CLINICAL MICROBIOLOGY SERVICE

VIRUS HUNTERS & RESPIRATORY TRACT DETECTIVES

DR. PHYLLIS DELLA-LATTA

LEARNING OBJECTIVES

- **Be familiar with viruses commonly associated with adult and pediatric patients**
- **Discuss methodologies available for the rapid detection of viral pathogens**
- **Distinguish common causes of community versus hospital associated pneumonia**

VIRUSES THE LOW DOWN

- **OBLIGATE INTRACELLULAR ORGANISMS**
- **REQUIRE METABOLICALLY ACTIVE CELLS FOR EFFICENT REPLICATION**
- **THERE IS NO UNIVERSAL CELL LINE**
- **CERTAIN VIRUSES CANNOT BE CULTURED IN TRADITIONAL CELL CULTURE MONOLAYERS**
  - ✓ **ROTAVIRUS**
  - ✓ **METAPNEUMOVIRUS**
  - ✓ **HEPATITIS**

VIROLOGY TESTING

THE MYTHS

- **TURN AROUND TIME [TAT] IS TOO LONG**
- **CAN'T TREAT A VIRUS**
- **ACADEMIC PURSUIT**
- **MINIMAL IMPACT ON PATIENT CARE**

BUSTING THE MYTHS

- **TAT CAN RANGE FROM MINUTES TO <72 HRS**
  - ✓ **DIRECT AG DETECTION**
  - ✓ **MOLECULAR ASSAYS**
- **MANY ANTI-VIRAL AGENTS AVAILABLE**
- **CLINICALLY RELEVANT INFORMATION**

PREDICTING THE PATHOGEN

- **AGE**
  - ✓ **PEDIATRICS**
  - ✓ **ADULTS**
- **SEASON**
  - ✓ **ENTEROVIRUSES: SUMMER/FALL**
  - ✓ **INFLUENZA & RSV: WINTER**
  - ✓ **ROTAVIRUS: WINTER/SPRING**
- **IMMUNE STATUS**
- **GEOGRAPHY**
- **SKIN**
  - ✓ **HSV, VZV, ENTERO**
- **EYE**
  - ✓ **ADENO, HSV, VZV, ENTERO**
- **CNS**
  - ✓ **ENTERO, HSV, CMV, VZV**
- **GI**
  - ✓ **ROTA, ADENO, ENTERO**
- **GENITAL**
  - ✓ **HSV**

RESPIRATORY VIRUSES CLINICAL SYNDROMES

- **COMMON COLD**
  - ✓ **RHINO, CORONA, ENTERO, PARA 1-3, ADENO**
- **PHARYNGITIS**
  - ✓ **ENTERO, ADENO, EBV, HSV**
- **CROUP**
  - ✓ **PARA 1 & 2, RSV**
- **BRONCHIOLITIS**
  - ✓ **RSV, PARA 3, INFLU A & B**
- **PNEUMONIA**
  - ✓ **RSV, PARA 1-3, INFLU A & B, ADENO, CMV,VZV**

MENU OF METHODOLOGIES

- ROUTINE CULTURE
- SHELL VIAL CULTURE [more rapid]
- RAPID ANTIGEN DETECTION
  - ✓ Enzyme Immunoassay [EIA]
    - HSV, RSV, INFLUENZA A & B, ADENO 40/41, ROTAVIRUS
  - ✓ Direct Fluorescent Antibody [DFA]
    - CMV, HSV, VZV, INFLUENZA A & B PARAINFLUENZA 1-3, RSV, ADENO
- NUCLEIC ACID AMPLIFICATION
  - ✓ REAL TIME POLYMERASE CHAIN REACTION [PCR]

VIRAL CULTURE

- TURNAROUND TIMES
  - ✓ROUTINE CULTURE
    - INCUBATE FOR UP TO 6 WEEKS
    - AVERAGE TAT
      - 5 TO 10 DAYS
  - ✓SHELL VIAL CULTURE
    - SPECIMENS ARE CENTRIFUGED ONTO CELLS TO FACILITATE RECEPTOR BINDING
    - RESULTS IN 1 – 5 DAYS

CUMC TEST METHODS & TAT

VIRUS	EIA	DFA	SHELL VIAL	CULTURE
ADENO 40/41	2 HRS			
ADENO		4 HRS	2 DAYS	7 DAYS
CMV			72 HRS	4 WKS
ENTEROVIRUS				21 DAYS
HSV		4 HRS	72 HRS	7 DAYS

CUMC TEST METHODS & TAT

VIRUS	EIA	DFA	SHELL VIAL	CULTURE
ROTAVIRUS	2 HRS			
VZV		4 HRS	5 DAYS	21 DAYS
FLU A & B	2 HRS	4 HRS		15 DAYS
PARAFLU 1,2,3		4 HRS		15 DAYS
RSV	2 HRS	4 HRS		15 DAYS

PEDIATRIC CASE

OCTOBER, 2003: A 3 MTH OLD INFANT PRESENTED TO THE PEDS ED A "CROUP-LIKE" ILLNESS WITH LOW-GRADE FEVER. THE CHILD DID NOT HAVE A RECENT TRAVEL HISTORY.

WHAT IS THE DIFFERENTIAL DX ?

- VIRAL INFECTION?
- BACTERIAL INFECTION?
- QUESTIONS TO CONSIDER
  - ✓ TIME OF YEAR?
  - ✓ SICK FAMILY MEMBERS?
  - ✓ IMMUNE STATUS?
  - ✓ FAMILY PETS?
- TESTS ORDERED?
- SPECIMENS COLLECTED?

PATIENT RESULTS

- EIA
  - ✓POSITIVE FOR FLU A
  - ✓NEGATIVE FOR RSV
- DFA
  - ✓POSITIVE FOR FLU A
  - ✓NEGATIVE FOR RSV
- CULTURE
  - ✓POSITIVE FLU A
    - ✓SENT TO CDC & WHO FOR SUBTYPING

- 1st CASE IN NYC-OCT
  - ✓COLUMBIA PRESBY CHONY (2002, 2003)
- 2004 & 2005 SEASONS BEGAN LATE (DECEMBER)
- RELIABILITY OF EIA DEPENDS ON CIRCULATING STRAIN OF FLU

EIA
FLU A+B

- Standard Membrane EIA
- Steps: 10-12
- Assay time: 15-20 min
- Hands on time: 7 min
- Specimen Storage: 2-8ºC
- Differentiates A & B

EIA
INFLUENZA A & B

Flu A	SEN	71-95%
	SPEC	91%
Flu B	SEN	70-87%
	SPEC	98%

DFA
DIRECT ANTIGEN DETECTION

- IMMUNOFLUORESCENCE
- VARIABLE SENSITIVITY
- CULTURE BACK-UP NECESSARY
- REQUIRES 10^3 – 10^6 VIRUSES/ML
- RELATIVELY RAPID
  - ✓2 - 3 hrs
- INTERPRETATION CAN BE SUBJECTIVE

DFA
INFLUENZA A & B

Flu A	SENS	84%
	SPEC	95%
Flu B	SENS	83%
	SPEC	99%

ADEQUATE SPECIMEN FOR DFA
- ✓> 200 CELLS/SLIDE
- ✓20 CILIATED EPITHELIAL CELLS

THE ABCs OF INFLUENZA

- 114,000 HOSPITALIZATIONS & 20,000 DEATHS/YR IN U.S.
- TYPES B & C
  - ✓ONLY HUMANS (C IS VERY RARE))
- INFLUENZA A
  - ✓ AQUATIC BIRDS ARE NATURAL HOSTS & SERVE AS RESERVOIRS
  - ✓INFECTS HUMANS, OTHER MAMMALS (SWINE, ETC.), & BIRDS
  - ✓PIGS PROPOSED AS “MIXING VESSELS” FOR GENETIC REASSORTMENT BETWEEN HUMAN & AVIAN FLU A

INFLUENZA SUBTYPES

- INFLUENZA SUBTYPES BASED UPON SURFACE GLYCOPROTEINS
  - ✓ Hemagglutinin Activity (HA)
  - ✓ Neuraminidase Activity (NA)
- NA CLEAVES CELL MUCIN BARRIER & HA FUSES TO CELLS SIALIC ACID RESIDUES, ENABLING VIRAL ADSORPTION & PENETRATION
- 15 HA & 9 NA SUBTYPES
  - ✓H1-H3 & N1-N2 CAUSE OF WIDESPREAD DISEASE IN HUMANS

INFLUENZA

- ANTIGENIC DRIFT
  - ✓Mutations in HA & NA
  - ✓Occurs during viral replication
- ANTIGENIC SHIFT
  - ✓Only occurs with Influenza A
  - ✓Trading of RNA segments between animal & human strains
- GENETIC REASSORTMENT BETWEEN SPECIES SPECIFIC VIRUSES HAS BEEN ASSOCIATED WITH PANDEMICS

ANTIGENIC DRIFT

GRADUAL ANTIGENIC CHANGE WITHOUT A CHANGE IN SUBTYPE

H3N2		H3N2	H3N2	H3N2
1968	→	1975	1993 →	2004
HONG KONG		VICTORIA	BEIJING	FUJIAN

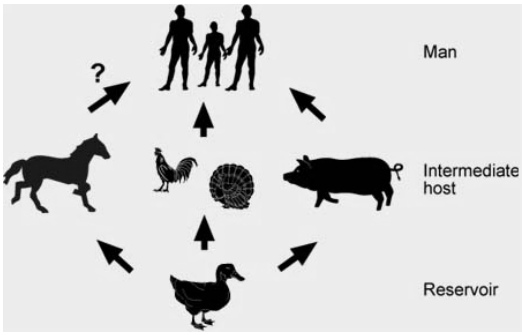
SMALL GENETIC CHANGES REQUIRE ANNUAL VACCINATION

ANTIGENIC SHIFT

SUDDEN COMPLETE ANTIGENIC CHANGE DUE TO HA AND/OR NA SUBTYPE SUBSTITUTION

H1N1		H2N2		H3N2
1918	→	1957	→	1968
SPANISH		ASIAN		HONG KONG

INTERSPECIES TRANSMISSION



FLU PANDEMICS 20TH CENTURY

1. "SPANISH FLU" (1918-1919)
   - ✓ H1NI STRAIN
   - ✓ KILLED 50 - 100 MILLION WORLD WIDE 500,000 U.S.
   - ✓ VERY VIRULENT
     - CYTOKINE STORM IN 15- 45 YO
   - ✓ GENETIC MATERIAL FROM 1918 BEING ANALYZED
     - CLOSELY RELATED TO SWINE VIRUSES
       - PIG TO HUMAN TRANSMISSION
     - GENE MUTATIONS OF AVIAN VIRUS
       - NOT REASSORTMENT
2. "ASIAN FLU" (1957)
   - ✓ H2N2 STRAIN
   - ✓ KILLED 1 MILLION GLOBALLY, 70,000 U.S.
   - ✓ 3 OF THE 8 RNA SEGMENTS WERE RELATED TO AVIAN INFLUENZA VIRUSES [REASSORTMENT]

FLU PANDEMICS 20TH CENTURY

3. "HONG KONG FLU" (1968)
   - H3N2 STRAIN
   - HA GENE SEGMENT – AVIAN ORIGIN
   - KILLED 40,000 U.S.
- LOWER MORTALITY DUE TO HA-ONLY SHIFT, NOT NA
- 2 DUCK-DERIVED GENES & 6 HUMAN

FLU
FROM CHICKENS TO HUMANS

1997 HONG KONG H5N1 INFLUENZA
18 CASES & 6 DEATHS

- ✓INDEX CASE – 3 Y.O. BOY
- ✓PATIENT DIED OF EXTENSIVE INFLUENZA PNEUMONIA COMPLICATED BY REYE'S SYNDROME
- ✓FIRST DOCUMENTED OUTBREAK OF AVIAN INFLUENZA A VIRUS IN HUMANS
- ✓ESTABLISHED THAT AVIAN INFLUENZA VIRUSES CAN INFECT HUMANS WITHOUT PASSAGE THROUGH INTERMEDIATE HOSTS
- ✓ALL GENE SEGMENTS WERE AVIAN, WHICH PROBABLY LIMITED ITS PANDEMIC POTENTIAL

FLU
FROM CHICKENS TO HUMANS

- H5N1 HONG KONG
  - ✓2003, 1 DEATH
- H7N7 NETHERLANDS
  - ✓2003
  - ✓HIGHLY PATHOGENIC
  - ✓INFECTED PIGS & HUMANS
  - ✓83 POULTRY WORKERS & FAMILY
    - ✓79 CONJUNCTIVITIS
    - ✓6 RESPIRATORY SYMPTOMS
    - ✓FIRST DEATH WITH THIS STRAIN
  - ✓ FIRST REPORT OF H7N7 CAUSING RESPIRATORY SYMPTOMS IN HUMANS

1st CASE OF HUMAN TO HUMAN TRANSMISSION 2004

An 11-YR OLD GIRL IN THAILAND

- DIED OF PNEUMONIA SEPT 8 (H5N1)
- RESIDED WITH 32-YEAR AUNT (ALSO INFECTED)
- BOTH HAD CONTACT WITH INF. CHICKENS
- GIRL'S MOTHER FROM BANGKOK PROVIDED BEDSIDE CARE FOR DAUGHTER UNTIL CHILD'S DEATH
- MOTHER FELL ILL & DIED UPON RETURN TO BANGKOK

PANDEMIC FLU
WAITING IN THE WINGS

- POULTRY DISEASE
  - ✓10 EUR-ASIAN COUNTRIES
- AFFECTS LARGE # OF ANIMAL SP.
- 252 HUMAN CASES, 148 DEATHS
- HIGHLY PROBABLE HUMAN TO HUMAN TRANSMISSION REPORTED
- RESISTANT TO OLDER CLASS OF ANTI-VIRALS

H5N1 TREATMENT

- AMANTADINE
- RIMANTIDINE

RECENT CASES RESISTANT

TWO NEW NEURAMINIDASE INHIBITORS FOR TREATMENT OF UNCOMPLICATED INFLUENZA A & B

- ZANAMIVIR
- OSELTAMIVIR
  - ✓ EFFECTIVE AGAINST H5N1
  - ✓ CURRENTLY BEING STOCKPILED IN PREPARATION FOR THE NEXT PANDEMIC
  - ✓ 2/05, 14 YO VIETNAMESE GIRL
    - 1ST CASE OF RESISTANCE
  - ✓ ZANAMIVIR ADDED TO STOCKPILE

HIGH ALERT

- RULE OUT INFLUENZA IS HIGH PRIORITY
- WHY? "FLU-LIKE" PRODROME
  - ✓INHALATIONAL ANTHRAX
  - ✓SARS
  - ✓H5 HONG KONG STRAIN !!!
- VACCINATION AGAINST INFLUENZA CRITICAL IN ORDER TO REDUCE CASES OF NON-AVIAN FLU

CASE HISTORY

- 74 YO FEMALE WITH SEVERE RESPIRATORY DISTRESS
- 5 DAY PRIOR TO ADMISSION DEVELOPED COUGH & RHINITIS
- 2 DAYS LATER BEGAN WHEEZING, DEVELOPED FEVER
- BROUGHT TO ED WHEN LETHARGIC

•ONE GRANDCHILD REPORTED TO BE COUGHING, AND HER SON HAD A "COLD"

• PUT IN RESPIRATORY ISOLATION IN MICU PENDING MICRO RESULTS

WHAT SPECIMENS SHOULD BE SENT TO R/O VIRAL INFECTION?

- NASOPHARYNGEAL WASH OR SWAB
  - ✓SENSITIVITY IS OPTIMAL
- THROAT SWAB (VIRAL TRANSPORT MEDIUM)
  - ✓SENSITIVITY IS SUBOPTIMAL
- TRACHEAL ASPIRATE
- SPECIMEN TRANSPORT
  - ✓HAND DELIVER IMMEDIATELY TO MICROBIOLOGY LAB

LABORATORY DIAGNOSIS

- NASOPHARYNGEAL SWAB WAS COLLECTED

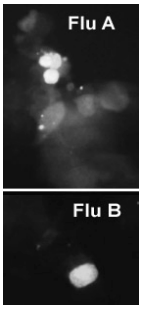
POSITIVE FOR RSV

- ASSUME THIS WAS NEGATIVE; DO YOU STOP THERE?
- WHAT OTHER TESTS WOULD YOU ORDER?

LAB DX RSV

TEST	SENSITIVITY	SPECIFICITY
EIA	52-98%	80-100%
DFA	75-97%	74-100%
SHELL VIAL	75-85%	100%

RSV EIA +



RSV FACTS

- RNA VIRUS
- 2 ANTIGENIC SUBTYPES A & B
- SPREAD THROUGH RESPIRATORY SECRETIONS BY CLOSE CONTACT WITH INFECTED PERSONS/OBJECTS
- CAUSE REPEATED INFECTIONS THROUGHOUT LIFE
- VIRUS UNSTABLE IN ENVIRONMENT
- CAUSES COMMUNITY OUTBREAKS (DAY CARE) & NOSOCOMIAL INFECTIONS

RSV INFECTION

- ADULTS
  - ✓MILD COURSE
- ELDERLY & PEDIATRICS
  - ✓LOWER RESPIRATORY INFECTIONS
- INFANTS & CHILDREN <2 YRS
  - ✓FIRST MONTHS OF LIFE
    - 40% PNEUMONIA
    - 90% BRONCHIOLITIS
  - ✓BY 2 YRS, NEARLY ALL HAVE HAD RSV INFECTION

MOLECULAR TESTING RESPIRATORY VIRUSES

- THOSE WE CANNOT GROW EASILY
  - ✓hMPV
- THOSE WE DON'T WANT TO GROW
  - ✓SARS
  - ✓AVIAN INFLUENZA
- LABILE VIRUSES
  - ✓RSV
- IMPACT ON CLINICAL MANAGEMENT
  - ✓INFLUENZA
  - ✓MENINGITIS/ENCEPHALITIS

REAL TIME MULTI-PLEXED PCR

CASE

- 78 YO MALE PRESENTS TO THE ED FEBRILE (103), TACHYPNIC WITH SHAKING CHILLS
- PUT IN RESPIRATORY ISOLATION IN MICU PENDING MICRO RESULTS
- CHEST RADIOGRAPH SHOWED INFILTRATE IN RIGHT LOBE

WHAT IS THE DIFFERENTIAL DX ?

- VIRAL INFECTION?
  - ✓RSV EIA & DFA TESTS WERE NEGATIVE
- BACTERIAL INFECTION?
- WHAT OTHER TESTS DO YOU ORDER?

- X-RAY FINDINGS INDICATE LOBAR PNEUMONIA
  - ✓DISCRETE LOBE IN LUNG IS AFFECTED

SUSPECT BACTERIAL PATHOGENS

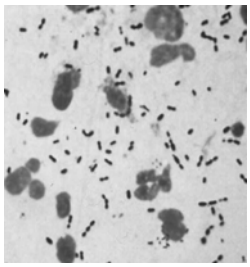
- GRAM- POSITIVE BACTERIA
  - ✓*S. pneumoniae* - community acquired
  - ✓*S. aureus* - nosocomial
- GRAM-NEGATIVE BACTERIA
  - ✓Enterobacteriaceae - nosocomial
    - *K. pneumoniae, E. coli, Serratia*
  - ✓*P. aeruginosa* – nosocomial
  - ✓*Acinetobacter sp.* - nosocomial
  - ✓*H. influenzae* - community acquired
  - ✓*Legionella sp.* - community & nosocomial

SPECIMENS SENT TO R/O BACTERIAL INFECTION

- SPECIMEN COLLECTION
  - ✓SPUTUM
  - ✓BRONCHOSCOPIC ASPIRATES
- MICROBIOLOGY TESTS
  - ✓GRAM STAIN & CULTURE
  - ✓ANTIMICROBIC SUSCEPTIBILITY
  - ✓URINE ANTIGEN TESTS
    - Strep pneumo
    - Legionella

MICROBIOLOGY RESULTS BRONCHIAL WASH

- DAY 1
  - ✓ GRAM POSITIVE COCCI PAIRS & CHAINS
  - ✓URINE ANTIGEN TEST POSITIVE FOR *S. PNEUMONIAE*
- DAY 2
  - ✓BIOCHEMICAL TESTS *STREPTOCOCCUS PNEUMONIAE*



S. PNEUMONIAE

	URINE AG	BLOOD CULTURE	SPUTUM CULTURE
SENS (%)			
SPEC (%)			
TAT			

PNEUMOCOCCAL URINE AG

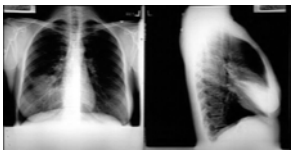
- DETECTS C-POLYSACCHARIDE CELL WALL ANTIGEN COMMON TO ALL SEROTYPES
- NASOPHARYNEAL COLONIZATION
  - ✓5-10% HEALTHY ADULTS
  - ✓20-40% HEALTHY CHILDREN
- ADULTS: BEST CORRELATION
  - ✓DETECTS BACTEREMIC & NONBACTEREMIC PNEUMONIA
- NEED SPUTUM FOR CULTURE FOR SUSCEPTIBILITY TESTING
  - ✓PENICILLIN CANNOT BE ASSUMED TO BE SUSCEPTIBLE

S. PNEUMONIAE

- MOST COMMON CAUSE OF BACTERIAL DISEASE
- OCCULT BACTEREMIA, MENINGITIS, PNEUMONIA – 17,000/YR; < 5 YEARS
- ACUTE OTITIS MEDIA, ACUTE BACTERIAL SINUSITIS
- PEAK AGE 6-12 MONTHS
- HIGH RISK GROUPS (ASPLENIA, HIV, DAY CARE, SICKLE CELL ANEMIA)

CASE

- RF IS A 46 Y.O. MALE 7 DAYS STATUS POST-RENAL TRANSPLANT
- WHILE STILL IN THE HOSPITAL
  - ✓FEVER TO 102
  - ✓CHEST X RAY C/W PNEUMONIA

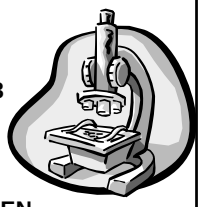


WHAT SPECIMENS DO YOU COLLECT?

WHAT TESTS DO YOU ORDER?

WHAT ANTIBIOTICS DO YOU INITIATE?

CASE



- BLOOD CULTURES NEG X 3
- SPUTUM CULTURES X 3
  - ✓GS = MANY POLYS NO ORGANISMS SEEN
  - ✓NORMAL RESPIRATORY FLORA ISOLATED
- AFB & FUNGAL CULTURES NEGATIVE
- CONDITION WORSENING

CASE

- **WHAT PATHOGENS DO WE THINK ABOUT 1 WEEK POST TRANSPLANT?**

CASE

- **ONE WEEK LATER A BAL IS OBTAINED**
  - ✓**GS = MANY POLYS/NOS**
  - ✓**CULTURE = GRAM NEGATIVE ROD**
    - **SLOW GROWING**
    - **NO GROWTH ON MACCONKEY**
    - **RECOVERED ON BCYE AGAR**

CASE

CASE

- **1 WEEK LATER: SAME UNIT**
  - ✓**53 Y.O. MALE STATUS POST-RENAL TRANSPLANT, DEVELOPS A HIGH FEVER AND PNEUMONIA-LIKE PICTURE**
  - ✓**48 Y.O. FEMALE STATUS POST-RENAL TRANSPLANT, DEVELOPS A HIGH FEVER AND PNEUMONIA-LIKE PICTURE**
  - ✓**SOUND FAMILIAR??**

CASE

- **EPIDEMIOLOGIC INVESTIGATION BEGINS IN CONJUNCTION WITH NYC AND NYS DOHs**
- **12 ADDITIONAL PATIENTS HAD SEROLOGIC EVIDENCE OF INFECTION**
- ***L. MICDADEI* WAS ISOLATED FROM SEVERAL HOT WATER SOURCES**
  - ✓**SHOWERS & SINKS IN PATIENT ROOMS**
  - ✓**HEATED WATER RECIRCULATION LOOP**
- **PULSED FIELD GEL ELECTROPHORESIS (PFGE) CONFIRMED CLONALITY**

LEGIONELLA

- **SEVERE INFECTION & DEATH ASSOCIATED WITH**
  - ✓**CIGARETTE SMOKING**
  - ✓**IMMUNOSUPPRESSION**
  - ✓**COPD**
  - ✓**RENAL FAILURE**
  - ✓**ALCOHOLISM**
- **TREATMENT**
  - ✓**LEVOFLOXACIN**
- **ENVIRONMENTAL SOURCES**
  - ✓**FAUCETS**
  - ✓**SHOWERHEADS**
  - ✓**FOUNTAINS**
- **MAY REQUIRE A BIOFILM FOR COLONIZATION**
  - ✓**MULTIPLY WITHIN AMOEBAE**

RESPIRATORY INFECTIONS SUMMARY

- **THINK BACTERIAL VS VIRAL**
- **ORDER APPROPRIATE ASSAYS TO ENSURE DIAGNOSIS**
- **CONSIDER AGGRESSIVE TREATMENT OPTIONS FOR NOSOCOMIAL PNEUMONIA**