER
 - TEST MENU & METHODS
 - PITFALLS & SAFETY NETS
 - TURNAROUND TIME TO DETECTION (TAT)
 - ✓ SPECIMEN COLLECTION
 - APPRECIATE IMPORTANCE OF APPROPRIATE SPECIMEN COLLECTION, HANDLING & TRANSPORT PRACTICES
- ANALYTICAL PARAMETERS
 - ✓ BACKSTAGE AT THE LAB.....REVIEW SALIENT ISSUES
 - TEST METHODS
 - CULTURE & MOLECULAR
 - CONSIDER ASSAY STRENGTHS & LIMITATIONS
- POST ANALYTICAL
 - ✓ CRITICAL INTERPRETATION & REPORTING OF TESTS

TIME PERMITS JUST A BIRD'S EYE VIEW



**IT'S A GERM'S WORLD
AFTER ALL**

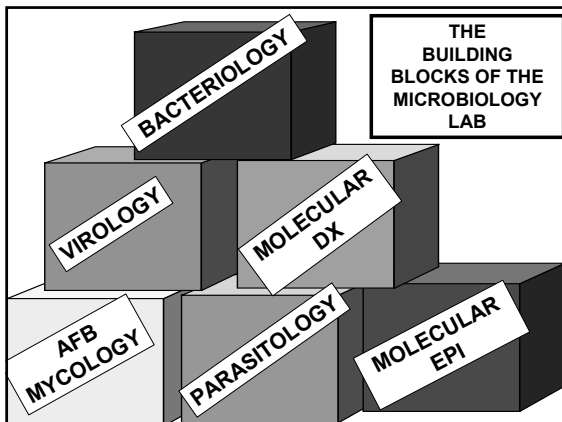
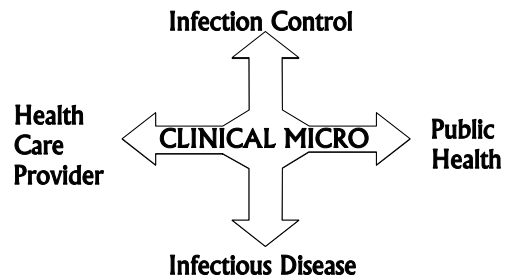


Microbes were the first & will be the last living forms on earth.
Human body: 10- fold more microbial cells than human cells.

INFECTIOUS PATHOGENS

- ✓ 1st CAUSE OF DEATH WORLDWIDE
- ✓ TOP KILLERS GLOBALLY
 - RESPIRATORY DISEASES
 - TUBERCULOSIS
 - MALARIA
 - DIARRHEA
- ✓ 3rd CAUSE OF DEATH U.S.
 - REGARDLESS OF SPECIALITY.....YOUR PTS WILL DEVELOP INFECTIONS
- ✓ NEW INFECTIOUS DISEASES
 - ABOUT 30 IN LAST 20 YRS
 - AVIAN FLU, WEST NILE, SARS
 - HOW ABOUT M.S., CARDIAC DISEASE, ULCERS???

**CLINICAL MICROBIOLOGY
ESSENTIAL LINK IN
HEALTH CARE CHAIN**



**BACKSTAGE AT THE LAB
JOURNEY OF A SPECIMEN**

BEDSIDE

Specimen Collection




SPECIMEN TRANSPORT

Pneumatic Tube
&
Sneaker Power




CLINICAL MICRO LAB

PATHOGEN DETECTION

THE SPECIMEN GARBAGE IN GARBAGE OUT 

SPECIMEN	PITFALLS	SAFETY NETS
BLOOD	<ul style="list-style-type: none"> •NONCOMPLIANCE WITH SKIN PREP PROTOCOL •SKIN CONTAMINATION ↑ 	<ul style="list-style-type: none"> •EDUCATION •QA MONITORS
	<ul style="list-style-type: none"> •LOW VOLUME < 10 ML/ BOTTLE •ONLY 1 SET 	<ul style="list-style-type: none"> •EDUCATION

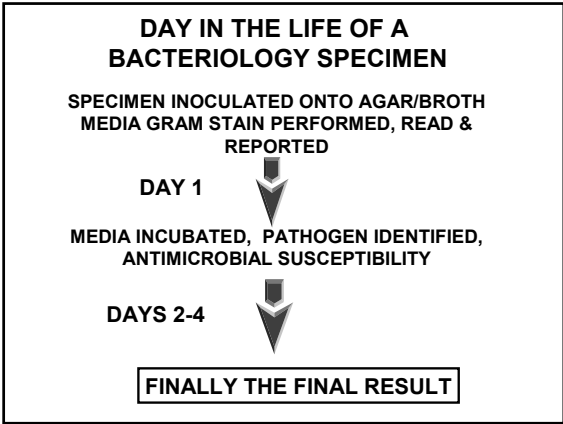
THE SPECIMEN MORE GARBAGE RECYCLING 

SPECIMEN	PITFALLS	SAFETY NETS
STOOL	<ul style="list-style-type: none"> •RAW SEWAGE •OVERGROWTH OF COMMENSALS 	<ul style="list-style-type: none"> •PARA-PAK SAF FIXATIVE FOR O & P •PARA-PAK C & S FIXATIVE FOR ENTERICS
SURGICALS	<ul style="list-style-type: none"> •SWAB ONLY •TISSUES SENT TO PATHOLOGY & NOT MICRO 	<ul style="list-style-type: none"> •STERILE CONTAINER •BLOOD CULTURE BOTTLE

- SPECIMEN TRANSPORT CHALLENGES**
- SPECIMEN DELAY TO MICROBIOLOGY
 - ✓ COMPROMISES PATHOGEN VIABILITY
 - ✓ INCREASES TURN-AROUND-TIME TO RESULTS
 - TRANSPORT MEDIA
 - ✓ REDUCE OVERGROWTH OF CONTAMINANTS
 - ✓ MAXIMIZES PATHOGEN/ NA/ TOXIN RECOVERY
 - TRANSPORT MEDIA EXAMPLES
 - ✓ LIM SELECTIVE MEDIA FOR GROUP B STREP
 - ✓ URINE TRANSPORT MEDIA WITH BORIC ACID PRESERVATIVE
 - ✓ PARA-PAK FOR PARASITES & ENTERIC-TM BACTERIAL ENTERIC PATHOGENS
 - ✓ PRECISION SPUTUM COLLECTORS FOR MYCOBACTERIA
 - ✓ ANAEROBIC BD VACUTAINER

- HOW ARE TESTS CHOSEN? PATHOGEN DETECTION**
- CLINICAL NEED
 - ✓ PATIENT POPULATION SERVED
 - TEST METHODS AVAILABLE
 - ✓ CULTURE VS NON GROWTH DEPENDENT
 - PERFORMANCE CHARACTERISTICS
 - ✓ SENSITIVITY, SPECIFICITY, PPV, NPV
 - RAPIDITY OF RESULTS
 - ✓ OPTIMUM: SAME DAY DETECTION
 - EASE OF PERFORMANCE BY TECHNOLOGIST & FITS INTO WORKFLOW
 - VOLUME OF TESTS PERFORMED
 - ✓ PERFORM IN-HOUSE OR SEND OUT
 - COST OF THE TEST
 - ✓ CONSIDER COST BY PT OUTCOME, LOS NOT JUST COST TO THE LAB BUDGET

- TEST MENU**
- MICROSCOPY - GRAM STAIN
 - GROWTH DEPENDENT
 - ✓ GOLD STANDARD
 - ✓ CULTURE & ANTIMICROBIC SUSCEPTIBILITY (C & S)
 - NON GROWTH DEPENDENT
 - ✓ MOLECULAR DIAGNOSTICS
 - NUCLEIC ACID AMPLIFICATION
 - STRAIN FINGERPRINTING
 - ✓ RAPID NON-MOLECULAR ASSAYS
 - ANTIGEN DETECTION
 - LATEX AGGLUTINATION



IDENTIFICATION OF PATHOGENS

- ♦ MICROSCOPY
 - ✓ GRAM STAIN
- ♦ PATHOGEN DETECTION & IDENTIFICATION
 - ✓ CULTURE (GROWTH DEPENDENT)
 - ✓ RAPID METHODS, E.G. ANTIGEN DETECTION
- ♦ ANTIMICROBIC SUSCEPTIBILITY TESTS
 - ✓ E.g. MICROSCAN & VITEK
- ♦ MOLECULAR TESTS
 - ✓ STRAIN FINGERPRINTING
 - ✓ NUCLEIC ACID AMPLIFICATION TESTS
 - CHLAMYDIA/GC
 - S. AUREUS & MRSA
 - GROUP B STREPTOCOCCI
 - TB
 - ✓ DNA PROBES



THE GRAM STAIN CLINICAL UTILITY



- | | |
|---|--|
| <p>ADVANTAGES</p> <ul style="list-style-type: none"> ♦ GROUPS BACTERIA BY CELL WALL DIFFERENCES <ul style="list-style-type: none"> ✓ GRAM POS OR NEG <ul style="list-style-type: none"> • CLUE TO EMPIRIC RX ♦ CLUE TO PATHOGEN IDENTITY <ul style="list-style-type: none"> ✓ MICROSCOPIC MORPHOLOGY <ul style="list-style-type: none"> • SHAPE & SIZE ♦ ASSESS SPECIMEN QUALITY <ul style="list-style-type: none"> ✓ WBCS, e.g. SPUTUM ♦ INEXPENSIVE, FAST ♦ ROUTINELY PERFORMED ON <ul style="list-style-type: none"> ✓ CSF, RESPIRATORY, WOUNDS, STERILE BODY FLUIDS & TISSUES | <p>LIMITATIONS</p> <ul style="list-style-type: none"> ♦ INTERPRETIVE SKILL <ul style="list-style-type: none"> ✓ ATYPICAL GRAM REACTIONS & MORPHOLOGY ✓ REQUIRES EXPERIENCED MICROBIOLOGISTS ♦ MODERATELY COMPLEX TEST ♦ ASSAY SENSITIVITY LIMITED TO HIGH BACTERIAL LOAD <ul style="list-style-type: none"> ✓ >10⁴ PER ML ♦ POOR SPECIFICITY <ul style="list-style-type: none"> ✓ GIVES CLUES <ul style="list-style-type: none"> • NO DEFINITIVE ID • FALSE POSITIVES |
|---|--|

GRAM -NEGATIVE MORPHOTYPES NAME THAT BUG!

MORPHOTYPE	GROUP
SHORT RODS	ENTERIC <i>E. coli</i>
SHORT, PLUMP RODS BIPOLAR STAINING	ENTERIC <i>Klebsiella</i>
SLENDER, LONG FAINT STAINING	NON FERMENTER <i>Pseudomonas</i>
POINTED ENDS, FILAMENTOUS RODS FAINT STAINING	ANAEROBE <i>Fusobacterium</i> <i>Bacteroides</i>

PATHOGEN DETECTION FROM CULTURE

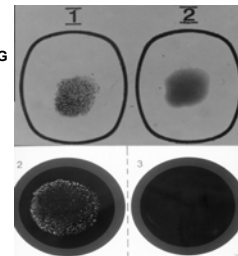
- ♦ GROWTH DEPENDENT AUTOMATED SYSTEMS
 - ✓ MICROSCAN, VITEK, PHOENIX
 - ✓ IDENTIFICATION & ANTIMICROBIAL SUSCEPTIBILITY
- ♦ DIFFERENTIAL GROWTH ON MEDIA
 - ✓ BIOCHEMICAL TESTS
- ♦ ANTIGEN DETECTION SYSTEMS
 - ✓ LATEX AGGLUTINATION TESTS
 - ✓ ENZYME IMMUNOASSAYS (EIA)
- ♦ MOLECULAR ASSAYS
 - ✓ PROBES & PCR

RAPID ID METHODS

- ♦ ANTIGEN DETECTION
 - ✓ DETECTION OF PATHOGENS FROM CULTURE
 - ✓ IMMUNOCHROMATOGRAPHIC
 - URINE PNEUMO & *LEGIONELLA*
 - ✓ ENZYME IMMUNOASSAY
 - *C. DIFFICILE*, FLU A & B, RSV
 - ✓ LATEX AGGLUTINATION
 - ✓ 15-30 MIN
- ♦ MOLECULAR – PCR FROM SPECIMEN

LATEX AGGLUTINATION ANTIGEN DETECTION

- ♦ STREPTOCOCCI
 - ✓ SURFACE POLYSACCHARIDE AG
 - ✓ TYPES GRPS A, B, C, D, F, G
- ♦ GENERAL PROCEDURE
 - ✓ BACTERIAL SUSPENSION FROM CULTURE PLATE
 - ✓ PLACE SAMPLE ON TEST CARD
 - ✓ ADD CONTROLS TO SEPARATE CIRCLES
 - ✓ ROCK & ROLL FOR 10 MIN



+ AGGLUTINATION - AGGLUTINATION

URINE AG TEST

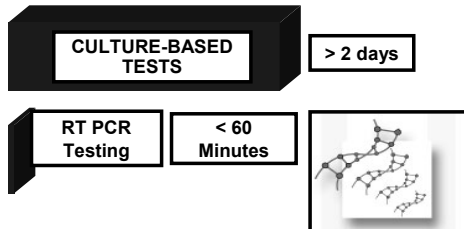
- | | |
|--|--|
| <p>PNEUMOCOCCAL</p> <ul style="list-style-type: none"> ♦ DETECTS C-POLYSACCHARIDE CELL WALL ANTIGEN ♦ SENSITIVITY 86-90% ♦ SPECIFICITY 71-94% ♦ PEDS: COLONIZATION VS. DISEASE? ♦ ADULTS: BEST CORRELATION <ul style="list-style-type: none"> ✓ BACTEREMIA & PNEUMONIA | <p>LEGIONELLA</p> <ul style="list-style-type: none"> ♦ <i>L. PNEUMOPHILA</i> TYPE 1 <ul style="list-style-type: none"> ✓ 70-80% OF CASES ♦ DETECTS LIPO POLYSACCHARIDE CELL WALL ANTIGEN <ul style="list-style-type: none"> ✓ 1-3 DAYS AFTER ONSET OF SYMPTOMS ✓ ANTIGEN PERSISTS DAYS-WKS POST TX ♦ SENSITIVITY 70-99% ♦ SPECIFICITY 91-98% |
|--|--|

PCR MOLECULAR TEST GROUP B STREP

- | | |
|---|--|
| <p>♦ SMARTCYCLER IDI KIT</p> <ul style="list-style-type: none"> ✓ REAL-TIME PCR ✓ TARGETS <i>cfg</i> gene <ul style="list-style-type: none"> • Extracell GBS protein ✓ SENSITIVITY 98% ✓ SPECIFICITY 99% ✓ PPV 98%, NPV 99% | <p>♦ CULTURE</p> <ul style="list-style-type: none"> ✓ SENSITIVITY 69% ✓ SPECIFICITY 100% ✓ PPV 99%, NPV 86% ✓ INTERNAL STUDY <ul style="list-style-type: none"> • Poster AMP 2004 |
|---|--|



TURNAROUND TIME GP B STREP



BRIEF CASE



A ventilated 53 year old male was transferred from an outside hospital directly to the MICU. On admission he was found to be hypotensive and tachycardic, with a temperature of 39.3 C, a heart rate of 130 beats/minute and a systolic blood pressure of 44 – 80mm Hg

EXAM

- ♦ TEMPERATURE (39.3 C)
- ♦ TACHYCARDIA, CHEST (CLEAR)
- ♦ CREATININE = 2.7mg/dl
- ♦ WBC ct = 13,000/ul, 45% PMNs & 45% bands

WHAT IS THE CLINICAL DX IN THIS CASE?

Septicemia

WHAT SPECIMENS WILL YOU SEND TO THE LAB?

Blood, Urine

HOW DO YOU COLLECT THE SPECIMENS?

♦ SEPTICEMIA IS A MEDICAL EMERGENCY

- ♦ >200,000 CASES/YR
- ♦ MORTALITY 20-50%

BLOOD CULTURES DOING IT RIGHT THE FIRST TIME EVERY DROP COUNTS

BLOOD CULTURE COLLECTION

- ♦ SKIN PREPARATION ?
- ♦ WHAT TO COLLECT & WHEN?
- ♦ WHAT SITE WAS DRAWN?
 - ✓ Antecubital? Central Line?

MOST IMPORTANT VARIABLE!

- ♦ BLOOD VOLUME
 - ✓ What volume is drawn & why?
- ♦ Septic Adults only 1-10 colonies/ml

Central Line Bundle

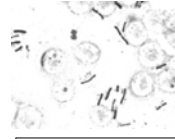
- **BUNDLE DEFINITION**
Group of evidence based interventions for patients with central venous catheters that when implemented together results in better outcomes.
 - ✓ Hand hygiene
 - ✓ Maximal barrier precautions for insertion
 - ✓ Chlorhexidine skin antisepsis
 - ✓ Optimal site selection
 - ✓ Daily review of line necessity with prompt removal if necessary
- **RELIABILITY DEPENDS ON INFRASTRUCTURE USING**
 - ✓ Central line checklists (insertion, maintenance, necessity)
 - ✓ Multi-disciplinary rounds

LAB RESULTS FROM PT BLOOD DAY 1

- CONTAMINANT OR PATHOGEN?
- What are the GPR morphotypes?
 - How many sets were positive?

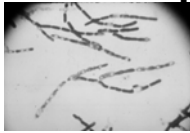
INTERPRETATION OF POSITIVE BLOOD CULTURES- TRUE POSITIVE OR CONTAMINANT?

- ✓ CONSIDER
 - PROPORTION OF BLOOD CULTURE SETS POS TO # SETS OBTAINED
 - TIME FOR GROWTH DETECTION IN BLOOD CULTURE
 - IDENTITY OF MICROORGANISM

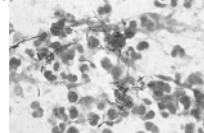


GPR
from Blood Bottle
Day 1

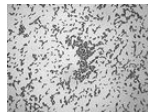
DIFFERENTIATE FROM



BACILLUS ANTHRACIS
NON-HEMOLYTIC ON BAP
SHORT CHAINS, SQUARE ENDS,
NON-MOTILE, ENCAPSULATED
BACILLUS CEREUS
CAN CAUSE PEDIATRIC SEPSIS
BACILLUS spp. CONTAMINANTS



NOCARDIA CAN MIMIC
MYCOBACTERIA (NTM) OR
DIPHTHEROIDS



DIPHTHEROID
OR
CORYNEBACTERIA

BLOOD CULTURE RESULTS

BLOOD CULTURE SIGNALS POSITIVE BY SEMIAUTOMATED INSTRUMENT

DAY 1



GRAM STAIN



GRAM- POSITIVE RODS CALLED TO DOC

DAYS 2



GRAM STAIN &
MORPHOLOGY
ON MEDIA INDICATES
GRAM NEGATIVE ROD

DOES THIS ALTER EMPIRIC THERAPY?

LAB RESULTS FROM PT BLOOD DAY 2

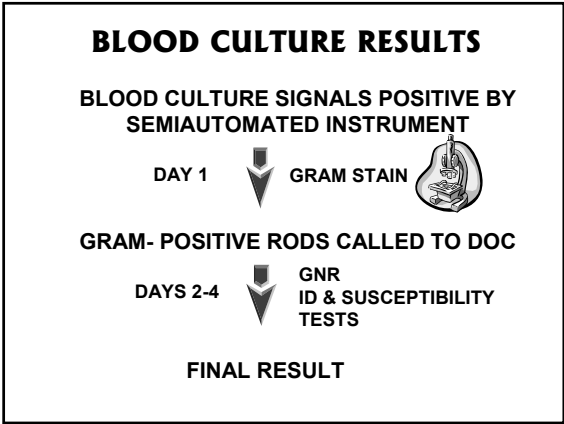
- Empiric Therapy changes from Vanco + Pip/Tazo to
- Pip/Tazo + tobramycin



GNR
from Culture Media
Day 2

BACTERIAL MASQUERADE BALL GRAM-STAIN IMPERSONATORS

BACTERIAL CLASSIFICATION		RESULT OFTEN APPEARS
<i>Acinetobacter</i>	GNR	GPR, GPC
<i>Bacillus</i>	GPR	GNR
<i>Listeria</i>	GPR	Diphtheroids (corynebacteria)




GROWTH METHODS BLOOD CULTURES			
SYSTEMS	METHOD	DETECTION	TAT
BACTEC ADULTS: 8-10 ml/bottle 2 bottles/ set PEDS: 1-3 ml in 1 bottle	Continuous monitoring every 10 minutes Signals positives 24/7	CO ₂ ↑ bacteria & yeast reacts with dye in sensor Fluorometrics	1- 5 d
ISOLATOR ADULTS: 10 ml PEDS: 1-5 ml	Saponin lyses WBC Centrifugation & plating on media	Conventional growth media	1 - 2 d MAC 1- 8 wk

- ### BLOOD CULTURES PREDICTING THE PATHOGEN
- ♦ KNOW YOUR MEDICAL CTRS PREVALENT BLOOD CULTURE ISOLATES
 - ♦ CUMC 2006 BLOOD CULTURE DATA
 - ✓ TOP 3 GRAM POSITIVES
 - *Staphylococcus* (coagulase negative)
 - *S. aureus*
 - *Enterococcus faecalis*
 - ✓ TOP 3 GRAM NEGATIVES
 - *E. coli*
 - *Klebsiella pneumoniae*
 - *P. aeruginosa*




- ### BLOOD CULTURE FROM SEPTIC PATIENT
- ♦ DAY 2
 - ✓ CORRECTION OF GRAM STAIN FROM GPR TO GNR
 - ✓ HOW DO YOU CHANGE THE ANTIBIOTIC REGIMEN?
 - ♦ DAY 3
 - ✓ BOTH SETS OF BLOOD CULTURES GREW
ACINETOBACTER BAUMANII
 - ✓ DO YOU CHANGE YOUR ANTIBIOTIC REGIMEN?
 - ♦ DOES THIS ORGANISM HAVE A PREDICTABLE ANTIBIOGRAM?
 - ✓ KNOW PATHOGEN'S TYPICAL ANTIBIOGRAM PATTERN

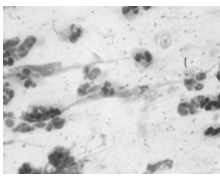
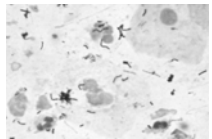
BRIEF CASE

- ♦ A 46 YO MALE DEVELOPED RT MIDDLE LOBE PNEUMONIA WHILE IN THE SURGICAL ICU
- ♦ HIS RECENT MEDICAL HISTORY WAS SIGNIFICANT FOR A ORTHOTOPIC HEART TRANSPLANT 1 WK PRIOR TO THIS EVENT
- ♦ LOWER RESPIRATORY SECRETIONS FROM FIBEROPTIC BRONCH SENT TO MICROBIOLOGY FOR ANALYSIS



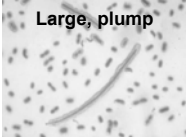
RESULTS FROM LAB

- ♦ Gram Stain 
 - ✓ >10-25 polys & <10 Epithelial Cells
 - ✓ Polys are GRAM-NEG
- ♦ Interpretation
 - ✓ Quality Sputum
- ♦ Consequences
 - ✓ Delay in Dx & Tx
 - ✓ Repeat Specimens collected after Tx
- ♦ Gram Stain 
 - ✓ <10-25 polys & >10 Epithelial Cells 
- ♦ Interpretation
 - ✓ Spit, not sputum
 - ✓ Specimen Rejected

ICU CASE GRAM STAIN CLUES

- ◆ LAB CALLS WITH GRAM STAIN RESULT
 - MANY GNR



Large, plump

**TRANSLATION ?
NOSOCOMIAL
PNEUMONIA**

- ◆ DIFFERENTIAL...CONSULT FOR MORPHOTYPE
 - *KLEBSIELLA* spp.
 - *ACINETOBACTER* spp.
 - *PSEUDOMONAS* spp.
 - *LEGIONELLA* spp.
- ◆ HALLMARKS OF NOSOCOMIAL PNEUMONIA
 - MOST OFTEN GNRs
 - ANTIBIOTIC RESISTANT
 - > 5 DAYS AFTER ADMISSION
 - DISTINGUISH INFECTION VS COLONIZATION

NOSOCOMIAL PNEUMONIA

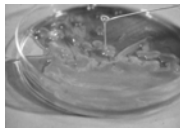
- ◆ DEFINED AS NEW PULMONARY INFILTRATE THAT USUALLY OCCURS >1 WEEK OF HOSPITALIZATION
- ◆ MOST PATIENTS HAVE FEVER & LEUKOCYTOSIS

NOSOCOMIAL PNEUMONIA	<i>P. AERUGINOSA</i> <i>KLEBSIELLA</i> <i>ACINETOBACTER</i>
COMMUNITY ASSOCIATED	<i>LEGIONELLA</i> <i>S. PNEUMONIAE</i> <i>H. INFLUENZAE</i> <i>M. CATARRHALIS</i>

ICU CASE

- ◆ ADDITIONAL LAB DATA
 - LEGIONELLA
 - ✓ URINE AG TEST NEG
 - ✓ RESPIRATORY CULTURE NEG
 - ROUTINE CULTURE
 - ✓ MUCOID LACTOSE FERMENTING GNR ON MACCONKEY AGAR
- ◆ THINK.....
 - *KLEBSIELLA PNEUMONIAE*

MACCONKEY



NOTE
BEAUTIFUL
CAPSULE
FORMATION

KLEBSIELLA


- ◆ ANTIMICROBIAL RESISTANCE TESTING
 - SUSCEPTIBLE
 - ✓ IMIPENEM
 - ✓ POLYMYXIN B
 - RESISTANT
 - ✓ CEPHALOSPORINS
 - ✓ BETA LACTAMS
 - ✓ AMINOGLYCOSIDES
- ◆ Think:
 - ✓ EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCER?
 - ✓ CARBAPENEMASE PRODUCER? KPC

**SUPER BUGS,
DUMB DRUGS**


**FORECASTING
PRE
ANTIBIOTIC
ERA**

NOSOCOMIAL PNEUMONIA

- ◆ EMPIRIC THERAPY
 - MONOTHERAPY
 - ✓ IMIPENEM
 - ✓ PIP/TAZO
 - COMBINATION REGIMENS
 - ✓ CARBAPENEM PLUS
 - LEVOFLOXACIN
 - AZTREONAM
 - AMIKACIN



NEONATAL ICU CASE



- ◆ PT IS 13 DAY OLD EX-24 WK BABY GIRL
- ◆ APGAR SCORE 6.7
- ◆ INTUBATED ON FIRST DAY OF LIFE
- ◆ SEPSIS WORKUP AT BIRTH WAS NEGATIVE
- ◆ 11TH DAY OF LIFE, YOU NOTED MORE FREQUENT DESATURATIONS, HYPOTENSION & INCREASING WBCs TO 21.7

DECISIONS ON THE FLOOR

- ♦ WHAT MICROBIOLOGY SPECIMENS ARE COLLECTED?
 - ✓ BLOOD CULTURE
 - ✓ URINE
- ♦ HOW IS BLOOD CULTURE SITE PREPARED?
 - ✓ <2 MTHS USE ALCOHOL
- ♦ WHAT IS THE EMPIRIC THERAPY?
 - ✓ VANCO, GENTA & PIP/TAZO

MICRO LAB TESTS

CULTURE ONLY

Blood Bottle

DAY 1

- ✓ GRAM STAIN- GPC clusters
- ✓ DAY 2 – GROWTH
- Rapid Ag test - *S. aureus* +
- PBP2a latex agglutination for oxacillin-resistance +
- ✓ DAY 3 – MICROSCAN OR VITEK ID SYSTEM
- MIC ≥ 4 $\mu\text{g/ml}$ by antibiotic susceptibility test
- DAY 4 – FINAL REPORT
MRSA

GRAM STAIN REPORTS DAY 1



REPORT

- ♦ GPC, clusters, tetrads, bunches
- ✓ Rapid test available, distinguishes *S. aureus* from coagulase-negative staph



HALLMARK TETRAD

TRANSLATION

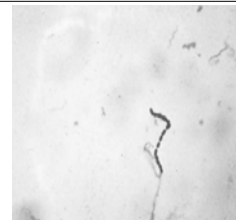
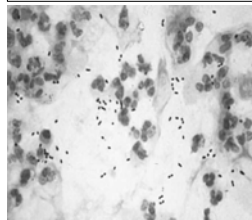
- ♦ If *S. aureus*
 - ✓ Consider: Methicillin Resistance
 - ✓ Consider: Vancomycin Susceptibility Pattern
- GPC
GPC, PRS, CHAINS & CLUSTERS
Lab speaking in tongues!

GPC MORPHOTYPES



REPORT: LANCET-SHAPED
GPC
THINK: *S. PNEUMONIAE*
CONSIDER: PEN RESISTANT?

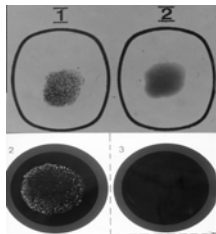
REPORT: ROUND GPC
CHAINS
THINK: STREPTOCOCCI
CONSIDER: PEN SUSC



MRSA AG TEST FROM CULTURE DAY 2

LATEX AGLUTINATION ASSAY

- ♦ PBP2a low-affinity penicillin binding protein
- ♦ Latex beads sensitized with monoclonal Antibody vs PBP2a
- ♦ PURE CULTURE ONLY
 - ✓ Not directly from specimen
 - ✓ Need 10^8 cells
- ♦ 1 hr test



MICRO LAB TESTS

CULTURE & PCR

Blood Bottle

DAY 1

- ♦ GRAM STAIN- GPC clusters
- ♦ PCR TEST
 - ✓ *Nuc* + = *S. aureus*
 - ✓ *mecA* += oxacillin- resistant
- ♦ FINAL RESULT IN ONE DAY
MRSA



MOLECULAR DX MRSA DETECTION

- ♦ PCR – NEW GOLD STANDARD ?
 - ✓ *mecA* & *nuc* genes – coamplification
 - ✓ SAMPLES
BLOOD CULTURES, NASAL SWABS OR PURE CULTURE
- ♦ SmartCycler
 - ✓ AMPLIFICATION & DETECTION
 - Thermocycler & fluorimeter
 - ✓ CLOSED INSTRUMENT SYSTEM
 - Minimizes contamination
 - ✓ 1 ½ HR TEST
 - ✓ EXPENSIVE, TECHNICALLY CHALLENGING



TURNAROUND TIMES

CULTURE-BASED
TESTS

> 2 days

PCR-Based
Testing

< 90
Minutes

MOLECULAR ASSAY FORMATS
PCR, SDA, TMA, bDNA,
Sequencing, Chip Technology

MRSA PROFILE

- ♦ NOSOCOMIAL MRSA 1970s
 - ✓ Resistant to penicillins, cephalosporins, carbapenems & monobactams
 - ✓ Vanco-1st line
 - ✓ Often multiply resistant to gentamicin, rifampin, clindamycin & T/S
 - ✓ Staph Chromosomal Cassette (SCC) *mec* 1-III
 - ✓ Multiple Clones
 - ✓ MRSA infections vs MSSA
 - ↑LOS 12 days + \$5000
- ♦ COMMUNITY ASSOC MRSA 1990s
 - ✓ Usually susceptible to genta, clinda, tetra, T/S
 - ✓ SCC *mec* IV
 - Smaller more mobile lacks R genes
 - ✓ +/- Panton-Valentine Leukocidin (PVL)
 - Recurrent furuncles
 - ✓ More virulent than MSSA
 - ✓ 2 Major Clones

RAPID DETECTION OF VIRULENCE FACTORS (SUPERANTIGENS*)

- ♦ WHAT ARE SUPERANTIGENS?
 - ✓ PROTEINS THAT ELICIT POWERFUL IMMUNE RESPONSES
 - ✓ MASSIVE CYTOKINE RELEASE OR CASCADE
 - ✓ 5 TO 30% OF T CELLS
 - ✓ MASSIVE ORGAN FAILRUE
 - ♦ PANTON VALENTINE LEUKOCIDIN (*pvl*)
 - ✓ 2-3% of SA
 - ✓ CA-MRSA
 - ♦ STAPH ENTEROTOXIN ALPHABET, e.g.
 - ✓ B (*seb*)
 - ✓ C & H (*sec* & *seh*)
 - ♦ TOXIC SHOCK
 - ✓ TSST-1 TOXIN (*tstI*)
- *McCormick, Yarwood and Schlievert (2001) Toxic Shock Syndrome and Bacterial Superantigens: Update
Annu Rev Microbiol 55:77-104

CA-MRSA STUDY

- ♦ 8 POSTPARTUM WOMEN
 - SKIN/SOFT TISSUE INFECTIONS
 - ✓ MASTITIS, ABSCESS, CELLULITIS, PUSTULOSIS
 - ✓ MEAN TIME AFTER DELIVERY: 23 DAYS
 - RESULTS SHOW VIRULENT CA STRAIN IN NORTHEAST
 - ✓ SPREAD FROM MIDWEST (STRAIN MW2)
 - ✓ 1ST REPORT TO DOCUMENT HOSP TRANSMISSION CA-MRSA
- ♦ MICROBIOLOGY STUDIES
 - MOLECULAR ANALYSIS
 - ✓ SCC TYPE IV & PVL PRESENT
 - ✓ PFGE CLONE "I" SAME AS MW2 PROTOTYPE
 - SURVEILLANCE
 - ✓ EMPLOYEES, ENVIRON, NEONATES NEG FOR OUTBK STRAIN & ROUTE OF TRANSMISSION UNKNOWN

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