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, GIMSA
- 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

THE SPECIMEN GARBAGE IN GARBAGE OUT

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

ALL TESTS ARE NOT CREATED EQUAL

<table>
<thead>
<tr>
<th>TEST RESULT</th>
<th>GOLD STANDARD</th>
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<tbody>
<tr>
<td></td>
<td>POSITIVE +</td>
<td>NEGATIVE -</td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>TRUE POSITIVE</td>
<td>FALSE POSITIVE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(TP) +/ +</td>
<td>(FP) +/ -</td>
<td></td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>FALSE NEGATIVE</td>
<td>TRUE NEGATIVE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(FN) -/ +</td>
<td>(TN) -/ -</td>
<td></td>
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TEST PERFORMANCE PARAMETERS

<table>
<thead>
<tr>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
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<tbody>
<tr>
<td>TP X 100</td>
<td>TN X 100</td>
</tr>
<tr>
<td>TP+ FN</td>
<td>TN+ FP</td>
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</tbody>
</table>

THE HIGHER THE TEST SENSITIVITY... THE LOWER THE FALSE-NEGATIVES

THE HIGHER THE TEST SPECIFICITY... THE LOWER THE FALSE-POSITIVES

TP = true positive
FP = false positive
TN = true negative
FN = false negative
**Test Performance Parameters**

<table>
<thead>
<tr>
<th>Positive Predictive Value (PPV)</th>
<th>Negative Predictive Value (NPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP / (TP + FP) x 100</td>
<td>TN / (TN + FN) x 100</td>
</tr>
</tbody>
</table>

Indicates % that test will predict a true-positive 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

**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%

**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

**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% bacteremias 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

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

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 >10^4/ML
- FALSE NEGATIVES <10^4
- POOR SPECIFICITY
- NO DEFINITIVE ID
- FALSE POSITIVES

BACTERIAL MASQUERADE BALL

GRAM-STAIN IMPERSONATORS

<table>
<thead>
<tr>
<th>BACTERIAL CLASSIFICATION</th>
<th>RESULT OFTEN APPEARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td>GPR, GPN, GPR, GPN</td>
</tr>
<tr>
<td>Bacillus</td>
<td>GPR</td>
</tr>
<tr>
<td>Listeria</td>
<td>GPR</td>
</tr>
</tbody>
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Diphtheroids (corynebacteria)

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

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**BRIEF CASE**

- A 46 YO male developed RT middle lobe pneumonia while in the surgical ICU
- His recent medical history was significant for an orthotopic heart transplant 1 wk prior to this event
- Lower respiratory secretions from fiberoptic bronch sent to microbiology for analysis

**ICU CASE**

- **GRAM STAIN CLUES**
  - Lab calls with Gram stain result
  - Many GNR
  - Large, plump
  - Interpretation quality sputum

**GRAM STAIN CLUES**

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**RESULTS FROM LAB**

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

**GRAM –NEGATIVE MORPHOTYPES NAME THAT BUG!**

<table>
<thead>
<tr>
<th>MORPHOTYPE</th>
<th>GROUP</th>
</tr>
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<tbody>
<tr>
<td>SHORT RODS</td>
<td>ENTERIC</td>
</tr>
<tr>
<td>SHORT, PLUMP RODS</td>
<td>ENTERIC</td>
</tr>
<tr>
<td>BIPOLAR STAINING</td>
<td>Klebsiella</td>
</tr>
<tr>
<td>SLENDER, LONG</td>
<td>NON FERMENTER</td>
</tr>
<tr>
<td>FAINT STAINING</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>POINTED ENDS, FILAMENTOUS</td>
<td>ANAEROBE</td>
</tr>
<tr>
<td>RODS FAINT STAINING</td>
<td>Fusobacterium</td>
</tr>
<tr>
<td></td>
<td>Bacteroides</td>
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**NOSOCOMIAL PNEUMONIA**

- Defined as new pulmonary infiltrate that usually occurs >1 week of hospitalization
- Most patients have fever & leukocytosis

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<tr>
<th>NOSOCOMIAL PNEUMONIA</th>
<th>P. AERUGINOSA</th>
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<tbody>
<tr>
<td>KLEBSIELLA</td>
<td></td>
</tr>
<tr>
<td>ACINETOBACTER</td>
<td></td>
</tr>
<tr>
<td>LEGIONELLA</td>
<td>S. PNEUMONIAE</td>
</tr>
<tr>
<td>H. INFLUENZAE</td>
<td>M. CATARRHALIS</td>
</tr>
</tbody>
</table>
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

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
    - IMPENEM
    - POLYMIXIN B
  - RESISTANT
    - CEPHALOSPORINS
    - BETA LACTAMS
    - AMINOGLYCOSIDES

- Think:
  - EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCER?
  - CARBAPENEMASE PRODUCER?

SUPER BUGS, DUMB DRUGS

FORECASTING PRE ANTIBIOTIC ERA

GRAM STAIN REPORTS
WHAT DO THEY MEAN?

REPORT
- GPC, clusters, tetrad, bunches
- Rapid test available, distinguishes S. aureus from coagulase-negative staph

TRANSLATION
- If S. aureus
  - Consider:
    - Methicillin Resistance
  - Consider:
    - Vancomycin Susceptibility Pattern
- GPC
- PCR, PRS, CHAINS & CLUSTERS

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 > 4 ug/ml by antibiotic susceptibility test
- DAY 4 – FINAL RESULT MRS

PCR
Blood Bottle
DAY 1
- GRAM STAIN- GPC clusters
- PCR TEST
- Nuc + = S. aureus
- mecA oxacillin resistant
- FINAL RESULT MRS
- MRSA
- DAY 1!
**MRSA AG DETECTION FROM CULTURE**

**LATEX AGGLUTINATION ASSAY**
- PBPa low-affinity penicillin binding protein
- Latex beads sensitized with monoclonal Antibody vs PBPa
- PURE CULTURE ONLY
  - Not directly from specimen
  - Need 10^6 cells
  - 1 hr test

**GPC MORPHOTYPES**

- **REPORT: LANCET-SHAPED**
  - GPC CHAINS
  - THINK: S. PNEUMONIAE
  - CONSIDER: PEN RESISTANT?
- **REPORT: ROUND GPC**
  - GPC CHAINS
  - THINK: STREPTOCOCCI
  - CONSIDER: PEN SUSC

**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

**MRSA DETECTION CULTURE VS PCR**

**CULTURE**
- Blood Bottle
  - Day 1
    - GRAM STAIN-GPC clusters
  - Day 2 – Growth
    - Rapid Ag test for S. aureus +
    - PBPa 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
    - MRSA

**PCR**
- Blood Bottle
  - Day 1
    - GRAM STAIN-GPC clusters
    - PCR TEST
      - Nuc + = S. aureus
      - mecA + = oxacillin-resistant
    - FINAL RESULT
      - MRSA

**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

**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)