Micro 101

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Objectives

➢ Describe basic morphology of microorganisms

➢ Review methods of detection and sensitivity testing of microorganisms

➢ Demonstrate practical application of Microbiology results to clinical practice
Introduction

Clinical Microbiology is the scientific study of microorganisms and the diseases they cause in humans.
Microorganisms

- **Bacteria** - single-celled or noncellular spherical or spiral or rod-shaped organisms lacking chlorophyll that reproduce by fission
- **Fungi** - Molds, mildews, yeasts, mushrooms: a group of organisms lacking in chlorophyll and which are usually non-mobile, filamentous, and multicellular.
- **Protozoa** - A single-cell organism that can only divide within a host organism.
- **Viruses** - ultramicroscopic infectious agent that replicates itself only within cells of living hosts
Bacteria

Physical conditions
- Nutrition – types of media
- Temperature

Atmospheric conditions
- **Aerobic** - survive and grow in an oxygenated environment – most common clinical bacteria
- **Obligate aerobe** - requires oxygen to grow – *P. aeruginosa, M. tuberculosis*
- **Obligate anaerobes** – die in presence of oxygen – *Clostridium*
- **Facultative anaerobes** – aerobic respiration if O$_2$ is present but are also capable of switching to fermentation; e.g. *Staph, E. coli*
Bacteria

- Two general categories:
  - **Gram Positive** cocci and rods
  - **Gram Negative** cocci and rods

- Classification based on the organisms gram stain reaction, shape, and arrangement

- Staining reaction is based on organisms cell wall structure
Gram Stain

The following characteristics should be noted by the technologist:

- presence of a single type or several types of organisms

- predominant type of organism if more than one is present

- staining characteristics (gram positive or gram negative)
Gram Stain

- Shape of the organisms, rods (bacilli) or round (cocci)

- Relation to inflammatory cells because some organisms are characteristically inside inflammatory cells (intracellular) or adherent to them

- Gram stain: only ‘stat’ Micro procedure: CSF, joints
Gram Stain

- Divides all bacteria providing direction for microbiologic testing
- Quality of specimens: squamous epithelial cells vs neutrophils

Many squamous cells - unacceptable

Neutrophils, no squamous cells; paired, slightly elongated lancet shaped gram-positive cocci; halo = capsule
Gram Stain

Gives initial direction for therapy or intervention
Bacilli

Diphtheroids

Pseudomonas
Curved Vibrio

Gram stain of gastric mucus smear from a patient with duodenal ulcer (X 1000). Many curved H.pylori are seen (A) as well as a polymorphonuclear leucocyte engulfing some bacteria (B).
Spinal Fluid Gram Stain

Initiate Isolation – or not?

Neisseria

Haemophilus

Listeria
Media

- **Nutrient** - blood agar

- **Enrichment** - chocolate agar (blood heated to release growth factors for Haemophilus and Neisseria)

- **Enriched and selective** – Thayer Martin (abx inhibit oral and genital bacteria)

- **Differential and selective** – MacConkey (inhibit gram positive organisms)
Throat Culture
Sputum Culture
Normal Respiratory Flora with *Streptococcus*
S. pneumoniae
- **Hemolysis on blood agar**: color surrounding colony on blood agar used to classify Strep.

  - **Alpha** (partial hemolysis)
    - *viridans Strep, S. pneumoniae*
  - **Beta**
    - Complete lysis
    - *Strep pyogenes (GAS), Strep aggalactiae (GBS)*
  - **Gamma**
    - No change
    - *Strep bovis*
**Staphylococcus aureus**

- Gram positive cocci in clusters
- Pathogen from any site
- Most common wound isolate
**Escherichia coli**

- Lactose fermenting gram negative rod
- Normal flora of GI tract
- Common pathogen in UTIs
Pseudomonas aeruginosa
Candida albicans

Sabaroud agar – inhibits bacterial growth
Mixed Infection

- skin ulcer shows many gram-negative coccobacilli; short, plump gram-negative bacilli; and gram-positive cocci singly, in pairs, and in clusters.

- mixture of organisms commonly colonizes specimens from skin ulcers
Specimen Collection

- Antimicrobial therapy
- Appropriateness of specimen
- Sufficient quantity
- Prompt delivery to lab
- Sufficient clinical information provided
- Specimen rejection
Body Sites

**Blood**

- Most common isolates: Coag negative Staph, *S. aureus*, *Enterococcus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*

- Certain bacterial species indicate presence of an underlying neoplasm; e.g. *C. septicum*, *S. bovis*, *A. hydrophilia*

- *Streptococcus anginosus* → consider an abscess

- Site prep extremely important to reduce risk of contamination
Body Sites

*Respiratory*

- Sputum, not saliva
- First morning specimen for TB
- Sputum specimens offer minimal assistance in diagnosing pneumonia since many patients are colonized with gram negative rods
- Correlate with gram stain
- Obtain blood cultures
Body Sites

Urine

- Urethra has resident microflora that colonize the epithelium in the distal portion.
- *Enterobacteriaceae* and yeast may be transient colonizers.
- All areas above the urethra are sterile
- Community acquired – *E. coli*
- Hospital acquired – *E. coli*, *Klebsiella* spp., *Proteus* spp., *P. aeruginosa*, *Enterococci*, *Candida* spp.
- Do not obtain cultures routinely from asymptomatic catheterized patients.
Body Sites

Pressure Ulcers

- Aspirated fluid or purulent drainage will more likely yield a causative agent

- Surface swabbing not recommended $\rightarrow$ skin flora

- Remove superficial debris

- *S. aureus*, anaerobes, gram negatives
Colonization vs. Infection

- **Colonization** - microorganisms inhabit a specific body site (such as the skin) but don't cause signs and symptoms of infection. Colonized pathogens have the potential to cause infection if they spread to a different site on the same patient (for example, from the skin to the urinary tract) or to another person.

- **Infection** - the pathogen actually invades the host's tissues and causes signs and symptoms of illness.
Lab Report

- **Sputum specimen**
  - Gram stain
    - No wbc's (or neutrophils)
    - 2+ epithelial cells
    - Many (4+) gram positive cocci in clusters
    - Moderate (3+) gram negative rods
  - Culture results
    - Moderate normal respiratory flora
    - 3+ *P. aeruginosa*
Identification and Susceptibility

- Specimen collected $\rightarrow$ Micro $\rightarrow$ plated and incubated
- Culture read $\rightarrow$ colony morphology
- Isolate potential pathogen for pure culture
- Automated system for ID and sensitivity
Identification

➢ Coagulase
  • Coag positive = *S. aureus*
  • Coag negative = *S. epidermidis*, etc.

➢ Catalase
  • Staph vs Strep
Identification and Susceptibility Methods

- API biochemical strips for identification – 24 hrs.

- Kirby Bauer for susceptibility – 24 hrs.
Automated Testing

- General ID and sensitivity
  - Biochemical and antibiotic susceptibility panels are supplied with well plates of dried reagents
  - Results within 48 hrs, depending on original culture
  - Marker wells for Extended Spectrum Beta-lactamase (ESBL) detection
ESBL

- Beta-lactamases - primary mode of resistance to beta-lactam antibiotics

- Mid 1980s - a new type of beta-lactamase was being produced by *Klebsiella & E. coli* that could hydrolyze the extended spectrum cephalosporins. These are collectively termed the 'extended spectrum beta-lactamases'.

- Hydrolysis of beta-lactam ring of basic penicillin structure: opens up the ring, thus making the drug ineffective.
Plasmids

- Rings of extrachromosomal DNA
- Can be transferred between different species of bacteria
- Carry resistance
Beta-lactamase Inhibitor

- Clavulanic acid + amoxicillin = Augmentin
- Clavulanic acid + ticarcillin = Timentin
- Sulbactam + ampicillin = Unasyn
- Tazobactam + piperacillin = Zosyn

**Good News:** Beta-lactamase inhibitors inhibit the beta-lactamase thereby not allowing the molecule to hydrolyze the antibiotic. Most ESBLs remain susceptible to Beta-lactamase inhibitors

**Bad News:** some ESBL producing bacteria produce large amounts of beta-lactamase thereby overwhelming the beta-lactamase inhibitors
Blood Cultures

- Refinements in blood culture media and detection methods

- Automated systems capable of monitoring microbial growth continuously

- Measures CO$_2$ produced by microbial metabolism by fluorescence or colorimetric sensors

- Continuous monitoring of every bottle

- Non-invasion of bottles eliminates potential for cross contamination.

- Once viable for gram stain → 48 hrs to ID and sensitivity
Final Results

Specimens from different sites are held for a designated time

- Blood – 5 days
- Respiratory routine culture – 2 days
- Wound – 4 days
- Urine – 18 hours
- Fluids – 4 days
- TB – up to 8 weeks
  - Probes for *M. tuberculosis* and *M. avium* within 1-2 weeks
- Fungal – 4 weeks
Labeling

Include both the source
(i.e., abscess, incision, synovial fluid, etc.)

and

the site (i.e., axilla, right knee, left cheek, etc.)

If specimen is only labeled “fluid”, Micro has no idea if it is CSF, peritoneal, pleura, etc.

Note location of wound specimen: hip, knee, sternum, sacral ulcer, etc.
Case Study I

- Patient is a 78-year-old male with a past medical history of diabetes mellitus, COPD and hypothyroidism. He is admitted to the hospital after an outpatient cardiac catheterization that revealed three-vessel coronary artery disease. A CABG is performed on day 1 of his hospitalization.

- The patient received appropriate antibiotic prophylaxis preoperatively and three doses post-op ending within 24 hours. The patient is extubated in the ICU on post-op day 1. He developed severe bronchospasm requiring reintubation on post-op day 2 and then again on post-op day 6.

- He remained intubated in the ICU on post-op day 7. He had a Foley catheter that was placed in the OR and a PICC line was placed on post-op day 3.

- On post-op day 7, the patient spikes a fever to 102°. He has hypotension and tachycardia. While on tube feedings, his stools are loose and frequent.
Case Study I

Diagnostic Testing – which should be done?

A. BC x 2
B. UA and C&S (must have UA with C&S)
C. CXR portable
D. Stool for C. *diff*
E. Sputum for gram stain C&S (must have gram stain)
F. All of the above
Case Study I

What is/are the most likely source(s) of this patient’s fever and hypotension on post-op day 7?

A. Central line bloodstream infection (CLABSI)
B. Catheter associated urinary tract infection (CAUTI)
C. *Clostridium difficile* associated diarrhea (C. *diff*)
D. Ventilatory associated pneumonia (VAP)
E. All of the above
Case Study I

After diagnostic testing is completed, the appropriate therapeutic intervention would be:

A. Remove PICC line and Foley catheter

B. Initiate broad-spectrum antibiotics

C. Begin therapy with Xigris-activated protein C (for the reduction of mortality in adult patients with severe sepsis)

D. Exploration of the clean/dry/intact sternal wound
Laboratory findings available to you on post-op day 8 include a CXR with a new LLL pneumonia, WBC 18,000, blood cultures negative x 24 hours, C. diff negative, UA negative, sputum gram stain positive for WBC and four plus gram negative rods. The differential diagnosis should now be streamlined. The most likely diagnosis is:

A. CLABSI  
B. CAUTI  
C. C. diff  
D. VAP  
E. DVT
Case Study I

On post-op day 9, the patient has clearly improved while on intravenous Vancomycin and Zosyn with a TMAX of 99.8° and the sputum is growing *Pseudomonas aeruginosa* sensitive to the antibiotic Zosyn, which has been administered since he spiked a fever. He remains on Vancomycin IV. Blood cultures remain negative. The next therapeutic step would be to:

A. Continue same antibiotics  
B. Discontinue antibiotics since he has improved  
C. Narrow the spectrum of antibiotics to treat the *Pseudomonas* with Zosyn alone  
D. Add a third antibiotic  
E. Culture the ventilator equipment
Case Study II

A 60-year-old female is admitted for colon resection due to colon cancer. A right hemicolectomy is performed on day 1 of hospitalization.

She develops abdominal pains on post-op day 4 and is found to have an anastomotic leak.

A right subclavian line is placed on that day for TPN, antibiotics and fluids on post-op day 11.

She experiences chills, rigors with fevers of 102°.

Her WBC that day is 18,000. Her Foley catheter had been removed on post-op day 1.

A CXR shows only atelectasis.

Her UA was 0-2 WBC.
Case Study II

The differential diagnosis for this patient’s fever and elevated WBC’s on post-op day 11 is (choose one or more):

A. CA-UTI
B. Post-op atelectasis
C. DVT
D. CLABSI
E. Intra-abdominal abscess
Blood cultures have been drawn peripherally, a CT scan of the abdomen reveals no changes from a CT on post-op day 4 - no abscess; CLABSI is the most likely diagnosis. Which organisms are most commonly responsible for this type of HAI?

A. Staphylococcus aureus/epidermidis; Enterococcus / yeast

B. Pseudomonas / Klebsiella/ E. coli

C. Streptococci (viridans, Group A), diphtheroids

D. Mycobacterium tuberculosis
The blood cultures are positive at 24 hours revealing gram positive cocci in clusters. What is the next therapeutic step?

A. Begin Amphotericin B for a fungal infection

B. Change the right subclavian line over a wire leaving the device in the same site

C. Change the line from a 3-port access line to a single lumen line

D. Remove the right subclavian line, culture the tip of the line, assure the patient is on Vancomycin or an appropriate antibiotic to cover *Staph aureus*
Electron micrograph of internal surface of central venous catheter. Note thick biofilm after only 24 hours. Cocci (arrows) are present in biofilm
Case Study II

True or false?

- Factors that increase the rates of CLABSI:
  - the number of ports (lumens) on a line increases the risk of a CLABSI \textbf{True}
  - The frequency of blood drawing from a port of a line increases the risk of CLABSI \textbf{True}
  - The insertion of a line in the femoral region is associated with the least risk of CLABSI \textbf{False}
Case Study III

A 68-year-old male had a pacemaker placed in the left anterior chest two months prior to the present admission.

He now is experiencing chills, fevers to 102°, rigors and pain, redness and swelling at the pacer site.

Upon examination of the patient, his temperature is 101°, the pacemaker site is erythematous, tender to palpation and purulent drainage exists from a sinus tract.

The gram stain reveals gram positive cocci in clusters.
Case Study III

The most likely type of organism causing this patient’s device related infection is:

A. Streptococcal species

B. Diphtheroids

C. Staphylococcal species

D. Pseudomonas species

E. Fungal organisms
The final culture result is positive for MRSA. The drug of choice is:

A. Vancomycin
B. Ancef
C. Nafcillin
D. Imipenem
E. Rocephin
Community acquired MRSA strains most commonly cause what clinical illness(es)?

A. Gastroenteritis

B. Urinary tract infections and bacteremia

C. Meningitis

D. Carbuncles (skin abscess) and necrotizing pneumonia
FIGURE 1. A lesion that tested positive for MRSA on the thigh of a recreational athlete, about 2 days after initiating treatment with warm compresses, NSAIDs, and mupirocin cream (A). The pustule ruptured 3 days into treatment, and painful induration lasted about 10 days. Another MRSA infection, 6 days after initiating treatment, on the knee of a college football player (B) who developed the infection after scraping his knee on artificial turf. Treatment consisted of warm compresses, a drying agent, NSAIDs, and double-strength TMP-SMZ. Once opened, the lesion was treated with silver sulfadiazine cream for an additional week.
Case Study III

True or False

- Greater than 50% of all healthcare workers are colonized with MRSA  False

- MRSA strains have shown a decreasing sensitivity (MIC) to Vancomycin  True
Case Study IV

A 79-year-old female is admitted to the hospital from a LTC facility after a fall in the nursing home causing a fractured pelvis.

Her past medical history includes a CVA, seizures and diabetes. Because of her limited mobility, a urinary catheter is inserted on hospital day 1.

Five days later she spikes a temperature to 101.4°C.

A physical examination reveals suprapubic pain, clear lungs, intact skin and no inflamed I.V. sites. The nurses report no diarrhea.
The differential diagnosis for this patient’s fever on hospital day 5 include(s):

A. Neuroleptic malignant syndrome

B. Line sepsis

C. Catheter-associated urinary tract infection

D. Fever of unknown origin

E. Nosocomial acquired meningitis
At this point, what diagnostic test(s) should be ordered?

A. CAT scan of the head

B. CAT scan of the chest-abdomen-pelvis

C. BC x 2, U/A C&S, chest x-ray, CBC and differential

D. Lumbar puncture

E. HIDA scan to rule out cholecystitis
The urinalysis shows pyuria (> 20 WBC) and the WBC is 14,000 with a left shift. The CXR reveals no new pneumonia. A urine gram stain which shows gram negative rods. The best empiric antibiotic option would be:

A. Oral Penicillin

B. Intravenous Vancomycin and Gentamicin

C. Oral Levaquin

D. Intravenous Imipenem

E. Intravenous Fortaz and a dose of Gentamicin
Case Study IV

The laboratory studies now available to you include negative blood cultures and a urine culture growing *E. coli* resistant to ampicillin, ciprofloxacin and cephalexin. How would you complete this patient’s antibiotic therapy?

A. 7 days of I.V. Fortaz

B. Convert to oral Bactrim – the organism was sensitive to this antibiotic; 7-10 days of antibiotics

C. 7 days of I.V. Gentamicin

D. Convert to oral Ciprofloxacin even though the organism was resistant

E. Re-insert foley catheter to prevent skin breakdown along with 7 days of I.V. imipenem
Roger crams for his microbiology midterm.
Websites

- http://www.kscience.co.uk/as/module1/bacteria.htm
- http://www.ann-clinmicrob.com/content/4/1/18/figure/F5?highres=y
- en.wikipedia.org/wiki/Costophrenic_angle