Antimicrobial Susceptibility Testing (AST) of Bacteria That Cause Gastroenteritis!

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“and consultant with the Association of Public Health Laboratories”
Faculty Disclosure

The Association of Public Health Laboratories adheres to established standards regarding industry support of continuing education for healthcare professionals. The following disclosures of personal financial relationships with commercial interests within the last 12 months as relative to this presentation have been made by the speaker(s):

Janet Hindler:

- bioMerieux Speaker Honorarium
- Siemens Healthcare Diagnostics Speaker Honorarium
- Merck Advisor
At the conclusion of this talk, you will be able to......

- Describe appropriate AST testing and reporting on bacteria that cause gastroenteritis.
- Discuss the incidence of resistance among bacterial stool pathogens and options for conveying resistance data to clinicians.
Bacteria to Discuss

- *Salmonella* spp.
- *Shigella* spp.
- *Campylobacter* spp.

*also...*

- *Aeromonas* spp., *Plesiomonas shigelloides*
- *Vibrio* spp.
- *Yersinia* spp.
- *Clostridium difficile*
Includes:

**Threat level of urgent:**
Drug-resistant *Clostridium difficile*

**Threat level of serious:**
Drug-resistant *Campylobacter*
Drug-resistant *Salmonella*
Drug-resistant *Shigella*

Non-typhoidal *Salmonella* (serotypes other than Typhi, Paratyphia A, Paratyphia B, and Paratyphi C) usually causes diarrhea (sometimes bloody), fever, and abdominal cramps. Some infections spread to the blood and can have life-threatening complications.

**RESISTANCE OF CONCERN**

Physicians rely on drugs, such as ceftiraxone and ciprofloxacin, for treating patients with complicated *Salmonella* infections. Resistant infections are more severe and have higher hospitalization rates. Non-typhoidal *Salmonella* is showing resistance to:

- ceftiraxone
- ciprofloxacin
- multiple classes of drugs

**PUBLIC HEALTH THREAT**

Non-typhoidal *Salmonella* causes approximately 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States. Direct medical costs are estimated to be $365 million annually. CDC is seeing resistance to ceftiraxone in about 3% of non-typhoidal *Salmonella* tested, and some level of resistance to ciprofloxacin in about 3%. About 5% of non-typhoidal *Salmonella* tested by CDC are resistant to five or more types of drugs. Costs are expected to be higher for resistant than for susceptible infections because resistant infections are more severe, those patients are more likely to be hospitalized, and treatment is less effective.

<table>
<thead>
<tr>
<th>Resistance Type</th>
<th>Percentage of all non-typhoidal <em>Salmonella</em></th>
<th>Estimated number of illnesses per year</th>
<th>Estimated illnesses per 100,000 U.S. population</th>
<th>Estimated number of deaths per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftiraxone resistance</td>
<td>3%</td>
<td>16,000</td>
<td>12.0</td>
<td>13</td>
</tr>
<tr>
<td>Ciprofloxacin resistance or partial resistance</td>
<td>3%</td>
<td>33,000</td>
<td>10.9</td>
<td>12</td>
</tr>
<tr>
<td>Resistance to 5 or more antibiotic classes</td>
<td>5%</td>
<td>66,000</td>
<td>21.9</td>
<td>24</td>
</tr>
<tr>
<td>Any resistance pattern above</td>
<td>8%</td>
<td>100,000</td>
<td>34.1</td>
<td>38</td>
</tr>
</tbody>
</table>

*2-year average (2009-2011)
For more information about data methods and references, please see technical appendices.
CDC’s human surveillance data

- non-typhoidal *Salmonella*
- typhoidal *Salmonella* spp.
- *Shigella* spp.
- *Campylobacter* spp.
- *E. coli* O157
- *Vibrio* species other than *V. cholerae*

http://www.cdc.gov/narms/
NARMS Isolate Sources

- Participating public health laboratories forward to CDC for AST:
  - Every 20th non-typhoidal *Salmonella*, *Shigella*, and *E. coli* O157 isolate
  - Every isolate of *Vibrio* species other than *V. cholerae* (≈ 5 *V. cholerae* in USA / year)
  - *Campylobacter jejuni/coli* from PH labs in states participating in CDC’s Foodborne Diseases Active Surveillance Network (FoodNet)
Salmonella and Shigella spp. Antibiogram Preparation

- Most US clinical labs isolate small numbers of *Salmonella* / *Shigella*
  - 30 isolate minimum suggested in CLSI M39-A3 for antibiogram preparation
    - Combine data from several years?
    - Combine data from multiple geographically close facilities?
  - Provide accessibility to national surveillance data (NARMS)
Diarrhea

IDSA Guidelines

"Practice Guidelines for the Management of Infectious Diarrhea"

The widening array of recognized enteric pathogens and the increasing demand for cost-containment sharpen the need for careful clinical and public health guidelines based on the best evidence currently available. Adequate fluid and electrolyte replacement and maintenance are key to managing diarrheal illnesses. Thorough clinical and epidemiological evaluation must define the severity and type of illness, exposures, and whether the patient is immunocompromised, in order to direct the performance of selective diagnostic cultures, toxin testing, parasite studies, and the administration of antimicrobial therapy. Link to full text guideline

*Projected Publication, Fall 2014

http://www.idsociety.org
Salmonella spp.
Salmonella – Current Taxonomy

- Two major species
  - *Salmonella bongori* (uncommon in human infections)
  - *Salmonella enterica*
    - Six subspecies including *Salmonella enterica* subsp. *enterica*
- >2500 serovars

*Salmonella enterica* subsp. *enterica* serovar Typhi
*Salmonella enterica* serovar Typhi
*Salmonella* ser. Typhi
*Salmonella* Typhi or *S. Typhi*

**Typhoidal** Salmonella = *S. Typhi* and *S. Paratyphi* A-C

WHO Collaborating Centre for Reference and Research on Salmonella
Salmonella Infections

♦ **Typhoidal**
  – Require antimicrobial therapy from any source
  – Usually ceftriaxone or fluoroquinolones in adults

♦ **Non-typhoidal**
  – Systemic sources require antimicrobial therapy
  – Gastroenteritis
    • Usually self-limiting
    • Therapy **NOT** recommended due to prolongation of carrier state
    • Therapy indicated for:
      – Severe diarrhea
      – Patients with underlying medical conditions (e.g., immunosuppression)
Specimen: Stool
Diagnosis: Diarrhea
(35 yo otherwise healthy sales clerk)

*Salmonella* spp. (non-typhoidal)

Should we do more?
Salmonella spp.
AST and Reporting (1)

“(2) Susceptibility testing is indicated for typhoidal Salmonella (S. Typhi and Salmonella Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal Salmonella spp. isolated from intestinal sources.”

CLSI M100-S23. Table 2A.
Specimen: Stool
Diagnosis: Diarrhea
(35 yo otherwise healthy sales clerk)

*Salmonella* spp. (non-typhoidal)

“Gastroenteritis due to non-typhoidal *Salmonella* spp. is generally self-limiting in patients without underlying medical issues.”
Salmonella and Shigella spp.
AST and Reporting

“(2) When fecal isolates of Salmonella and Shigella spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of Salmonella spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested.”

Also reminder… WARNING: For Salmonella spp., first- and second-generation cephalosporins, cephemycins and aminoglycosides may appear active in vitro, but are not effective clinically and should not be reported as susceptible.

CLSI M100-S23. Table 2A.
Salmonella and Shigella spp. AST and Reporting

<table>
<thead>
<tr>
<th>Report</th>
<th>Do Not Report*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; or 2&lt;sup&gt;nd&lt;/sup&gt; generation cephalosporins</td>
</tr>
<tr>
<td>Ciprofloxacin (fluoroquinolone) not for children</td>
<td>Cephamycins</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>Ceftriaxone (extraintestinal)</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol (if requested)</td>
<td></td>
</tr>
</tbody>
</table>

* May test susceptible in vitro but not effective clinically

CLSI M100-S23. Table 2A.
Why do we need reliable ASTs for fluoroquinolones (FQs) and Salmonella

- *Salmonella* big global health concern
- Widespread resistance to ampicillin, chloramphenicol, TMP-SMX in many parts of world
  - WHO recommends FQ (oral) or ceftriaxone (parenteral) for uncomplicated typhoid fever
  - FQ usually = ciprofloxacin or ofloxacin
  - Azithromycin sometimes used (empirically)

Salmonella spp. Fluoroquinolones (FQs) Issue

- Clinical response rates to ciprofloxacin are poorer for isolates with “decreased ciprofloxacin susceptibility” (MICs of 0.12 – 0.5 µg/ml)
  

- MICs of 0.12 – 0.5 µg/ml are “S” with standard “Enterobacteriaceae” breakpoints

- CLSI M100-S23
  - Separate “lower” breakpoints for all Salmonella spp.
    (ciprofloxacin, levofloxacin, ofloxacin)
  - Nalidixic acid does not detect all mechanisms of fluoroquinolone resistance
### Salmonella spp. Fluoroquinolone Resistance

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ciprofloxacin MIC (µg/ml)</strong></td>
<td><strong>Nalidixic Acid</strong></td>
</tr>
<tr>
<td>Wild type (No resistance)</td>
<td>0.008-0.06</td>
</tr>
<tr>
<td>Chromosomal gyrA (single mutation)</td>
<td>0.12 - 2.0</td>
</tr>
<tr>
<td>Chromosomal gyrB (single mutation)</td>
<td>0.12 – 0.5</td>
</tr>
<tr>
<td>Chromosomal gyrA, gyrB (multiple mutations)</td>
<td>≥4.0</td>
</tr>
<tr>
<td>PMQR (e.g. qnr or aac(6’)-lb-cr)</td>
<td>0.12 - 2.0</td>
</tr>
</tbody>
</table>

PMQR, plasmid-mediated quinolone resistance - newer mechanism and less common than chromosomal gyrase mutations
### Salmonella spp. Fluoroquinolone AST and Reporting

<table>
<thead>
<tr>
<th>CLSI Standard</th>
<th>Fluoroquinolone Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>M100-S21 (2011)</td>
<td>One set of breakpoints for all Enterobacteriaceae including <em>Salmonella</em> spp. Nalidixic acid screen for reduced ciprofloxacin susceptibility in extraintestinal isolates of <em>Salmonella</em> spp. that are ciprofloxacin “S”</td>
</tr>
<tr>
<td>M100-S22 (2012)</td>
<td>Lower ciprofloxacin breakpoints for <em>S. Typhi</em> and extraintestinal <em>Salmonella</em> spp.</td>
</tr>
<tr>
<td>M100-S23 (2013)</td>
<td>Lower ciprofloxacin, levofloxacin and ofloxacin breakpoints for use with all <em>Salmonella</em> spp.</td>
</tr>
</tbody>
</table>

## Enterobacteriaceae

### FLUROQUINOLONES

NOTE: Reevaluation of fluoroquinolones is ongoing. See comment (2).

<table>
<thead>
<tr>
<th>Test/Report Group</th>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter Interpretive Criteria (nearest whole mm)</th>
<th>MIC Interpretive Criteria (µg/mL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>B</td>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>16-20</td>
<td>≤15</td>
<td>≤1</td>
</tr>
<tr>
<td>B</td>
<td>Levofloxacin</td>
<td>5 µg</td>
<td>14-16</td>
<td>≤13</td>
<td>≤1</td>
</tr>
<tr>
<td>B</td>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>21-30</td>
<td>≤20</td>
<td>≤0.06</td>
</tr>
<tr>
<td>B</td>
<td>Levofloxacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Ofloxacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Nalidixic acid

<table>
<thead>
<tr>
<th>Test/Report Group</th>
<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter Interpretive Criteria (nearest whole mm)</th>
<th>MIC Interpretive Criteria (µg/mL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>Nalidixic acid</td>
<td>30 µg</td>
<td>14-18</td>
<td>≤13</td>
<td>≤0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Salmonella* spp. and Fluoroquinolones

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CLSI M100-S23. Table 2A.
### Salmonella spp. - Nalidixic Acid Test

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>DD (mm)</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susc</td>
<td>Int</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>≥19</td>
<td>14-18</td>
</tr>
</tbody>
</table>

“(37) Until laboratories can implement the current interpretive criteria for ciprofloxacin, levofloxacin, and/or ofloxacin, nalidixic acid may be used to test for reduced fluoroquinolone susceptibility in Salmonella. Strains of Salmonella that test resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis.

Note that nalidixic acid may not detect all mechanisms of fluoroquinolone resistance.”

CLSI M100-S23. Table 2A.
Salmonella spp.
2011 Distribution of Ciprofloxacin MICs

- Wild Type
- Decreased Susc
- Res

% Isolates

MIC (mcg/ml)

- Typhi (n=383)
- Paratyphi A (n=146)
- non-typhoidal (n=2344)

CDC NARMS
## Salmonella spp. % Susceptible 2011

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>Non-typhoidal <em>Salmonella</em> spp. (n=2344)</th>
<th>S. Typhi (n=383)</th>
<th>S. Paratyphi A (n=146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≤8.0</td>
<td>90.8</td>
<td>88.8</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1.0</td>
<td>97.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td>99.7</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.06</td>
<td>97.0</td>
<td><strong>28.5</strong></td>
<td><strong>2.8</strong></td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>≤2/38</td>
<td>98.8</td>
<td>88.3</td>
<td>100</td>
</tr>
</tbody>
</table>

* CDC NARMS
Increasing Resistance or Partial Resistance to Ciprofloxacin in *Salmonella*, 1999-2011

Graph showing the percentage of resistant S. Typhi and non-typhoidal Salmonella strains from 1999 to 2011.

- **CDC NARMS**
- **S. Typhi**
- **non-typhoidal**
**Appendix B. Intrinsic Resistance**

Intrinsic resistance is defined as inherent or innate (not acquired) antimicrobial resistance, which is reflected in wild-type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that susceptibility testing is unnecessary. For example, *Citrobacter* species are intrinsically resistant to ampicillin.

These tables can be helpful in at least three ways: 1) they provide a way to evaluate the accuracy of testing methods; 2) they aid in the recognition of common phenotypes; and 3) they can assist with verification of cumulative antimicrobial susceptibility test data. In the tables, an "R" occurring with an organism-antimicrobial combination (1% to 3%) may appear susceptible due to method variation, mutation, or low levels of resistance.

A "susceptible" result should be viewed with caution. Ensure antimicrobial susceptibility test results and identification are accurate and reproducible. See Appendix A, footnote "a."

### B.1 Enterobacteriaceae

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Ampicillin</th>
<th>Amoxicillin-clavulanic acid</th>
<th>Ampicillin-subbactam</th>
<th>Ticarcillin</th>
<th>Ceftazidime: Cefotaxime</th>
<th>Cephalosporin II: Cefotaxim</th>
<th>Cephamycins: Ceftazidim, Cefotaxim</th>
<th>Cephalosporin II: Cefotaxim, Ceftriaxone</th>
<th>Imipenem</th>
<th>Tetraacyclines</th>
<th>Nitrofurantoin</th>
<th>Polymyxin B Collatin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Citrobacter freundii</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td></td>
</tr>
<tr>
<td><strong>Citrobacter koseri</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><strong>Enterobacter aerogenes</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><strong>Enterobacter cloacae</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>There is no intrinsic resistance to β-lactams in this organism.</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Escherichia hermannii</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hafnia alvei</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morganella morganii</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteus mirabilis</strong></td>
<td>There is no intrinsic resistance to penicillins and cephalosporins in this organism.</td>
<td></td>
<td></td>
<td>*</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteus penneri</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><strong>Proteus vulgaris</strong></td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Providencia rettgeri</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
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</tr>
<tr>
<td><strong>Salmonella and Shigella spp.</strong></td>
<td>There is no intrinsic resistance to β-lactams in these organisms; see Table 2A, comment (6) for reporting.</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Yersinia enterocolitica</strong></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td></td>
</tr>
</tbody>
</table>
Salmonella and Shigella spp.

“There is no intrinsic resistance to β-lactams in Salmonella / Shigella. See Table 2A comment 6 for reporting.”

(6) WARNING: For Salmonella spp., first- and second-generation cephalosporins, cephemycins and aminoglycosides may appear active in vitro, but are not effective clinically and should not be reported as susceptible.
Salmonella spp. - β-Lactams

♦ ESBLs (including CTX-M) and ampC (e.g., CMY) β-lactamases have been described in Salmonella spp.

♦ NARMS 2011
  - 58 of 2334 isolates had ceftriaxone MICs ≥4 µg/ml (R) and/or ceftiofur MICs ≥8 µg/ml
    • Ceftiofur used in veterinary medicine
  - 1 NDM producer and therefore a CRE (carbapenem-resistant Enterobacteriaceae)
Salmonella enterica ser. Senftenberg

NDM-1 – Positive

- 60 year old American previously hospitalized in India (cranial bleed)
- Isolated *K. pneumoniae* (sputum) and *Salmonella* (perirectal surveillance) both positive for *bla*<sub>NDM-1</sub>
  - Both isolates MHT and Etest metallo β-lactamase (MBL) positive
  - Plasmids had different restriction profiles
- *K. pneumoniae* – only S to colistin
- *Salmonella enterica*
  - Imipenem = MIC 4 µg/ml (R)
  - Isolate also had *bla<sub>TEM</sub>* and *bla<sub>CMY</sub>*
  - Only S to tetracycline, tigecycline, trimethoprim-sulfamethoxazole

**Specimen:** Stool  
**Diagnosis:** Diarrhea  
*Salmonella* ser. Newport

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ampicillin</td>
<td>&gt;16 R</td>
</tr>
<tr>
<td>ceftriaxone</td>
<td>32 R*</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>S</td>
</tr>
<tr>
<td>trimeth-sulfa</td>
<td>&gt;2/38 R</td>
</tr>
</tbody>
</table>

## Appenix A. Suggestions for Confirmation of Resistant (R), Intermediate (I), or Nonsusceptible (NS) Antimicrobial Susceptibility Test Results and Organism Identification

<table>
<thead>
<tr>
<th>Organism or Organism Group</th>
<th>Resistance Phenotype Detected&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Category I</th>
<th>Category II</th>
<th>Category III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Enterobacteriaceae</td>
<td>Carbapenem – I or R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Uncommon in most institutions</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin, gentamicin, and tobramycin – R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Extended-spectrum cephalosporin&lt;sup&gt;1&lt;/sup&gt; – I or R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella and Shigella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Confirm ID and susceptibility<sup>2</sup>. Report to infection control. Send to public health laboratory. Save isolate.

Note: May be appropriate to notify infection control of preliminary findings before confirmation of results.

Action Steps:

- Confirm ID and susceptibility if uncommon in your institution<sup>3</sup>.
- Check with infection control in your facility to determine if special reporting procedures or further action are needed.
- Check with your local public health department to determine which isolates should be reported to them and when isolates should be sent to the public health laboratory.

- Confirm ID and susceptibility if uncommon in your institution<sup>3</sup>.
- Check with infection control in your facility to determine if special reporting procedures or further action are needed.
Salmonella spp. - CLSI Appendix A
“Suggestions for Confirming Results”

If Salmonella spp. is:
- I or R to 3rd-generation cephalosporin and/or
- I or R to fluoroquinolone or R to nalidixic acid

Then
- Confirm ID/AST
- Inform Infection Control
- Discuss with local Public Health staff and send AST results to them with isolate

CLSI M100-S23. Appendix A.
When should we perform AST on *Salmonella* spp.?
- Extraintestinal isolates
- Typhoidal Salmonella from all sources
- Other when requested (select patient populations?)

*How can we test Salmonella spp. and fluoroquinolones?*
- Of commercial AST systems, only Etest currently encompasses new low MIC breakpoints for ciprofloxacin
- Ciprofloxacin disk diffusion
- Nalidixic acid doesn’t capture all isolates with reduced fluoroquinolone susceptibility
Shigella spp.
Shigella Infections

- **Systemic infections** require antimicrobial therapy
- **Gastroenteritis**
  - Mild infections usually self-limiting
  - **Antimicrobial therapy** may shorten duration of illness and decrease the spread of infection – treatment recommended
**Shigella spp.**
% Susceptible 2011 (n=293)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>% Susceptible*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≤8.0</td>
<td>65.5</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1.0</td>
<td>98.3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1.0</td>
<td>97.6</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>≤2/38</td>
<td>33.1</td>
</tr>
</tbody>
</table>
**Shigella spp. - β-Lactams**

- ESBLs (including CTX-M) and ampC (e.g., CMY) β-lactamases have been described in *Shigella* spp.
Azithromycin and *Salmonella/Shigella* (1)

- **Azithromycin** recommended or alternative “empiric” drug for *Salmonella*, *Shigella* and other types of gastroenteritis (off label)
  - American Academy of Pediatrics and the IDSA recommend for treatment of MDR *Shigella*

- Common GNR (e.g. *E. coli*, etc.) generally resistant to macrolides (outer membrane limits drug uptake)

- Unique activity of azithromycin vs. *Salmonella* / *Shigella* due to intracellular concentration in neutrophils and macrophages (>100 times the concentration in serum)

- Azithromycin has been shown to perform better than ceftriaxone or ciprofloxacin for typhoid fever
  
  Effa et al. 2008. Cochrane Database Syst. Rev. CD006083
Azithromycin and *Salmonella/Shigella* (2)

- No CLSI recommendations for azithromycin testing for GNR (other than *V. cholera*)…. but under investigation!
  - EUCAST - “Azithromycin has been used in the treatment of infections with *Salmonella* Typhi (MIC ≤16 mg/L for wild type isolates) and *Shigella* spp.” (EUCAST.org)
  - CDC notes wild type isolates have MICs ≤16 µg/ml

- Los Angeles County *S. sonnei* outbreak May 2012
  - “S” ampicillin, ciprofloxacin, ceftriaxone
  - “R” trimeth-sulfa, azithromycin (MIC >16 µg/ml)
  - First USA outbreak with high azithromycin MICs
**UCLA Salmonella - Azithromycin**  
Mixed bloodstream infection in pediatric patient

<table>
<thead>
<tr>
<th>Method</th>
<th>Azithromycin MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. paratyphi A</td>
</tr>
<tr>
<td>Broth microdilution</td>
<td>16</td>
</tr>
<tr>
<td>Etest inner zone</td>
<td>4</td>
</tr>
<tr>
<td>Etest outer zone</td>
<td>1</td>
</tr>
<tr>
<td>Etest 80% inhibition</td>
<td>2</td>
</tr>
</tbody>
</table>

Specimen: Stool
Diagnosis: Diarrhea
(5 yo otherwise healthy girl) – ciprofloxacin in appropriate
Shigella sonnei

**MIC (μg/ml)**

- ampicillin      >32 R
- cefixime        S
- ceftriaxone     ≤0.5 S
- trimeth-sulfa   >4/76 R

Option for testing - cefixime = oral extended-spectrum cephalosporin (oral administration)
### E. coli O157

**% Susceptible 2011 (n=132)**

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>% Susceptible*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≤8.0</td>
<td>96.3</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1.0</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1.0</td>
<td>99.4</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>≤2/38</td>
<td>97.5</td>
</tr>
</tbody>
</table>

Note: NO TREATMENT with antimicrobial agents or anti-motility drugs as may enhance toxin release and increase risk of HUS.
CLSI M45-A2 Guideline

“Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria”

M45-A3 In preparation
M45-A2 August 2010
## CLSI M45-A2 Guideline

<table>
<thead>
<tr>
<th>Potential Bacterial Agents of Bioterrorism</th>
<th>Disk Diffusion Method Described in Addition to MIC Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotrophia / Granulicatella</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>*Aeromonas / Plesiomonas</td>
<td>Leuconostoc</td>
</tr>
<tr>
<td>Bacillus spp. (not anthrax)</td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>*Campylobacter jejuni / coli</td>
<td>*Moraxella catarrhalis</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>*Pasteurella</td>
</tr>
<tr>
<td>Erysipelothrix</td>
<td>Pediococcus</td>
</tr>
<tr>
<td>HACEK Group</td>
<td>*Vibrio spp. (incl. cholera)</td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>Potential Bacterial Agents of Bioterrorism</td>
</tr>
</tbody>
</table>

* Disk diffusion method described in addition to MIC method
When should we test the CLSI M45-A2 organisms?

- **Only** on clinically significant isolates (e.g., sterile site isolates)
- **Only** if empiric therapy is not deemed appropriate
- **Only** with close communication with Infectious Diseases specialist so that the data will be understood and used appropriately
Campylobacter jejuni / coli
Campylobacter jejuni/coli Infection

♦ Systemic infections require antimicrobial therapy

♦ Gastroenteritis
  – Mild infections usually self-limiting
  – Antimicrobial therapy indicated for:
    • Prolonged and severe diarrhea
    • Patients with underlying medical conditions (e.g., immunosuppression)

♦ Antimicrobial therapy:
  – Erythromycin or ciprofloxacin
  – Doxycycline (alternative)
  – Note: C. fetus and C. lari are intrinsically resistant to quinolones
Campylobacter spp.
% Susceptible 2011
(N=1275 C. jejuni; 148 C. coli)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>%Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≤1.0</td>
<td>75.8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤8.0</td>
<td>98.2</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>≤2.0</td>
<td>98.2</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤4.0</td>
<td>54.8</td>
</tr>
</tbody>
</table>

CDC NARMS
Campylobacter jejuni/coli
AST Methods

CLSI Reference Methods

- Media & Incubation Conditions
  - MIC - CAMHB w/ 2-5% (v/v) lysed horse blood
  - Disk diffusion - MHA w/ 5% sheep blood
  - 36-37 °C for 48 hrs; 42 °C for 24 hrs (10% CO₂, 5% O₂, and 85% N₂)

- QC
  - MIC - C. jejuni ATCC 33560
  - Disk diffusion - S. aureus ATCC 25923

- Interpretive criteria available:
  - MIC – ciprofloxacin, erythromycin, doxycycline, tetracycline
  - Disk diffusion – ciprofloxacin, erythromycin (both for R only)

Etest – MHA w/ 5% sheep blood


Concerns

- Poor growth of some isolates; equivocal endpoints

CLSI M45-A2. Table 4.
Specimen: Stool
Diagnosis: Prolonged diarrhea

Campylobacter jejuni

Ciprofloxacin * (18 mm zone)
Erythromycin R (no zone)

“*Results for ciprofloxacin inconclusive; contact lab if additional testing needed.”

- Tested by disk diffusion
- No zone = R
- Any zone requires MIC
Can I use a commercial system for AST of an M45-A2 organism?

- Yes, but must verify and qualify results as appropriate
  - Most not FDA cleared for M45 organisms (e.g., commercial AST lysed horse blood MIC panels not FDA cleared for *Campylobacter* spp.)

Include comments on report as decided by your laboratory director, for off label use of FDA-cleared test; eg....

“This test was performed using reagents not approved or cleared by the U.S. Food and Drug Administration. The analytical performance characteristics of this test have been determined by XYZ Health System Clinical Laboratories. Results are for Research Use Only. This test should not be used for diagnostic purposes.”
Other
*Aeromonas* spp., *Plesiomonas* spp.
*Vibrio* spp. (including *V. cholera*)
*Yersinia* spp.
*Clostridium difficile*
**Aeromonas spp. / Plesiomonas shigelloides**

- **Antimicrobial therapy:**
  - Ciprofloxacin, trimethoprim-sulfa, 3\textsuperscript{rd} generation cephalosporin
- **AST Method – CLSI M45-A2**
  - Test like Enterobacteriaceae (*P. shigelloides* now grouped with Enterobacteriaceae)
- **All R to ampicillin; most are S to ciprofloxacin, 3\textsuperscript{rd}-generation cephalosporins**
  - One study (n=43) showed %S: ciprofloxacin 100%; cefotaxime 100%; trimethoprim-sulfa 80%
**Vibrio spp. including V. cholera**

 جميلة

**Antimicrobial therapy:**
- Tetracycline / doxycycline, azithromycin, ciprofloxacin

**AST Method – CLSI M45-A2**
- Test like Enterobacteriaceae; prepare inoculum in 0.85% NaCl (normal saline)
- Doxycycline and azithromycin – MIC breakpoints only

<table>
<thead>
<tr>
<th>MIC (µg/ml)</th>
<th>Susc</th>
<th>Int</th>
<th>Res</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin (V. cholerae only)</td>
<td>≤2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Azithromycin ref:
Saha et al. 2006. New Eng J Med. 354:2452
**Vibrio spp. other than V. cholera**

% Susceptible 2011 (N=400)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>%Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≤8.0</td>
<td>35.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1.0</td>
<td>100</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>≤2/38</td>
<td>99.7</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤4.0</td>
<td>99.7</td>
</tr>
</tbody>
</table>

*V. parahaemolyticus* (201), *V. alginolyticus* (103), *V. vulnificus* (n=63), *V. fluvialis* (n=18), Other (n=15)

**CDC NARMS**
Yersinia enterocolitica

- **Antimicrobial therapy:**
  - Ciprofloxacin, trimethoprim-sulfa, 3rd generation cephalosporin

- **AST Method** – CLSI M100-S23 method for Enterobacteriaceae

- **Intrinsic resistance:**
  - Ampicillin, amoxicillin, ticarcillin, cefazolin/cephalothin

- One study (n=80) showed %S: ciprofloxacin 100%; trimethoprim-sulfa 100%
  
Although resistance to the antibiotics used to treat *C. difficile* infections is not yet a problem, the bacteria spreads rapidly because it is naturally resistant to many drugs used to treat other infections.
Clostridium difficile (1)

- **Antimicrobial therapy:** vancomycin, metronidazole, fidaxomicin
- **AST Method CLSI M11-A8:**
  - Anaerobic agar dilution – Brucella agar with Vitamin K and hemin
  - CLSI Investigating broth microdilution
  - Etest
Clostridium difficile (2)

No need to test (generally research only):
- Resistance to metronidazole, vancomycin or fidaxomicin not identified (or very rare) in USA
- Current thinking - failures / recurrences due to host factors not drug resistance
- Difficult to define “R”; what breakpoint?
  - Fecal concentrations of vancomycin and fidaxomicin extremely high and much higher than the organisms’ MICs
  - Fecal concentrations of metronidazole close to MICs of organism
- AST results may be misleading

Suggestion (JH) of AST strategy for isolates causing gastroenteritis among patients without underlying medical problems....

<table>
<thead>
<tr>
<th>Organism</th>
<th>Report AST on fecal isolates?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhoidal Salmonella spp.</td>
<td>Always</td>
</tr>
<tr>
<td>Non-typhoidal Salmonella spp.</td>
<td>On request</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>Yes</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>On request</td>
</tr>
<tr>
<td>Aeromonas spp., Plesiomonas shigelloides</td>
<td>Yes</td>
</tr>
<tr>
<td>Vibrio spp. (not V. cholerae)</td>
<td>On request</td>
</tr>
<tr>
<td>Yersinia spp.</td>
<td>On request</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>On request (with Infectious Diseases input)</td>
</tr>
</tbody>
</table>
Diarrhea

Overview | Common Causes | Tests | Prevention | Treatment | Related Pages

Common Causes

There are many infectious and non-infectious causes of acute and chronic diarrhea. Viral, bacterial, and parasitic infections are associated with diarrhea that lasts several days to a few weeks, although some cases may linger — causing chronic diarrhea in those with suppressed immune systems (such as those who have HIV/AIDS, cancer, or organ transplants). These sources of diarrhea are infectious, with the virus, bacteria, or parasite shed into the stool and passed from person to person through oral contact with a contaminated surface. Eating food or drinking water that has been contaminated is the most frequent route of infection.

Once someone is infected, the person may pass it on to others around them unless careful sanitation practices (especially thorough handwashing) are followed. This is especially a challenge in households with infected infants, in daycare centers, and in nursing homes. Sometimes an outbreak of bacterial or parasitic infection can be traced back to a particular restaurant or a single food item at a picnic. Sometimes it may be due to a contaminated water source.
CDC NARMS report contains valuable antimicrobial susceptibility test data for enteric bacteria.

Routine AST is not indicated for nontyphoidal *Salmonella* spp. isolated from gastrointestinal sources.

Drugs recommended for routine reporting for *Salmonella* (if indicated) and *Shigella* include ampicillin, ciprofloxacin and trimethoprim-sulfamethoxazole… and a 3rd gen cephalosporin for extraintestinal source isolates.

Neither *Salmonella* nor *Shigella* spp. have intrinsic resistance to agents commonly used for treatment of infections due to GNR.

The new fluoroquinolone breakpoints should be used for all *Salmonella* spp.
Fluoroquinolone resistance in *Salmonella* spp. is a much greater concern for typhoidal strains vs. non-typhoidal strains.

Azithromycin may be given empirically for *Salmonella* and *Shigella* gastroenteritis but there are no standard AST testing methods for azithromycin and GNRs (other than *V. cholerae*).

Extended-spectrum β-lactam resistance is uncommon but has been reported in *Salmonella* and *Shigella* spp. (including a non-typhoidal *Salmonella* strain with *bla<sub>NDM-1</sub>*)

CLSI M45-A2 describes AST methods for *Aeromonas/Plesiomonas, Campylobacter jejuni/coli*, and *Vibrio* spp.

If AST for *Campylobacter jejuni/coli* is necessary, MIC testing is best.
Currently, disk diffusion testing of ciprofloxacin and erythromycin for *Campylobacter jejuni/coli* can only detect resistance (not susceptibility).

AST testing of *C. difficile* is rarely indicated for patient care and results might be misleading.

Treatment failures/recurrences of *C. difficile* infection are believed to be due to host factors and not drug resistance.

Infections due to many gastrointestinal pathogens are self-limiting in otherwise healthy individuals.
Thank you!