Basic Microbiology

Basics of Infection Prevention
2-Day Mini-Course
May 2017
Objectives

• Describe role of the laboratory in infection prevention; emphasis on microbiology
• Describe factors that can adversely affect reliable lab results
• Interpret gram stains
• Discuss common HAI pathogens for HAI
• Understand laboratory testing methods to for confirming infections
Microbiology and Infection Prevention

Microbiology has two important functions related to infections

- **Clinical**: diagnosis and management of infections

- **Epidemiological**: understand infectious microbes in patients (and populations), to find sources and routes of transmission necessary for prevention efforts
Clinical Microbiology

Physician’s perspective:

– What’s growing?
– What antibiotic can be used?
  • Determined either by predictive value of the organism type (e.g. gram negative bacillus) or by complete result with sensitivities

IP or Epidemiologist’s perspective:

– Surveillance for determining clusters/outbreaks and assessing trends
– Need to know organism so can implement proper transmission-based precautions as needed in a timely fashion
Primary Rules on Microbiology Cultures

Rule 1: No Lab Test is 100% Accurate

Rule 2: Positive Cultures Do Not Make an Infection
Assessing Accuracy of Lab Results

**Rule #1: No lab test is 100% accurate 100% of the time**

Many factors can affect accuracy of laboratory tests

1. **Collection Error**
   - How was specimen collected, handled, transported, preserved prior to arrival in the lab?

2. **Lab Error**
   - Were correct agar plates used? Was the specimen incubated at correct temp? Lab protocols followed? Skill of the micro tech? Accuracy of biochemicals and instrument system?

3. **Reporting Error**
   - Accurate result transcription in computer systems? Did results get communicated to the doctor accurately?
Rule #2: Positive Culture Does Not Mean Infection

Bacteria must invade tissue to cause an infection.

- For some tests such as polymerase chain reaction (PCR), because an organism is present does not mean it is viable (transmissible).
- Pseudo-outbreaks due to lab contamination of samples can occur.
What might indicate invasion into Tissue???
White Blood Cell (WBC) Terminology

- PMNs (polymorphonuclear leukocytes) made in bone marrow; provide general response to threat
  - Neutrophils (~50-60% wbcs) are first line of response to infection; may also be called ‘segs’
  - Eosinophils (1-7% wbcs); allergic reactions and parasites
  - Basophils (<1%); allergic reactions, help mediate strength of immune response
- Left shift: presence of immature neutrophils (called ‘bands’ or ‘stabs’) in blood count; are indicative of acute infection or inflammatory process

www.rnceus.com/cbc/cbcdiff.html
Lymphocytes

• Lymphocytes (lymphs) mature in the lymphatic portion of the immune system
  • Include pathogen-specific immune response (B cells, T cells)
  • Increase may be indicative of viral infection

• Monocytes (or macrophages) phagocyte function (or eat) cellular debris and foreign pathogens from the immune system

www.rnceus.com/cbc/cbcdiff.htm
Immunoglobulins are **Specific** Lymphocytes

- Immunoglobulins (antibodies) are proteins that bind to viruses and bacteria
  - IgM – produced immediately after exposure
  - IgG – most abundant, is long term response to disease
  - IgA – secretory, present in mucosal linings
  - IgE – plays a role in hypersensitivity reactions
**Gram Staining**

- Method of classifying bacteria into 2 large groups: positive (+) and negative (-)
- Differentiates bacteria by the chemical and physical properties of their cell walls
- Helpful in guiding initial empiric therapy
  - results should get to physician ASAP
Bacterial Groups

**Gram Stain** identifies four basic groups of bacteria:

1. Gram positive cocci (Staphylococcus, Streptococcus, Enterococcus)
2. Gram negative cocci (Neisseria, Moraxella)
3. Gram positive bacilli (Clostridium, Listeria, Corynebacterium)
4. Gram negative bacilli (Pseudomonas, Escherichia coli, Haemophilus, Bacteroides)

**Acid-fast stain**

Distinguishes bacteria that retain the stain even in the presence of an acid decolorizer.

Used to show the presence of Mycobacterium species (tuberculosis, avium and others)
Sputum Gram Stain

Quality of sputum specimen:
- Squamous epithelial cells (SEC)
  - <10  excellent, no appreciable
  - 10-25  equivocal but acceptable
  - >25  reject due to unacceptable contamination
- WBC
  - <10  no infection (or poor immune response)
  - 10-25  equivocal
  - >25  purulence indicates presence of infection

Bacteria (list of common respiratory pathogens on slide 14)
Lower Respiratory Cultures

- Sputum and bronchial wash: often contaminated with oral flora
- Protected brush specimen: not contaminated with oral flora
  - semi-quantitative method recommended
  - put brush into 1.0mL TSI* broth; vortex; inoculate agar with urine loop
  - reported as number of CFU/ml**
- Tracheal aspirates: often show colonizers

*TSI (triple sugar iron) helps distinguish between certain enteric pathogens
**CFU/ml = colony forming units per milliliter
Common Lower Respiratory Tract Pathogens

• Community-acquired pneumonia (CAP)
  - *S. pneumoniae*
  - *H. influenzae*
  - Mycoplasma

• Either CAP or hospital-acquired pneumonia
  - *Staphylococcus aureus* (MRSA or MSSA)
    - ↑ mortality; must be recognized quickly
  - *Moraxella catarhalis* (most often CAP)

• Hospital-acquired, most often ICU or ventilator-associated
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*

Note: Yeast is NOT usually an infecting organism for pneumonia or other lower respiratory tract infections unless it constitutes >70% of organisms in a specimen and specimen is not contaminated with oral flora
Cerebrospinal Fluid (CSF) Bacteria

• Source: often upper respiratory flora

• Meningitis due to gram negative rods or *Staphylococcus* usually associated with predisposing factors such as trauma

• Adult, most common: *Strep pneumo* (gram positive cocci in pairs)
  • generates increased WBC response

• Meningococcemia: gram stain showing gram-negative diplococci is diagnostic
  • a single case is a true infection emergency
Meningitis

Onset of Symptoms

Patient presents for medical evaluation
Lumbar Puncture (LP)

Bacterial
- CSF cloudy
- Elevated protein
- Decreased glucose
- WBC; positive neutrophils
- Organisms on gram stain

Viral (aseptic)
- CSF clear
- Normal or elevated protein
- Normal glucose
- No organisms on gram stain
Blood Cultures

• A single blood culture consists of two bottles
  • Bottles designed to recover aerobes and anaerobes
  • Irrelevant which bottle has growth or if both or only one bottle has growth

• Adults: low numbers of bacteria in blood ($\leq 30$/mL)
  • Can lead to negative gram stain and false negative
  • Volume is important; usual 4 bottles/40cc blood
  • Less blood needed for children due to larger number of bacteria per cc of blood/don’t normally have anaerobes
Blood Culture Contaminants

Partial list of common contaminants

- Coag neg staphylococci
- Diphtheroids
- Bacillus
- Propionibacteria
- Viridans strep
- Aerococcus
- Micrococcus

For these bacteria to be interpreted as causing infection, two sets of blood cultures are required PLUS specific signs and symptoms such as fever; refer to your NHSN definitions and for a more comprehensive list of contaminants.
Common Pathogens of Deep and Organ Space SSI

- Anaerobic (does not require $O_2$ for growth)
  - *B. fragilis*
  - Clostridium
  - *Peptostreptococcus*
  - *Propionibacterium* (septic arthritis, endocarditis, suture sites for craniotomy)

- Aerobic examples
  - Staphylococcus
  - Streptococcus
  - Gram negative rods (GNR)
Common UTI Pathogens

• Gram negatives
  • *E. coli*: Causes 80% of all UTI
  • Proteus, Klebsiella, Enterobacter, Pseudomonas, Gardnerella cause 5-10%

• Gram positives
  • Staph, Enterococcus, *Staph saprophyticus*, 10-20%

• Positive leukocyte esterase and/or nitrite found on a UA can be helpful in determining infection status.

• Increased WBC in urine w/ negative cultures may indicate infection w/ chlamydia or gonorrhea.

• Presence of yeast are not part of the NHSN definition for a urinary tract infection
Common Bowel Flora

- Normal mix of bacterial flora keeps numbers of yeast, *C. difficile*, and other potential pathogens in the gut in check.
- With altered flora, yeast, *C. difficile*, pseudomonas species, VRE, and others can proliferate.

Of note: Stool samples contain digestive enzymes; enzymes continue to work after collection, necessitating addition of a preservative and/or prompt processing of specimens.
Antibiotic Resistance

- Emerges when some or all of a species/subspecies of bacteria survive exposure to an antibiotic
  - Can be intrinsic or transferred
  - Multi-drug resistance organisms (MDRO) - resistant to multiple antibiotic agents; defined by organism type/specific agents
Sensitivity Testing: Dilution in liquid broth

Tubes containing increasing antibiotic concentrations
Incubation during 18 hr at 37°C
URINE CULTURE WITH MIC
* SOURCE: URINE-CYSTO
* STATUS: FINAL
* COMPLETED CULTURE RESULTS
* ESCHERICHIA COLI - GREATER THAN 100,000 ORGANISMS PER ML

SUSCEPTIBILITY RESULTS:
S = Susceptibility  I = Intermediate  R = Resistant
Minimum Inhibitory Concentration (MIC) expressed in ug/mL

<table>
<thead>
<tr>
<th>ORGANISM(S):</th>
<th>ECOLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIKACIN</td>
<td>S &lt;=2</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>R &gt;=32</td>
</tr>
<tr>
<td>AUGMENTIN</td>
<td>R &gt;=32</td>
</tr>
<tr>
<td>CARBENICILLIN</td>
<td>R &gt;=512</td>
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<tr>
<td>CEFOTAXIME</td>
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<tr>
<td>CEFTAZIDIME</td>
<td>S &lt;=8</td>
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<tr>
<td>CEFTIOFUR</td>
<td>S &lt;=1</td>
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<tr>
<td>CEFTRIAXONE</td>
<td>S &lt;=8</td>
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<tr>
<td>CEPHALOTHIN</td>
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<tr>
<td>CHLORAMPHENICOL</td>
<td>S 4</td>
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<tr>
<td>CIPROFLOXACIN</td>
<td>R &gt;=4</td>
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<tr>
<td>DOXYCYCLINE</td>
<td>R &gt;=16</td>
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<tr>
<td>ENROFLOXACIN</td>
<td>R &gt;=2</td>
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<tr>
<td>GENTAMICIN</td>
<td>R &gt;=16</td>
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<tr>
<td>IMIPENEM</td>
<td>S &lt;=4</td>
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<td>NITROFURANTOIN</td>
<td>S &lt;=32</td>
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<tr>
<td>OFLOXACIN</td>
<td>R &gt;=8</td>
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<tr>
<td>PIPERACILLIN</td>
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<td>TETRACYCLINE</td>
<td>R &gt;=16</td>
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<td>TICARCILLIN</td>
<td>R &gt;=256</td>
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<tr>
<td>TOBRAMYCIN</td>
<td>S 2</td>
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<td>TRIBRISSEN</td>
<td>R &gt;=320</td>
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### Blood Culture, Aerobic + Anaerobic #2

<table>
<thead>
<tr>
<th>Report Status</th>
<th>Final report 01/09/2017</th>
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<tbody>
<tr>
<td>Specimen Description</td>
<td>Blood, Peripheral</td>
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<tr>
<td>Special Requests</td>
<td>Label SEPSIS</td>
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<tr>
<td>Culture Result</td>
<td>Both bottles have growth: Escherichia coli 2 morphological types, same sensitivity</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Meropenem</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Trimeth-Sulfamethoxazole</td>
<td>Resistant</td>
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<tr>
<td>Method</td>
<td>MIC</td>
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<table>
<thead>
<tr>
<th>Organism</th>
<th>Extra drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>Susceptible</td>
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<tr>
<td>Method</td>
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### Urine Culture C&S Only

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<th>Report Status</th>
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<tbody>
<tr>
<td>Specimen Description</td>
<td>Urine, Void</td>
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<tr>
<td>Special Requests</td>
<td>None</td>
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<tr>
<td>Culture Result</td>
<td>Over 100,000 colonies/ml Escherichia coli 2 morphological types, same sensitivity 50,000 colonies / ml Mixed gram positive growth</td>
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<td>Resistant</td>
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<tr>
<td>Levofloxacin</td>
<td>Resistant</td>
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<tr>
<td>Meropenem</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Nitrofurantion</td>
<td>Susceptible</td>
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<td>Piperacillin/Tazobactam</td>
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Sensitivity Testing: Dilution in liquid broth

Tubes containing increasing antibiotic concentrations
Incubation during 18 hr at 37°C

Focus on the interpretation **not** the number
S = Sensitive
I = Less sensitive
R = Resistant

Bacterial growth

MIC

Inhibition

0 (Control) 0,25 0,50 1 2 4 8 mg/l
Antibiotic Resistance

- An antibiogram shows the proportion of bacteria resistant to specific antibiotics in a hospital or region
  - Used for clinical decision-making

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<thead>
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<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
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<tbody>
<tr>
<td>Amoxicillin-Clavulanate</td>
<td>116 100.0%</td>
<td>0 0.0%</td>
<td>0 0.0%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>93  80.2%</td>
<td>23 19.8%</td>
<td>0 0.0%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>116 100.0%</td>
<td>0 0.0%</td>
<td>0 0.0%</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>104 89.7%</td>
<td>2 1.7%</td>
<td>10 8.6%</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>115 99.1%</td>
<td>1 0.9%</td>
<td>0 0.0%</td>
</tr>
</tbody>
</table>
Extended Spectrum Beta-lactamase (ESBL)-producing Gram-negative Bacteria

- Cephalosporins: class of antibiotics developed to combat emergence of $\beta$-Lactamase producing GNR
- Resistance to cephalosporins began in ~1990s
- ESBLs now resistant to 3rd generation Cephalosporins (eg: cefotaxime, ceftazidime, ceftriaxone) and monobactams (e.g.: aztreonam)
- ESBL remain susceptible to cephamycins (cefoxitin, cefotetan, cefmetazole) and carbenapenems (meropenem, imipenem)
ESBL (continued)

- Carbapenems are the last β-Lactam antibiotic class for treatment of ESBL infections
  - e.g. imipenem, meropenem, doripenem, ertapenem
- New Delhi metallo-beta-lactamase 1 (ndm-1) CRE detected in 2008; susceptible only to polymyxins and tigecycline
- Carbapenemase-resistant Enterobacteriaceae (CRE) beginning to emerge, leaving few treatment options
  - Seen in 47 states by Feb 2014

See 2013 CDC guidance for management of CRE infected patients at [www.cdc.gov/hai/organisms/cre](http://www.cdc.gov/hai/organisms/cre)
Hepatitis A Viral Markers

Hepatitis A Virus (HAV)

- HAV, total – current or past HAV
- HAV, IgM – definitive diagnosis of active HAV infection

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health

**Acute hepatitis A requires immediate notification**
<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td>Immune due to hepatitis B vaccination**</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HbeAG</td>
<td>positive</td>
<td>Highly infectious</td>
</tr>
</tbody>
</table>

Ag = antigen  c = core  Ab = antibody  s = surface
Hepatitis C Viral Markers

Hepatitis C Virus (HCV)

- **Anti-HCV**
  - Presence of antibodies to the virus, indicating exposure to HCV
  - Active vs. chronic vs. resolved

- **HCV RIBA** *(recombinant immunoblot assay)*
  - Confirmatory test of antibodies to the virus
  - Demonstrates if HCV was true positive (present or past is unanswered)

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health
Laboratory Tests of Interest to IP

- Acid Fast Bacillus (AFB) test of sputum for diagnosis of TB
  - First morning specimen or bronch lavage are best
  - Rarely negative smear, positive culture (must follow up exposures)
- Direct fluorescent antibody (DFA) tests for identification of respiratory viruses such as legionella
- Rapid diagnostic testing: provides quick diagnosis
  - HIV: detects antibodies, has high sensitivity/specificity but because of false positives, confirmatory testing should be done
  - Influenza: very fast antigen detection; false positives 51-82% of time, so should not be used alone
  - Strep: antigen detection w/ 95% sensitivity; will also detect carriers
Nucleic Acid Amplification Tests (NAAT)

Molecular technique that detects viruses or bacterium

- Polymerase chain reaction (PCR) assays amplify gene segments specific to organism of interest; available for a number of bacterial and viral pathogens
  - Uses alternating step and temperature cycle process to detect molecules
  - Highly sensitive; may not indicate viability of organism
  - Expensive but getting cheaper, more rapid
- Ligase chain reaction (LCR) uses DNA polymerase (enzymes that build DNA and an enzyme that helps repair DNA. Because two targets are used, the test has greater specificity
- Loop-mediated isothermal amplification (LAMP) can be performed using a constant temperature and fewer primers
  - Newer, faster, expensive, less versatile, best for use with a single target
Laboratory Tests of Interest to IP - continued

- Serology testing to look for antibodies (see Slide 9) that demonstrate exposure/infection
  - Indicates patient immunity
  - Testing can also look for antigens

- Antibiotic susceptibility testing performed on bacterial cultures to test the susceptibility or resistance to specific antimicrobial agents (see Kirby Bauer, Slide 22)

- Viral load testing for HIV, HCV

- Microscopic evaluation for fungal infections such as wet mounts for vaginal organisms, CSF, skin

- Antigen tests for cryptococcal meningitis
Role of Microbiology in HAI Prevention

Microbiology support is critical to

- Outbreak management
- Performing additional tests for epidemiologic analyses
- Infection surveillance
- Knowledge of new microbes or unusual resistance
- Design of antibiotic formulary (antibiogram)
- Interpretation of microbiological results
- Education of health care staff
Questions?

Thank you