

**LABORATORY MEDICINE COURSE
2005
ROLE OF THE CLINICAL
MICROBIOLOGY LAB IN DX OF
INFECTIOUS DISEASES**



Dr. Phyllis Della-Latta, Director, 52929
Clinical Microbiology Service, CHC 3-325

THE TESTS

- ♦ MICROSCOPY
 - ✓ GRAM, AFB, GIEMSA
- ♦ GROWTH DEPENDENT
 - ✓ CULTURE & ANTIMICROBIC SUSCEPTIBILITY
- ♦ NON GROWTH DEPENDENT
 - ✓ MOLECULAR DIAGNOSTICS
 - NUCLEIC ACID AMPLIFICATION TESTS
 - STRAIN FINGERPRINTING
 - ✓ RAPID NON-MOLECULAR ASSAYS
 - ANTIGEN DETECTION
 - LATEX AGGLUTINATION

**PATHOGEN DETECTION
HOW ARE TESTS CHOSEN?**

- ♦ CLINICAL NEED
 - ✓ PATIENT POPULATION SERVED
- ♦ 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

**MEASURING QUALITY
ALL TESTS ARE NOT CREATED EQUAL**

TEST RESULT	GOLD STANDARD	
	POSITIVE +	NEGATIVE -
POSITIVE +	TRUE POSITIVE (TP) +/ +	FALSE POSITIVE (FP) +/ -
NEGATIVE -	FALSE NEGATIVE (FN) -/ +	TRUE NEGATIVE (TN) -/ -

**THE SPECIMEN
GARBAGE IN GARBAGE OUT**

SPECIMEN	PROBLEMS	SOLUTIONS
URINE	•>2-3 hr transit time •Overgrowth of commensal flora - False Positives	•Transport Tube with Boric Acid for inhibition
STOOL	•Raw Sewage- Loss of Pathogen Viability False Negatives	•PARA-PAK fixative for enterics
SURGICAL	•Swab - False Negatives •Tissues Sent Only to Pathology – No Pathogen Identification	•Sterile Container •Blood Culture Bottle

**TEST PERFORMANCE
PARAMETERS**

SENSITIVITY $\frac{TP}{TP+ FN} \times 100$ <p>THE HIGHER THE TEST SENSITIVITY.... THE LOWER THE FALSE- NEGATIVES</p>	SPECIFICITY $\frac{TN}{TN+ FP} \times 100$ <p>THE HIGHER THE TEST SPECIFICITY.... THE LOWER THE FALSE-POSITIVES</p>
TP = true positive FP = false positive	TN = true negative FN = false negative

TEST PERFORMANCE PARAMETERS

POSITIVE PREDICTIVE VALUE (PPV)

$$\frac{TP}{TP + FP} \times 100$$

INDICATES % THAT TEST WILL PREDICT A TRUE-POSITIVE RESULT

NEGATIVE PREDICTIVE VALUE (NPV)

$$\frac{TN}{TN + FN} \times 100$$

INDICATES % THAT TEST WILL PREDICT A TRUE-NEGATIVE RESULT

COLLECTION & TIMING BLOOD CULTURES

- ♦ SKIN PREPARATION
 - ✓ CHLORHEXIDINE
 - ✓ 70% ALCOHOL + TINCTURE OF IODINE
 - ✓ DO NOT USE IODOPHORS (BETADINE)
 - Need 2 min exposure to iodophor compared to only 35 sec for 1% iodine for skin disinfection
- ♦ TIMING – SPECIMEN COLLECTION & RESULTS
 - ✓ COLLECT SPECIMEN ASAP AFTER FEVER SPIKE
 - ✓ BEFORE ADMINISTRATION OF ANTIBIOTICS
- ♦ THINKING MYCOBACTERIA OR FILAMENTOUS FUNGI?
 - ✓ INOCULATE ISOLATOR TUBE WITH LYTIC AGENT (SAPONIN) TO RELEASE INTRACELLULAR MICROBES

BRIEF CASE

A 21-year old migrant farm worker, 27 wks pregnant presented with fever, headache, chills, frequency & urgency on urination, decreased appetite, and a 1-d history of diarrhea.

The next morning the pt complained of rt costovertebral tenderness & abdominal pain. Ultrasound no fetal movement & intrafetal demise was suspected. Labor was induced & a stillborne infant was delivered vaginally.

PHYSICAL EXAM

- ♦ TEMPERATURE (38.3 C)
- ♦ TACHYCARDIA, CHEST (CLEAR)
- ♦ ABDOMINAL TENDERNESS
- ♦ PELVIC EXAM: NO DISCHARGE OR TENDERNESS

DOING IT RIGHT THE FIRST TIME EVERY DROP COUNTS

- ♦ # BLOOD CULTURE SETS
 - ✓ 2-3 sets over 24 hr
 - 1 set = 1 aerobic & 1 anaerobic bottle
 - Each set drawn from separate venipuncture site
 - ✓ Pathogen Recovery
 - Second set gives 65% greater yield than first set
 - Third set gives 96% greater yield than first
- ♦ BLOOD VOLUME - MOST IMPORTANT VARIABLE
 - ✓ Septic Adults only 1-10 colonies/ml
 - ✓ 20 ml blood per culture set (10 ml per bottle)
 - ✓ CAP survey-mean culture vol/venipuncture-10 ml
 - 30 ml gives 47% greater yield than 10 ml

WHAT IS THE DIFFERENTIAL IN THIS CASE?

- ♦ *Streptococcus* Group B
- ♦ *Listeria monocytogenes*
- ♦ *Chlamydia trachomatis*
- ♦ *Neisseria gonorrhoea*

- ♦ DIAGNOSIS: SEPTICEMIA
- ♦ MEDICAL EMERGENCY
 - ♦ >200,000 CASES/YR
 - ♦ MORTALITY 20-50%

PEDIATRIC BLOOD CULTURES

- ♦ ONLY ONE BLOOD CULTURE BOTTLE NEEDED
 - ✓ 1 Peds Plus Bottle in Infants optimizes pathogen recovery
 - Bottle accepts up to 5 ml
 - Resins present to adsorb antibiotics
 - Only <0.1% bacteremia are due to anaerobes
 - ✓ Anaerobes suspected?
 - Inoculate 1 anaerobic bottle + Peds Plus bottle
- ♦ BLOOD VOLUME
 - ✓ 0.5-2 ml Neonates
 - ✓ 2-3 ml 1 Mth to 2 Yr
 - ✓ 5 ml Older Children
 - ✓ 10-20 ml Adolescents

BACTERIAL LOAD HIGHER IN CHILDREN THAN ADULTS

BLOOD CULTURE RESULTS

BLOOD CULTURE SIGNALS POSITIVE BY SEMIAUTOMATED INSTRUMENT

DAY 1 ↓ GRAM STAIN

GRAM POSITIVE RODS CALLED INTO DOC

DAYS 2-4 ↓ MEDIA INOCULATED
PATHOGEN IDENTIFIED
SUSCEPTIBILITY RESULTS

FINAL RESULT

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)

LAB RESULTS FROM PT BLOOD

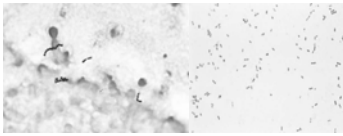
CONTAMINANT OR PATHOGEN?

- What are the GPR morphotypes?
- How many sets were positive?
- What site was drawn?
• Femoral, line

• INTERPRETATION OF POSITIVE BLOOD CULTURES- TRUE POSITIVE OR CONTAMINANT?

✓ CONSIDER

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



GP Coccobacillary from Blood Bottle

GNR from Culture Media

NAME THAT BUG! *LISTERIA*

- ♦ GI SYNDROME WITH FEVER, ABDOMINAL CRAMPS, DIARRHEA, FATIGUE, HEADACHE, MALAISE WITHIN (1-2) DAYS OF EXPOSURE
- ♦ MENINGITIS & BACTEREMIA
- ♦ SPONTANEOUS 2ND/3RD TRIMESTER ABORTIONS OR STILLBIRTHS
- ♦ FOOD-BORNE

GRAM STAIN CLUES ADVANTAGES & PITFALLS

ADVANTAGES

- EMPIRIC TX
 - ✓ GROUPS BACTERIA BY CELL WALL DIFFERENCES
- PATHOGEN IDENTITY
 - ✓ CONSULT MICRO LAB FOR MORPHOTYPES
- ASSESS QUALITY OF SPECIMEN & GRAM STAIN
- ROUTINELY PERFORMED ON
 - ✓ CSF, RESPIRATORY, WOUNDS, STERILE BODY FLUIDS & TISSUES
- INEXPENSIVE, FAST

PITFALLS

- ♦ INTERPRETIVE SKILL
- ♦ SENSITIVITY LIMITED TO HIGH BACTERIAL LOAD OF >10⁴/ML
 - ✓ FALSE NEGATIVES <10⁴
- ♦ POOR SPECIFICITY
 - ✓ NO DEFINITIVE ID
 - ✓ FALSE POSITIVES

TREATMENT

- ♦ AMPICILLIN + GENTAMICIN
- ♦ TMP-SMX (bactericidal, CSF penetration; resistance emerging)
- ♦ ERYTHROMYCIN/CLARITHROMYCIN (bacteriostatic, poor CSF penetration)
- ♦ VANCOMYCIN (poor CSF penetration, effective for endocarditis & bacteremia)

LISTERIA OUTBREAKS

- ♦ **FOODBORNE OUTBREAKS**
 - ✓ soft cheese, deli meats, hot dogs, milk, fish, vegetables, raw eggs, raw poultry
- ♦ 7.4 cases/million = 2,000 cases/year
- ♦ **HIGH MORTALITY**
 - ✓ meningitis (70%)
 - ✓ septicemia (50%)
 - ✓ perinatal/neonatal (>80%)
- ♦ **AT RISK:** pregnant, elderly, neonates, immunocompromised, antacid/ acid blockers

ICU CASE GRAM STAIN CLUES

- ♦ LAB CALLS WITH GRAM STAIN RESULT
 - MANY GNR
- ♦ DIFFERENTIAL...CONSULT FOR MORPHOTYPE
 - *KLEBSIELLA* spp.
 - *ACINETOBACTER* spp.
 - *PSEUDOMONAS* spp.
 - *LEGIONELLA* spp.



**TRANSLATION ?
NOSOCOMIAL
PNEUMONIA**

- ♦ HALLMARKS OF NOSOCOMIAL PNEUMONIA
 - MOST OFTEN GNRs
 - ANTIBIOTIC RESISTANT
 - > 5 DAYS AFTER ADMISSION
 - DISTINGUISH INFECTION VS COLONIZATION

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

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>

RESULTS FROM LAB

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

NOSOCOMIAL PNEUMONIA

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

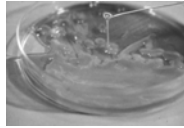
NOSOCOMIAL PNEUMONIA	<i>P. AERUGINOSA</i> <i>KLEBSIELLA</i> <i>ACINETOBACTER</i>
COMMUNITY ASSOCIATED	<i>LEGIONELLA</i> <i>S. PNEUMONIAE</i> <i>H. INFLUENZAE</i> <i>M. CATARRHALIS</i>

ICU CASE

♦ ADDITIONAL LAB DATA

- LEGIONELLA
 - ✓ URINE AG TEST NEG
 - ✓ RESPIRATORY CULTURE NEG
- ROUTINE CULTURE
 - ✓ MUCOID LACTOSE FERMENTING GNR ON MACCONKEY AGAR

MACCONKEY



NOTE
BEAUTIFUL
CAPSULE
FORMATION

♦ THINK.....

- *KLEBSIELLA PNEUMONIAE*

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
 - ✓ AMPICILLIN & GENTAMICIN PROPHYLAXIS DC'd
- ♦ 11TH DAY OF LIFE, YOU NOTED MORE FREQUENT DESATURATIONS, HYPOTENSION & INCREASING WBCs TO 21.7
- ♦ BLOOD CULTURES WERE COLLECTED
- ♦ BABY STARTED ON VANCO, GENTA & PIP/TAZO

KLEBSIELLA

♦ ANTIMICROBIAL RESISTANCE TESTING

- SUSCEPTIBLE
 - ✓ IMIPENEM
 - ✓ POLYMYXIN B
- RESISTANT
 - ✓ CEPHALOSPORINS
 - ✓ BETA LACTAMS
 - ✓ AMINOGLYCOSIDES

SUPER BUGS,
DUMB DRUGS

♦ Think:

- ✓ EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCER?
- ✓ CARBAPENEMASE PRODUCER?

FORECASTING
PRE
ANTIBIOTIC
ERA

GRAM STAIN REPORTS WHAT DO THEY MEAN?

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!

NOSOCOMIAL PNEUMONIA

♦ EMPIRIC THERAPY

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

MRSA DETECTION CULTURE VS PCR

CULTURE 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
- MIC \geq 4 μ g/ml by antibiotic susceptibility test
- DAY 4 – FINAL RESULT MRSA

PCR Blood Bottle DAY 1

- ✓ GRAM STAIN- GPC clusters
- ✓ PCR TEST
 - Nuc + = *S. aureus*
 - *mecA* += oxacillin-resistant
- ✓ FINAL RESULT MRSA DAY 1!

MRSA AG DETECTION FROM CULTURE

LATEX AGGLUTINATION ASSAY

- ♦ PBP2a low-affinity penicillin binding protein
- ♦ Latex beads sensitized with monoclonal Antibody vs PBP2a
- ♦ PURE CULTURE ONLY
 - ✓ Not directly from specimen
 - ✓ Need 10⁹ cells
- ♦ 1 hr test

CA-MRSA STUDY

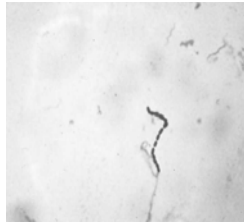
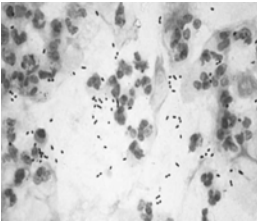
- ♦ 8 POSTPARTUM WOMEN
 - SKIN/SOFT TISSUE INFECTIONS
 - ✓ MASTITIS, ABSCESS, CELLULITIS, PUSTULOSIS
 - ✓ MEAN TIME AFTER DELIVERY: 23 DAYS
 - RESULTS SHOW VIRULENT CA STRAIN IN NORTHEAST
 - ✓ SPREAD FROM MIDWEST (STRAIN MW2)
 - ✓ 1ST REPORT TO DOCUMENT HOSP TRANSMISSION CA-MRSA
- ♦ MICROBIOLOGY STUDIES
 - 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

PUBLICATION – MAJOR ARTICLE - CLIN INFECT DIS 2003; 37:131

GPC MORPHOTYPES

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

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



MRSA DETECTION CULTURE VS PCR

- | CULTURE | PCR |
|---|--|
| Blood Bottle | Blood Bottle |
| Day 1 | DAY 1 |
| <ul style="list-style-type: none"> ✓ GRAM STAIN-GPC clusters ✓ DAY 2 – Growth • Rapid Ag test for <i>S. aureus</i> + • PBP2a latex agglutination test for oxacillin- resistance + ✓ DAY 3 - MicroScan • MIC ≥ 4 µg/ml by antibiotic susceptibility test • Oxacillin Screen Plate 6 µg/ml ✓ DAY 4 – FINAL RESULT | <ul style="list-style-type: none"> ✓ GRAM STAIN- GPC clusters ✓ PCR TEST • <i>Nuc</i> + = <i>S. aureus</i> • <i>mecA</i> += oxacillin- resistant ✓ FINAL RESULT |
| MRSA | MRSA |

MRSA PROFILE

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

WHY IS DNA FINGERPRINTING NEEDED?

- ♦ EPIDEMIOLOGY INVESTIGATION
 - ✓ Which clinical isolates are the result of patient-to-patient transmission?
 - ✓ Identify epidemic strain or index case
- ♦ INVESTIGATION AND CONTROL OF EPIDEMIC
 - ✓ Nosocomial infections in long stay patients
 - ✓ Contamination vs infection?
 - ✓ Isolate interrelationships
 - >Sequential blood isolates from same patient

THE POWER OF PULSED FIELD GEL ELECTROPHORESIS

- **GOLD STANDARD FOR MOST ORGANISMS**
 - ✓ Provides chromosomal overview
 - ✓ Separates very large DNA fragments (40-800 kb)
- **PFGE TECHNIQUE**
 - ✓ Microbe embedded in agarose & lysed
 - ✓ Endonucleases cleave chromosome into fragment patterns
 - ✓ Electrophoretic current "pulsed" in different directions for different lengths of time

INTERPRETING PFGE DATA

- **CLONES**
 - ✓ GENETICALLY RELATED ISOLATES
- **CATEGORIES OF DNA FRAGMENT RELATEDNESS**
 - ✓ INDISTINGUISHABLE (0)
 - ✓ CLOSELY RELATED (2-3)
 - ✓ POSSIBLY RELATED (4-6)
 - ✓ UNRELATED (>6)