Stool Culture Work Up
Someone's got to do it... at least for now

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• I do not have any financial relationships or affiliations to disclose related to this presentation
Objectives

- List the organisms most commonly associated with bacterial diarrhea in the US
- Describe emerging bacterial pathogens associated with diarrheal disease
- Discuss appropriate specimen collection, transport and processing for stool culture
- Discuss methods that can be used to streamline stool culture work up
- Discuss appropriate antimicrobial susceptibility testing and culture reporting
- Explore the impact of nucleic acid testing on performance of stool cultures
Let’s talk stool cultures...
Laboratory Diagnosis of Bacterial Gastroenteritis

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Impact of Bacterial Gastroenteritis

Global

- > 1.7 billion cases of diarrheal disease reported annually
  ▪ 22 million deaths
- Second leading cause of death in children <5 years of age

United States

- ~211-375 million episodes of diarrheal illness annually
  ▪ 1.8 million hospitalizations
  ▪ 3100 deaths
- 48 million cases – foodborne disease
  ▪ 128,000 hospitalizations
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Incidence of Foodborne Infection

FoodNet

Salmonella
Campylobacter
Shigella
STEC
Vibrio
Yersinia

Incidence/100,000 population
% infections associated with outbreaks

MMWR. 2015. 64:495-499
When is stool culture indicated?

From the patient perspective

- American College of Gastroenterology
  - Severe or persistent diarrhea or bloody diarrhea
  - Temperature > 38.5°C
  - Presence of fecal WBC/lactoferrin or occult blood
- Infectious Diseases Society of America
  - Diarrhea > 1 day
  - Fever or dehydration or systemic illness
  - Bloody stool

From the Public Health perspective

- Identify and track outbreaks of bacterial gastroenteritis

Specimen Collection and Transport

- Collect specimen in acute stage (5-7 days)
  - Clean, dry container
  - Rectal swabs less sensitive
- Transport
  - Fresh specimens
    - Clean, leakproof container
    - Transport and process within 2 hrs collection
  - Transport medium – Cary Blair
    - Buffered – prevent pH shifts
    - Low nutrient content – inhibit growth of other species
    - NaCl (Vibrio) and sodium thioglycollate (Campylobacter)
    - Transport and process within 48 hrs
Optimizing Stool Culture

• Fecal leukocyte testing
  ▫ Screen for evidence of inflammation
  ▫ Poor sensitivity – differentiating infectious and non-infectious diarrhea in inpatients

Methods
  ▫ Microscopy (Methylene blue/Gram stain)
  ▫ Fecal lactoferrin
    • Detects a glycoprotein component of neutrophilic granules
    • More stable (does not rely on detection of intact PMN); rapid

Optimizing Stool Culture

The Dos...
• Apply the 3 Day Rule
  ▫ Low yield of stool culture for patients developing diarrhea while hospitalized >3 days
  ▫ Think *C. difficile*-associated disease
• The Don'ts...
  ▫ Don’t process...
    • Fresh specimens not received within 2 hrs of collection
    • Specimens in Cary Blair after 48 hours
    • Specimens in Cary Blair if the indicator has turned yellow
    • Multiple specimens collected on the same day
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What should I look for in a stool culture?

- **Always**
  - *Salmonella, Shigella, Campylobacter, STEC*

- **Sometimes**
  - *Vibrio, Yersinia, Aeromonas and Plesiomonas*
    - Geography/patient population dependent; seasonal
    - Selective media used for optimal detection

- **Never - infrequently diagnosed by clinical laboratory**
  - *Bacillus cereus*
  - *Clostridium perfringens*
  - *Listeria monocytogenes*
  - *Staphylococcus aureus*
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Why test all stools for STEC?

- Selective testing strategies will miss many STEC infections
  - **Blood**
    - Not reliably present
    - Other pathogens can cause bloody stools
  - **Seasonality**
    - More common during summer months but infections and outbreaks occur year-round
  - **Age**
    - More frequent in children but almost half of all isolates are obtained from persons >12 years old
Why Culture and STEC-EIA?

• More effective for identifying STEC than either technique alone

• Early detection of O157 STEC
  ▫ High predictive positive value for severe disease
    • Almost all strains contain Stx2
  ▫ Prompt treatment with parental volume expansion decreases renal injury and improves outcomes
  ▫ Antibiotics should not be given for STEC
  ▫ Early recognition of public health problem

• Non-O157 STEC are important cause of infection
    • 5 yr study – detected additional 66 cases 47% non-O157
Should all stools be screened for STEC?

- Current CDC recommendation – Simultaneous culture for O157 STEC and toxin assay for STEC
- Selective testing approach
  - Screen all stools received for culture for a 12-month period to determine STEC prevalence in the population
  - Low incidence
    - Consider testing by request only
    - Apply combination of screening criteria

*MMWR.* 2009. 58 (RR-12):1-14  
What about emerging enteropathogens?

- Other less common bacteria can cause gastroenteritis
- Enterotoxigenic *Bacteroides fragilis*
- *Edwardsiella tarda*
- *Escherichia albertii*
- *Klebsiella oxytoca*
- *Providencia alcalifaciens*
What about emerging enteropathogens?

- Should I be looking for these organisms routinely?
  - **NO!**
    - Some can be found in the absence of symptoms
    - Difficult to differentiate from other resident fecal flora
- Culture only after discussion with clinicians to determine which patients are unique enough to look for these potential pathogens
What media should I use?

- Dependent on organisms you want to recover
  - Patient population
  - Organisms routinely isolated
- Suggested media
  - MacConkey
  - Selective/differential for *Salmonella/Shigella*
  - Selective media for *Campylobacter*
  - Selective media for STEC O157 and/or enrichment broth for shiga toxin testing

What about enrichment broth?
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Enrichment Broth
Do we need it?

- **Yes**
    - 35% of *Salmonella* only recovered in Selenite
    - 41% of newly identified *Salmonella* only recovered in Selenite

- **No**
  - Lue (*Clin Microbiol Newsl*. 1986. 8:5-6)
    - Appropriate subculture important (GN – 6-8 hr; Selenite – 18-24 hr)
    - Yield does not justify the cost

Review historical data to determine if enrichment broth provides additional recovery – if not, discontinue
What media should I use?

**Campylobacter**

- **Options**
  - Blood-free – Charcoal cefoperazone-desoxycholate agar (CCDA), charcoal based selective agar
  - Blood-containing – **Campy CVA**, Skirrow
- Avoid media with cephalothin, colistin, and polymyxin B – inhibitory to some strains of *C. jejuni* and *C. coli*, and are inhibitory to *C. fetus*
- Use of a combination of media, including one that is charcoal-based, increases yield 10-15%

What media should I use?

**Aeromonas, Yesinia, Vibrio**

- **Aeromonas**
  - Blood agar
  - Cefsulodin-irgasan-novobiocin (CIN) agar (35°C)

- **Yersinia enterocolitica**
  - Cefsulodin-irgasan-novobiocin (CIN) agar
    - 22-25°C – produces colonies with a more distinct "bull's-eye"

- **Vibrio**
  - Thiosulfate-citrate-bile salts-sucrose (TCBS) agar
    - *Grimontia hollisae* and *Vibrio metschnikovii* - inhibited
  - Blood agar
Stool Culture Work up

Screen plates for colorless or H₂S positive colonies

Screen suspicious colonies using biochemical tests
- Classic – TSI + LIA + urea
- Alternatives – MIO, MIL, MILS

Perform confirmatory biochemical and/or antigen testing

- Poor specificity
- False positive colonies
Optimizing Stool Culture Work up

**CHROMagar *Salmonella***

- Inhibits gram positives, yeast, *Proteus* spp., Non-glucose fermenters
- *Salmonella* – mauve/rose
  - *Salmonella enterica* subspecies *arizonae* (lactose +) = blue-violet to purple
- Coliforms – blue-green
- Others – colorless (white)

Incubate 24 hrs; if negative, reincubate additional 24 hr

Biochemical/serological confirmation
Optimizing Stool Culture Work up

CHROMagar *Salmonella*


- CHROMagar *Salmonella* vs. Hektoen ± enrichment
  \[\text{CHROMagar} - \text{higher specificity; reduced confirmatory testing} = \text{more economical than Hektoen}\]


- SS – XLD – HEK – GN vs. CHROMagar Sal ± enrichment
  \[\text{CHROMagar + XLD sensitivity} = 100\%; 27\% \text{ reduction in annual stool culture cost}; 78\% \text{ reduction in false positive results}\]
Optimizing Stool Culture Work up

**CHROMagar *Salmonella***

*Church et al. 2010. DMID 68:13-19*

- **Stool**
  - Selenite
  - CHROMagar
  - HEK
  - MAC + CHROMagar

- **n=2999; 51 (1.7%) *Salmonella***

<table>
<thead>
<tr>
<th></th>
<th>CHROMagar</th>
<th>CHROM + Sel</th>
<th>HEK + Sel</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>94.1</td>
<td>98.0</td>
<td>84.3</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>99.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>“Colony Picks” (CP)</td>
<td>114</td>
<td><strong>156 (+82%)</strong></td>
<td>880</td>
</tr>
<tr>
<td>CP not <em>Salmonella</em></td>
<td>66 (58%)</td>
<td>105 (67%)</td>
<td>841 (96%)</td>
</tr>
</tbody>
</table>

:. CHROMagar – higher sensitivity than HEK-Sel; 52% reduction in annual stool culture cost
Optimizing Stool Culture Work up

HardyCHROM *Salmonella Shigella*

- Facilitates detection of *Salmonella* and *Shigella*
  - *Salmonella* – teal blue colored colonies with/without black centers
  - *Shigella* – teal blue colored colonies
- Coliforms – pink colonies, with or without purple centers; dark blue; pink
Optimizing Stool Culture Work up

CHROMagar O157

- Facilitates detection of *E. coli* O157
  - Potassium tellurite
  - Antimicrobials (cefixime, cefsulodin)
- *E. coli* O157 – mauve
- Non *E. coli* O157 – blue/blue-green, colorless (white)

CHROMagar STEC (RUO)
Detects shiga toxin-producing *E. coli*
Optimizing Stool Culture Work up

CHROMagar O157

Church et al. 2007. *J Clin Microbiol* 45:3098-3100

- **CHROMagar O157 vs. sorbitol MAC**
  - 27 (0.9%) positive for *E. coli* O157
    - 26/27 (96.3%) on CHROMagar
    - 23/27 (85.2%) on sorbitol MAC

- **Costs with CHROMagar**
  - Labor – decreased 21%
  - Materials – decreased 64%
    - Less indole testing and O157 serotyping

\[ \therefore \text{CHROMagar} = \text{improved diagnostic efficiency compared to sorbitol MAC} \]
Optimizing Stool Culture Work up

MALDI-TOF

- Cost-effective alternative to screening of colonies and biochemical testing
  - Accurate identification of Aeromonas, Campylobacter, Plesiomonas, Salmonella, Vibrio spp. (including V. cholerae), Yersinia enterocolitica
- Caveats
  - Cannot differentiate Shigella and E. coli
  - Cannot differentiate E. coli from STEC
  - Media type may effect identification
    - Blood = MAC = XLD > HEK > SS

*J Clin Microbiol.* 2015. 53:329-31  
*J Thorac Dis.* 2014. 6:539-44  
*J Clin Microbiol.* 2012. 50:1008-13
Antimicrobial Susceptibility Testing

• Antimicrobials not routinely indicated in healthy patients with bacterial gastroenteritis
• Routine susceptibility testing of stool culture isolates not indicated
  ▫ Exceptions
    • Infants ≤ 6 mo of age
    • Elderly or immunocompromised
    • Prolonged disease
    • Isolation of *Salmonella* Typhi/Paratyphi A
Result Reporting

• Positive Cultures
  ▫ Pathogen with susceptibility testing, if appropriate

• Negative Cultures
  ▫ Include each organism routinely included in screening
    ☺ No *Salmonella*, *Shigella* or *Campylobacter* isolated
    ☠ No enteric pathogens isolated

• Verbal reporting/automated electronic alerts to healthcare providers – especially for STEC

• Rapid reporting to Public Health
  ▫ Forward isolates/broths to Public Health Lab as required
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The Future is now...
Nucleic Acid Amplification Testing

- Currently 5 FDA approved assays
- Will multiplex NAA assays replace culture and antigen/toxin testing?
  - High sensitivity
  - Rapid
  - Multiplex capability for parasites and viruses
- What is the impact of culture-independent testing on public health surveillance?
  - Lack of isolate for susceptibility testing or subtyping
Stool Culture Work up

Conclusions

- Number and types of agents cultured should be driven by geographic location and patient history
- Chromogenic media available to help make culture work up easier
- MALDI-TOF is a useful alternative to traditional work up algorithms
- Simultaneous culture for *E. coli* O157 and toxin assay for STEC EIA represent the best practice for detection of shiga toxin-producing *E. coli*
Stool Culture Work up

Conclusions

• Effective communication with physicians regarding need for AST and culture for “emerging pathogens” a must
  ▫ Includes physician understanding of organisms included in routine stool culture

• Still to be determined – the role of stool culture in the era of NAAT multiplex testing for detection of common pathogens
  ▫ Decisions on which method to choose
    • Cost
    • Expertise required/level of automation
    • Extent of testing required
    • Availability of organism isolate for additional testing
Questions??

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