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

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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>• &gt;2-3 hr transit time</td>
<td>• Transport Tube with Boric Acid for inhibition</td>
</tr>
<tr>
<td></td>
<td>• Overgrowth of commensal flora - False Positives</td>
<td></td>
</tr>
<tr>
<td>STOOL</td>
<td>• Raw Sewage- Loss of Pathogen Viability</td>
<td>• PARA-PAK fixative for enterics</td>
</tr>
<tr>
<td></td>
<td>• False Negatives</td>
<td></td>
</tr>
<tr>
<td>SURGICAL</td>
<td>• Swab - False Negatives</td>
<td>• Sterile Container</td>
</tr>
<tr>
<td></td>
<td>• Tissues Sent Only to Pathology – No Pathogen Identification</td>
<td>• Blood Culture Bottle</td>
</tr>
</tbody>
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# THE TESTS

- **MICROSCOPY**
  - GRAM, AFB, GIEOMSA
- **GROWTH DEPENDENT**
  - CULTURE & ANTIMICROBIC SUSCEPTIBILITY
- **NON GROWTH DEPENDENT**
  - MOLECULAR DIAGNOSTICS
    - NUCLEIC ACID AMPLIFICATION TESTS
    - STRAIN FINGERPRINTING
  - RAPID NON-MOLECULAR ASSAYS
    - ANTIGEN DETECTION
    - LATEX AGGLUTINATION
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>
</tr>
<tr>
<td>POSITIVE</td>
<td>TRUE POSITIVE</td>
</tr>
<tr>
<td></td>
<td>(TP) +/-</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>FALSE NEGATIVE</td>
</tr>
<tr>
<td></td>
<td>(FN) -/+</td>
</tr>
</tbody>
</table>

TP = true positive
FP = false positive
FN = false negative

TEST PERFORMANCE PARAMETERS

SENSITIVITY
\[
\frac{TP}{TP + FN} \times 100
\]
THE HIGHER THE TEST SENSITIVITY, THE LOWER THE FALSE-NEGATIVES

SPECIFICITY
\[
\frac{TN}{TN + FP} \times 100
\]
THE HIGHER THE TEST SPECIFICITY, THE LOWER THE FALSE-POSITIVES
**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

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**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%

**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
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% 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

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
**GRAM STAIN CLUES**

**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><strong>Acinetobacter</strong></td>
<td>GNR</td>
</tr>
<tr>
<td><strong>Bacillus</strong></td>
<td>GPR</td>
</tr>
<tr>
<td><strong>Listeria</strong></td>
<td>GPR</td>
</tr>
</tbody>
</table>
NAME THAT BUG!

*LISTERIA*

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

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

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

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

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ICU CASE
GRAM STAIN CLUES

- LAB CALLS WITH GRAM STAIN RESULT
  - MANY GNR

Large, plump

- 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

TRANSLATION? NOSOCOMIAL PNEUMONIA
## GRAM –NEGATIVE MORPHOTYPES
### NAME THAT BUG!

<table>
<thead>
<tr>
<th>MORPHOTYPE</th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHORT RODS</td>
<td>ENTERIC</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>SHORT, PLUMP RODS</td>
<td>ENTERIC</td>
</tr>
<tr>
<td>BIPOLAR STAINING</td>
<td><em>Klebsiella</em></td>
</tr>
<tr>
<td>SLENDER, LONG FAINT STAINING</td>
<td>NON FERMENTER</td>
</tr>
<tr>
<td>POINTED ENDS, FILAMENTOUS RODS</td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td>FAINT STAINING</td>
<td></td>
</tr>
<tr>
<td>ポイントED ENDS, FILAMENTOUS RODS</td>
<td>ANAEROBE</td>
</tr>
<tr>
<td>FAINT STAINING</td>
<td><em>Fusobacterium</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacteroides</em></td>
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## NOSOCOMIAL PNEUMONIA

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

<table>
<thead>
<tr>
<th>NOSOCOMIAL PNEUMONIA</th>
<th>P. AERUGINOSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KLEBSIELLA</td>
</tr>
<tr>
<td></td>
<td>ACINETOBACTER</td>
</tr>
</tbody>
</table>

| COMMUNITY ASSOCIATED               | LEGIONELLA                 |
|                                    | S. PNEUMONIAE               |
|                                    | H. INFLUENZAE               |
|                                    | M. CATARRHALIS              |
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

KLEBSIELLA

- ANTIMICROBIAL RESISTANCE TESTING
  - SUSCEPTIBLE
    - IMIPENEM
    - POLYMYXIN B
  - RESISTANT
    - CEPHALOSPORINS
    - BETA LACTAMS
    - AMINOGLYCOSIDES

- Think:
  - EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCER?
  - CARBAPENEMASE PRODUCER?
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
  - 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
GRAM STAIN REPORTS
WHAT DO THEY MEAN?

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

TRANSLATION
- If S. aureus
  - Consider: Methicillin Resistance
  - Consider: Vancomycin Susceptibility Pattern

HALLMARK TETRAD

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 μg/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 10^9 cells
• 1 hr test

GPC MORPHOTYPES

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

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

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
MRSA DETECTION
CULTURE VS PCR

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

PCR
Blood Bottle
DAY 1
✓ GRAM STAIN- GPC clusters
✓ PCR TEST
• Nuc + = S. aureus
• meca += oxacillin- resistant
✓ FINAL RESULT
  MRSA

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)