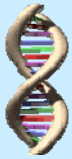
# LABORATORY MEDICINE COURSE
## 2005
## ROLE OF THE CLINICAL MICROBIOLOGY LAB IN DX OF INFECTIOUS DISEASES





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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

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

# 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

# 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) -/- |

# 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

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

# 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

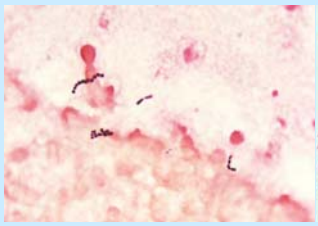
**FINAL RESULT**

# LAB RESULTS FROM PT BLOOD

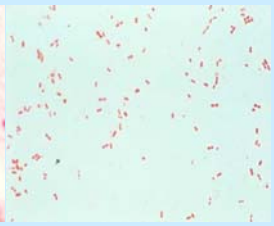
**CONTAMINANT OR PATHOGEN?**

- 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 & 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

| BACTERIAL CLASSIFICATION | | RESULT OFTEN APPEARS |
|---|---|---|
| *Acinetobacter* | GNR | GPR, GPC |
| *Bacillus* | GPR | GNR |
| *Listeria* | GPR | Diphtheroids (corynebacteria) |

# NAME THAT BUG!
## *LISTERIA*

- GI SYNDROME WITH FEVER, ABDOMINAL CRAMPS, DIARRHEA, FATIGUE, HEADACHE, MALAISE WITHIN (1-2) DAYS OF EXPOSURE
- MENINGITIS & BACTEREMIA
- SPONTANEOUS $2^{ND}/3^{RD}$ 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

# ICU CASE
## GRAM STAIN CLUES

- LAB CALLS WITH GRAM STAIN RESULT
  - ➢ MANY GNR


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

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

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

## NOSOCOMIAL PNEUMONIA

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

| | |
|---|---|
| NOSOCOMIAL PNEUMONIA | *P. AERUGINOSA*<br>*KLEBSIELLA*<br>*ACINETOBACTER* |
| COMMUNITY ASSOCIATED | *LEGIONELLA*<br>*S. PNEUMONIAE*<br>*H. INFLUENZAE*<br>*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*

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?

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
  - ✓ 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

HALLMARK TETRAD

**TRANSLATION**

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

GPC

GPC, PRS, CHAINS & CLUSTERS

Lab speaking in tongues!

# 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!

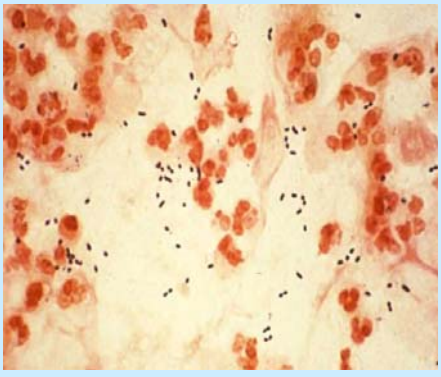
# MRSA AG DETECTION FROM CULTURE

## 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 | REPORT: ROUND GPC CHAINS |
|---|---|
| THINK: *S. PNEUMONIAE* | THINK: STREPTOCOCCI |
| CONSIDER: PEN RESISTANT? | 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*
- • *mec*A += 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)