LABORATORY MEDICINE COURSE

2004

CLINICAL MICROBIOLOGY ROLE IN DETECTION OF MYCOB

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MYCOBACTERIA MAIN PLAYERS

- **SPECIES NUMBER**
  - ✓ 30 species 25 yr ago
  - ✓ 100 species today
- **MAJOR PATHOGENS**
  - ✓ MTB complex (MTBC)
    - 30% of cases
    - Grows 1-2 mths
  - ✓ *M. avium* complex (MAC)
    - 60% of cases
    - Grows 2-4 wks
- **SLOW GROWERS**
  - ✓ *M. kansasii*
  - ✓ *M. xenopii*
  - ✓ Grows 4-6 wks
- **RAPID GROWERS**
  - ✓ *M. abscessus*
    - 50% of rapid growers
  - ✓ *M. chelonei*
  - ✓ *M. marinum*
  - ✓ *M. fortuitum*
  - ✓ Grows 1-2 wks

SHOULD WE STILL THINK TB?
THE BIG APPLE 2003

1140 CASES

14.2 CASES/100,000

3 X NATIONAL AVERAGE

5% CASE INCREASE SINCE 2002

67% IN FOREIGN BORN

43% IN HOMELESS

CLINICAL SITES OF INFECTION

- **PULMONARY INFECTIONS**
  - ✓ *M. tuberculosis*, MAC, *M. kansasii*, *M. abscessus*
  - ✓ Unilateral Noncavitary Lesion
  - ✓ Cavitary Lesions
- **SKIN & SOFT TISSUE INFECTIONS**
  - ✓ Rapid Growers
  - ✓ *Mycobacterium haemophilum*
- **FOREIGN MATERIAL**
  - ✓ Rapid Growers
- **DISSEMINATED DISEASE**
  - ✓ *M. tuberculosis*, MAC, *M. abscessus*

NON TUBERCULOUS MYCOBACTERIA NAME CALLING

- **Nontuberculous mycobacteria (NTM)**
  - ✓PREFERRED NAME
- **Mycobacteria Other Than Tuberculosis (MOTT)**
- **"Atypical" orginated from the mistaken belief that they were unusual MTB strains (old timers!!!)**

(NEVER USE THIS TERM)

NTM DISEASE, COLONIZATION, CONTAMINATION?

- **ATS RECOMMENDATIONS FOR CLINCAL SIGNIFICANCE OF NTM**
  - ✓ISOLATION FROM STERILE BODY SITE
  - ✓3 CULTURE Pos/AFB SMEAR Neg SPUTUM or BAL
  - ✓2 CULTURE Pos/1 AFB SMEAR Pos
  - ✓1 BAL CULTURE Pos/ AFB SMEAR Pos

QUALITY SPECIMEN = QUALITY RESULTS

- RESPIRATORY SPECIMEN COLLECTION
  - ✓ Kendel Precision Double Container
  - ✓ Reduces False Positives
- PATIENT WITH HIGH INDEX OF SUSPICION*
  - ✓ 75% Specimens Collected Were Culture Neg
  - ✓ 68% Normal Chest X-rays
- ADEQUATE NUMBER AND VOLUME
  - ✓ 3 Sputum Specimens
  - ✓ 5-10 ml/Specimen
- DIRECTLY SUPERVISED COLLECTION OR SPUTUM INDUCTION

*Ref: Della-Latta & Whittier (1999), Am J Clin Path 110:301-310

FROM SPECIMEN TO REPORTS

- SPECIMEN DIGESTION & DECONTAMINATION
  - ✓ ALL EXCEPT CSF & BLOODS
  - ✓ CENTRIFUGE, NALC/NAOH TREATMENT
  - ✓ TAKES ABOUT 3-4 HOURS
  - ✓ CONCENTRATED SEDIMENT IS THE INOCULUM
- AFB STAINS – SAME DAY
  - ✓ FLUORESCENT STAIN DIRECT FROM SPECIMENS
  - ✓ KINYOUN (FROM CULTURE)
- NUCLEIC ACID AMPLIFICATION TESTS- 3H to 2D
  - ✓ FOR MTBC ONLY
  - ✓ ROUTINE FOR ALL AFB SMEAR +
  - ✓ CONSULT FOR SMEAR NEGATIVES
- CULTURE TAT RESULTS 3-8 WEEKS
  - ✓ SOLID & LIQUID MEDIA
  - ✓ IDENTIFICATION
    - DNA PROBES & ROUTINE BIOCHEMICALS

FIRST DX TEST: AFB STAIN

- AFB STAINS
  - ✓ Stain Long-chain Fatty Acids (Mycolic Acids)
- PERFORMANCE
  - ✓ Poor Sensitivity & Specificity
    - MTB CULTURE POSTIVE
      - ✓ 60% SMEAR POSITIVE
    - NTM CULTURE POSITIVE
      - ✓ 19% SMEAR POSITIVE

AFB STAIN COMPARISON

- CARBOL FUCHSIN
  - ✓ From CULTURE
  - ✓ Kinyoun Stain
- REQUIREMENTS
  - ✓ 1,000x Magnification (Oil)
  - ✓ Negative Smear
    - 300 Microscopic Fields
    - 15 Min/Slide by Experienced Microscopist
- FLUORESCENT STAIN
  - ✓ From SPECIMEN
- REQUIREMENTS
  - ✓ 250x Magnification
    - High Power
  - ✓ Negative Smear
    - 30 Microscopic Fields
    - 3 Min/Slide by Experienced Microscopist

DNA PROBE FROM CULTURE

DNA PROBES (ACCUPROBE)

- ✓ Pure culture, not specimen
- ✓ Detects 16 S rRNA using labelled DNA probe
- ✓ Hybridization (NOT NUCLEIC ACID AMPLIFICATION)
- ✓ SENSITIVITY & SPECIFICITY: 99%

DETECTION

- ✓ Chemiluminescence

M. tuberculosis Complex (MTBC)

- ✓ *M. tuberculosis*
- ✓ *M. bovis*
- ✓ *M. africanum*
- ✓ *M. microti*
- ✓ *M. canetti*

M. avium Complex

- ✓ (28 serovars)
- ✓ *M. avium* 1-6, 8-11 & 21
- ✓ *M. intracellulare* 7, 12-20 & 25
- ✓ X cluster

M. kansasii

M. gordonae

TB OR NOT TB NUCLEIC ACID AMPLIFICATION

⇒ DIRECT AMPLIFICATION TESTS FOR MTBC ONLY

DIRECTLY FROM CONCEN SPECIMENS NOT CULTURE

- ✓ Pulmonary & Extrapulmonary Specimens
- ✓ TIME TO DETECTION
  - 3 Hrs
- ✓ TEST IS AMPLIFIED MTB DIRECT (AMTD)

AFB SMEAR POS SPECIMENS

- Sensitivity 89-99%
- Specificity 99%
- Pos Predictive Value 95.5%

AFB SMEAR NEG SPECIMENS

- Specificity 97.6%
- Neg Predictive Value 96.4%

IT'S NOT ALWAYS PCR

PARAMETERS	AMPLIFIED MTD
AMPLIFICATION METHOD	Transcription Mediated Amplification (NOT PCR)
TARGET	16S Ribosomal RNA
PROBE	DNA Acridinium ester labelled
DETECTION	Chemi-luminescence

AMTD FALSE -POSITIVES OCCUR

- TECHNICALLY CHALLENGING TEST
  - ✓ SELECT PERSONNEL
  - ✓ NO AUTOMATION
- REPEAT POSITIVES
- AMPLICON CONTAMINATION
  - ✓ ASSAYS NOT SELF-CONTAINED
  - ✓ LOTS OF BLEACH
  - ✓ DAILY CONTAMINATION CHECKS & MONITORS
- CONSULTATIONS PLEASE
- FASTER TIME TO RESULTS
- RAPID DX & TX
- 20% SMEAR +/AMTD
  - ✓ CASES ARE MAC
  - ✓ RULE OUT TB ??
  - ✓ MAC DRUGS STARTED
- 2003 NO FALSE + OR FALSE – PATIENTS
- NO TEST IS 100%
  - ✓ TB OR NOT TB IS A CLINICAL CALL

BRIEF & NOT SO BRIEF CASES

RAPID GROWING NTM

- CAUSE SKIN & SOFT TISSUE INFECTIONS
- COMMON SPECIES
  - ✓ *M. ABSCESSUS, M. CHELONAE*
  - ✓ *M. FORTUITUM, M. MARINUM*
- CULTURE GROWTH 1- 2 WKS
- UBIQUITOUS IN THE ENVIRONMENT
  - ✓ WELL WATER, OIL & DUST
  - ✓ EXTREMELY HARDY
- NO PROBE TEST AVAILABLE

M. ABSCESSUS NOSOCOMIAL INFECTIONS

- COSMETIC SURGERY
- CARDIAC SURGERY
  - ✓ STERNAL WOUND INFECTIONS, PROSTHETIC VALVE ENDOCARDITIS
- POSTINJECTION ABSCESSES
- DISSEMINATED INFECTIONS
- HEMODIALYSIS OUTBREAKS & PERITONEAL DIALYSIS
- CONTAMINATED BRONCHOSCOPES & ENDOSCOPES

PARTING THOUGHTS……

- EXPECT THE UNEXPECTED
- MTB ENDEMIC IN LARGE CITIES
- NTM ON THE RISE
- SEND BIOPSIES TO MICROBIOLOGY AS WELL AS PATHOLOGY
- THINK MTB IN YOUR DIFFERENTIAL

MYCOLOGY LAB 2004

FUNGI ON THE RISE 2003 CUMC

- 6% INCREASE IN SPECIMENS
- 4% INCREASE IN YEAST RECOVERY
- 32% INCREASE IN ANTIFUNGAL SUSCEPTIBILITY TESTS
  - ✓ASSAYS REQUIRE MICROBIOLOGY CONSULT

NO ANSWER WITHOUT A MICRO SPECIMEN

- BIOPSIES, LYMPH NODES, ETC OFTEN SENT TO PATHOLOGY BUT NOT MICRO
  - ✓ ASSUMPTION OF CANCER
  - ✓ UNAWARE THAT ID CANNOT BE MADE FROM PATH SMEAR ALONE
  - ✓ PATH SPECIMENS IN FORMALIN OR PARAFFIN – CANNOT BE CULTURED
- PROPENSITY OF PATH TO CALL ALL SEPTATE HYPHAE IN TISSUE AS "*ASPERGILLUS*"
- SOLUTIONS
  - ✓ COLLABORATION - PATHOLOGY & MICRO

DON'T FORGET MICRO SPECIMEN

MYCOLOGY LAB TESTS

- SMEARS & CULTURES
  - ✓KOH SMEAR ON ALL SPECIMENS
  - ✓FILAMENTOUS FUNGI
    - SOLID MEDIA, ID MORPHOLOGY
  - ✓YEAST
    - SEMIAUTOMATED ID SYSTEMS
- FUNGAL SUSCEPTIBILITY TESTS
  - ✓BROTH MICROTITER DILUTION
  - ✓ROUTINE FOR ALL BLOODS/CSFs

INVASIVE ASPERGILLOSIS RISK FACTORS

- GRANULOCYTOPENIA
  - ✓ HEMATOLOGIC MALIGNANCIES, ORGAN ALLOGRAFT, IMMUNE SUPPRESSION
- LEUKEMIA (10%- 20%)
- BMT RECIPIENTS (5-13%)
- HEART LUNG TRANSPLANT (5-25%)
- RELAPSE COMMON, EVEN AFTER A "CURE"

INVASIVE ASPERGILLOSIS DX

- CULTURE DX
  - ✓ SPECIMEN FROM STERILE BODY SITE IS BEST
    - TISSUE BX OR NEEDLE ASPIRATES NOT SENT FOR FUNGI OR SENT ON SWABS
  - ✓ CULTURE FROM NON STERILE SITE (SPUTUM) COULD BE A CONTAMINANT
- CULTURE ALONE HAS POOR SENSITIVITY
  - ✓ ISOLATION FROM BLOOD CULTURES NOT POSSIBLE USING CURRENT METHODS
- GALACTOMANNAN TEST FOR IA
- IA TX
  - ✓ FAVORABLE RESPONSE TO THERAPY (34%)
  - ✓ ABLC, VORICONAZOLE
  - ✓ VORICON + CASPO
    - CELL WALL & CELL MEMBRANE TARGETS
    - COMBINATION TX SURVIVAL ADVANTAGE WITH BMT

GALACTOMANNAN TEST *ASPERGILLUS* AG DETECTION

- EIA MONOCLONAL ANTIBODY TO GM POLYSACCHARIDE AG IN FUNGAL CELL WALL
  - ✓ 3 Hr Test
- SPECIMEN
  - ✓ Serum
- RECOMMENDATION
  - ✓ TRUE POSITIVE ONLY WHEN >1 SAMPLE POS

- PPV: 71%, NPV: 88%
- SENSITIVITY: 50-94%
- SPECIFICITY: 81-99%
  - ✓ False Positive
    - Other fungi
    - Translocation of GM antigen from food through damaged intestinal mucosa (e.g. bread, cereal, rice, turkey)
    - Mould-derived antibiotics e.g. penicillin

WHEN TO CONSIDER ANTIFUNGAL TX…..

- PROFOUND NEUTROPENIA
- INVASIVE FUNGAL DISEASE
  - ✓ THE MORTALITY RATE FOR CATHETER RELATED CANDIDEMIA APPROACHES 40%
- OROPHARYNGEAL CANDIDIASIS
- FEBRILE WITH POOR CLINICAL RESPONSE ON BROAD SPECTRUM ANTIBACTERIAL THERAPY
- EMPIRIC THERAPY
  - ✓ SUSPECT SYSTEMIC FUNGAL INFECTIONS
  - ✓ PROPHYLAXIS IN TRANSPLANT PTS

ANTIFUNGAL SUSCEPTIBILITY TESTING

- LYOPHOLIZED DRUGS IN BROTH DILUTION MICROTITRE PLATE:
  - ✓ OBTAIN MIC BREAKPOINTS
  - ✓ *CANDIDA* RESULTS IN 24 HRS
  - ✓ *CRYPTOCOCCUS* RESULTS WITHIN 72 HRS
  - ✓ FILAMENTOUS FUNGI – NOT STANDARDIZED EXCEPT FOR *ASPERGILLUS*

BREAKPOINT INTERPRETATIONS

- SUSCEPTIBILE
  - ✓ MOST OFTEN CORRELATES WITH SUCCESSFUL TX
- INTERMEDIATE
  - ✓ SUSCEPTIBILITY IS UNCERTAIN
  - ✓ SUSCEPTIBLE DOSE DEPENDENT (SDD)
  - ✓ HIGHER DOSES MAY BE REQUIRED , e.g. FLUCONAZOLE >400 MG/DAY
- RESISTANT
  - ✓ MOST OFTEN CORRELATES WITH TX FAILURE WITH THAT DRUG

PREDICTABLE SUSCEPTIBILITY PATTERNS

- *A. FUMIGATUS*
  - ✓ Most common cause of Invasive Aspergillosis
  - ✓ Susceptible to Amphotericin
- OTHER *ASPERGILLUS* SPECIES
  - ✓ *A. niger, A. flavus*
  - ✓ *A. terreus*
    - Only 25% Susceptible to Amphotericin
- OTHER FILAMENTOUS FUNGI
  - ✓ *FUSARIUM* & *MUCOR*
    - Triazole Resistant

THINK FUNGUS