Work up of Wound Cultures in the Microbiology Laboratory

“Watch your P’s and Q’s”

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Workup of Wound Cultures—Two approaches:

- Q-Score System
  - Q score system scores the Gram stain of the specimen giving a positive value to the number of PMN’s and negative values to the number of SEC seen in a direct Gram stain.
  - The above values are added to obtain the “Q score” which is used to determine the quality of the specimen and the extent of culture work-up. See following figure for this determination.

- Q234 System
  - This system does not score the Gram stain first but rather determines the number of potential pathogens in the specimen.

Work up of Respiratory and Wound Cultures

Q-Score System (RC Barlett, 1974)

<table>
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<th>-2</th>
<th>-3</th>
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- Up to 3 organisms can be considered potential pathogens (PP) and be worked up ID (identification) and AST (Antimicrobial susceptibility test) if from a good quality specimen (Q3)
- The lower quality of the specimen (e.g., the more SEC present) the fewer the organisms worked up (Q2, Q1)
- #PP in culture (2PP) ≤ Q-score (Q3): workup PP with ID/AST
- # PP in culture (3PP) > Q-score (Q2): look to Gram stain
  - Work up PP that were seen in Gram stain with ID/AST
  - If all PP in the culture are seen in Gram stain do not work up: morphologically identify (MID) (rapid test/observations: Gram stain, colony morphology-MacConkey (LF vs NLF), oxidase, catalase etc.

Q2-3-4 System

- Gram stain Quality Check: PMN and SEC
- Culture workup is based on the number of Potential Pathogens(PP) present:
• 2 PP = Work up (≤ 2PP)
• 4 PP= MID (morphologically identify)
• 3 PP= Look to Gram stain
  • Work up 2 PP if they are seen in the Gram stain
  • IF all 3 PP are seen in the Gram stain, MID all 3

• This is a simpler system-do not have to score the Gram stain first

Advantages for “Q” Systems
1. Offer a consistent approach for interpreting cultures
   a. Based on specimen quality (PMN’s vs SEC’s)
   b. Based on organisms seen in Gram stain (if see organism on smear, should be in a
      significant number in specimen
   c. Limits number of organisms worked up from mixed cultures, so that the reporting of
      misleading information can be minimized
2. No Potential Pathogen is ever ignored
   a. All Potential Pathogens listed out-may not be get a full ID/AST
   b. The pathogens that some believe should “ALWAYS BE WORKED UP”, such as S.
      aureus, Beta strep, P. aeruginosa are identified and always indicated on the report
   c. Criteria can be added to these systems
3. Guidelines:
   a. The Q-Systems offer “Guidelines” for a systematic culture interpretation approach
   b. Exceptions can be made if necessary
   c. Any concerned physician can consult with microbiology to have further work performed
      on any culture if clinically indicated
4. Q-Reference For cost Savings
      Microbiol. 44:1869-1872

Shiga Toxin Producing Escherichia coli (STEC)
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Quest Diagnostics

Shiga toxin-producing E. coli (STEC)
• Produce Shiga toxins type 1 and/or type 2 (stx1, stx2)
• Intimin (eae) –protein that is important in the attachment of the organism to the GI epithelium
• Most common STEC
  o E. coli serotype O157:H7
  o Accounts for 50-60% of STEC infections in US
• Non-O157 STEC
  o 100 serotypes
  o 40-50% of STEC infections in US but data is inadequate

STEC Infection: E.coli O157:H7
• Hemorrhagic colitis (bloody diarrhea), severe abdominal pain, no fever
• 8% develop hemolytic uremic syndrome (HUS)
• Virulence: 99% have stx2, 100% eae (attachment protein)
STEC Infection: Non-157 Serotypes

- Most illness is mild
- Nonbloody diarrhea
- Bloody diarrhea (<40%) and HUS (~2%)
- Some serotypes may not be pathogens
- Virulence: 40% have stx 2, 80% have eae
**STEC Infection: Hemolytic Uremic Syndrome**
- Children and elderly more vulnerable
- 3%-5% of HUS patients die
- Lifelong complications (blindness, paralysis, persistent kidney failure, effects from having bowel removed)

**STEC Infection: Treatment**
- Parenteral – volume expansion early in severe STEC infection may prevent HUS
- Avoid other treatments including:
  - Antibiotic treatment (inconclusive data)
  - Antibiotics don’t reduce risk of HUS
  - Some (Bactrim) may increase risk of HUS
- Antidiarrheal agents may increase risk of HUS
- Most patients recover in ~ 5 days without treatment

**STEC Diagnosis: *E.coli* O157:H7-Stool culture**
- Stool Media
  - Sorbitol MacConkey agar (SMAC)
  - Cefixime-tellurite SMAC (CT-SMAC)-more selective
  - Chrom agar O157
- Presumptive Identification
  - Select colony for agglutination in O157 latex reagent (test control latex also)
  - Biochemically identify as *E.coli*
- Send isolate to Public Health Laboratory

**STEC Diagnosis at the Public Health Laboratory**
- Isolates are confirmed as *E.coli* O157:H7
- Isolates are subtyped by pulse field gel electrophoresis (PFGE)
  - PFGE pattern uploaded to the PulseNet database
  - Outbreaks are detected when isolates have same patterns

**STEC Diagnosis Non-O157 STEC**
- Commercial Shiga toxin enzyme immunoassays (StxEIA) are the only practical method for diagnosis of non-O157 STEC
- STx EIA detects:
  - Shiga toxin in a broth inoculated from stool specimen
  - All STEC serotypes including *E.coli* O157:H7

**STEC Diagnosis disadvantages of STx EIA**
- STx EIA cannot differentiate:
  - Between *E. coli* O157:H7 and other STEC serotypes
  - Between STx1 and STX2 (more virulent)
- False positive reactions are not uncommon
  - Inadequate plate washing
  - Visual reading not accurate
  - Testing inappropriate specimens
- No Proficiency testing program
- Disadvantages for Public Health Laboratories
No E. coli O157:H7 isolates for outbreak detection by PFGE
No E. coli O157:H7 isolates for surveillance

CDC Recommendations for STEC Testing: Preferred Algorithm
- Test all stool specimens submitted for routine enteric bacterial testing (Salmonella, Shigella, Campylobacter) for STEC
- The ideal algorithm for testing:
  - Culture for E. coli O157:H7 on selective Media
  - Simultaneously inoculate a broth for STx EIA testing
- Send E. coli O157:H7 isolate or STx+/O157-negative broth to public health laboratory

CDC Recommendations for STEC Testing-Alternate Algorithm
- Test all stool specimens submitted for routine enteric bacterial testing (Salmonella, Shigella, Campylobacter) for STEC
- Alternate testing algorithm
  - Inoculate broth and run STx EIA after 18 hours
  - IF broth is STX+, culture for E. coli O157 on selective media
- Send E. coli O157:H7 isolate or STX+/O157 negative broth to public health laboratory

Summary
1. Timely laboratory diagnosis of STEC illness
   a. May prevent inappropriate treatment
   b. May guide appropriate treatment (e.g. IV fluids) to prevent HUS
2. Send E. coli O157:H7 isolate or STx+/O157-negative broth to public health laboratory
   a. Provides for prompt detection of outbreaks
   b. Allows public health actions (e.g. food product recall) to prevent additional infections

Emerging Pathogens
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University of North Carolina Hospitals

How do new pathogens emerge?
- Organisms that jump species barriers: HIV<SARS, avian Flu
- Changing ecosystems: Lyme disease
- Changes in food production techniques: EHEC
- Evolution of medical devices and care: long term survival of immunosuppressed
- Pathogens that are detected because of new technology: HCV, non-cultivable bacteria
- Misuse of microorganisms-biocrime/bioterrorism
- Organism evolution as a result of human intervention-antibiotic pressure

How emerging pathogens develop?
- Mutation drives evolution
  o Constantly occurring
  o Usually silent or lethal
  o Environmental pressure such as antibiotics may select “resistance” mutation
    - Key feature of success of antibiotic resistance strains is their genetic fitness
Recognition that certain bacteria may be hypermutators because of mutation in DNA repair genes
- These strains may not be as “fit” as wild-types but may predominant in certain chronic infections such as *P. aeruginosa* causing chronic pulmonary infections in cystic fibrosis patients

Recombination
- Resistance genes from antibiotic producing organisms
- Genetic exchange of resistant genes can occur among organisms which are genetically diverse
- Transfer of resistance/virulence genes can be mediated by plasmids/transposans/integrons

**Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA)**
- Strains are different than hospital acquired
- Have the mec A gene but it is packaged differently
  - Staphylococcal chromosome cassette (SCCmec type IV)
- More susceptible than hospital acquired strains

**Clinical presentation CA-MRSA**
- SSTIs (abscesses, cellulitis, folliculitis, impetigo, furunculosis)
  - Typically treated with excision and drainage; +/- oral antibiotics
  - Occasionally require IV antibiotics, hospitalization and surgical intervention
- Necrotizing pneumonia especially in young people secondary to influenza
  - Mortality was over 50%-median time death 3-5 days
  - Median age was 17.5 years
  - 5 isolates from Louisiana were CA-MRSA genotype of the same PFGE type
  - Both levofloxacin and inducible clindamycin resistance seen in these isolates

**Virulence of CA-MRSA**
- Panton-Valentine leukocidin (PVL)
  - Hemolysin reported in 1932 by Panton and Valentine
  - Located on mobile phage
  - 2 co-transcribed genes, lukS-PV and lukF-PV
  - The 2 subunits form a hexameric pore-forming cytolytic toxin with a high affinity for PMNs and macrophages
- PVL producing strains associated with skin and soft tissue infections and necrotizing pneumonia
- Rarely associated with osteomyelitis, septicemia or endocarditis
- Rare HA-MRSA strains with PVL have similar clinical symptoms
- Usually only 2% of all *S. aureus* isolates produce PVL but found in the majority of epidemic CA-MRSA strains

**Susceptibility Patterns-CA-MRSA**
- Resistant to penicillin, oxacillin, beta-lactams
- 93% resistant to erythromycin, 16% resistant to clindamycin
- Fluroquinolone resistance becoming more frequent

**Conclusions**
- CA-MRSA has emerged as an important cause of skin/soft tissue infections in the Carolinas
- The microbe quickly spread from the community into the hospital and is a significant cause of nosocomial bacteremia
• It is likely that it will become the pre-dominant strain of MRSA regardless of health care setting suggesting it is a highly genetically fit organism

*Clostridium difficile*-Is There an emerging pandemic of a highly virulent *C. difficile* strain?

• Recent outbreak in Canada, the regions of the US, Britain, and now the Netherlands have recognized the emergence of a highly virulent strain of *C. difficile*

• Characteristics of this new strain include:
  o Mutation in tcdC gene which down-regulates toxin production during logarithmic phase
  o Mutation results in approximately 20-fold increase in toxin production
  o Organism also produces binary toxin a 2nd virulence factor typically produced by a small number of isolates
  o Poor response to metronidazole has also been reported
  o Higher morbidity and mortality particularly among those over 75
Laboratory Detection-GDH Detection
- GDH (glutamate dehydrogenase) enzyme found in large amounts in cell membrane of C. difficile.
  - EIA for the detection of this organism has been developed
- Three assays using lateral flow devices: C diff Quik Chek, Tech labs, Immunocard, Meridian and Triage, Biosite (has accompanying toxin A assay)
- C diff Chek detects GDH only

Approach to using lateral flow devices (Triage, Biosite) that detect GDH and Toxin A
- GDH is highly sensitive but not specific
  - Produced by non-toxigenic strains and other organisms
- Toxin A assay is highly specific but not sensitive
- If GDH pos, toxin A pos-high specificity and PPV, report as positive
- If GDH neg, toxin neg, high NPV, report as negative
- If GDH positive, toxin negative, low PPV, must confirm with alternative method; approximately 5% specimens

UNC approach to detecting C. difficile
We have switched from Toxin A + B EIA to C. diff Quik. Check with confirmatory test with tissue culture cytotoxicity

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<th>C diff QUIKCHEK</th>
<th>Premier ToxinA/B EIA</th>
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<tr>
<td>Sensitivity</td>
<td>60%</td>
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<tr>
<td>Specificity</td>
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<tr>
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<tr>
<td>Confirmed Accuracy</td>
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Confirmed accuracy = TP + TN/all specimens using CTN as reference method

Rapidly Growing Mycobacteria (RGMs) - What makes them good candidates to become emerging pathogens?
- Ubiquitous in nature, able to survive under harsh environmental conditions including pH and temperature extremes and in nutritionally limited environments
- Grows as biofilm on a variety of surfaces
- Resistant to many first line antimicrobials including disinfectant
- Good news-comparatively low virulence

RGMs as Emerging Pathogens-Traditionally have been associated with:
- Skin and soft tissue infections
  - Following trauma with foreign objects
  - Following injections including acupuncture and natural medicines
  - Whirlpool associated infections
- Post-operative wound infections
  - Cosmetic and plastic surgery
  - Sterna wounds following cardiothoracic surgery
- Bronchitis in patients with chronic lung disease (non-CF)
- RGMs are being found with increasing frequency especially those belonging to the *M. fortuitum* and *M. chelonae-abscessus* groups
- We are finding them in three distinct patient populations with significant frequency
- *M. abscessus* in cystic fibrosis patients
- *M. chelonae* in ophthalmologic infections
- RGM in line related mycobacteremia

**Non tuberculous Mycobacterium following LASIK surgery**
- LASIK surgery is one of the most common surgical procedures done in the US
- Infectious complications are unusual
- RGM are responsible for 50/94 (53%) infections reported in the literature following this procedure:
  - 40/50 were in the *M.chelonae/abscessus* complex
  - With new surgical practices the number of infections due to RGM post-Lasik has declined significantly
- 14% with NTM infection were legally blind
- We supply 7H11 plates to our ophthalmology clinic routinely so they can plate specimens directly
  - So far 2 positives patients from plugs from dry eye patients

**Tips for Detecting RGMs bacteremia**
- Found in line related sepsis usually folks with “chemical AIDS”
- Organisms usually grows at 3-5 days from broth and then as faint growth at 2-3 days
- Often will be reported as “diphtheroids”
- We have begun doing Ziehl-Neelsen stains on poorly growing diphtheroids
  - Those that are AFB positive are identified by sequencing