Salmonella and Fluoroquinolones: Where are we now?

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Disclosures
Nothing to Disclose

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 subspecies enterica serovar Typhi
Salmonella enterica serovar Typhimurium
Salmonella enterica serovar 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
(29 yo otherwise healthy lab tech)

Salmonella spp. (non-typhoidal)

Should we do more?

Salmonella spp.
AST and Reporting

“(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-S24: Table 2A.
Specimen: Stool  
Diagnosis: Diarrhea  
(29 yo otherwise healthy lab tech)  
Final Report with Optional Comment

Salmonella spp. (non-typhoidal)

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

Salmonella spp. AST and Reporting

Table 2A

<table>
<thead>
<tr>
<th>Report</th>
<th>Do Not Report*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>1st or 2nd generation cephalosporins</td>
</tr>
<tr>
<td>Ciprofloxacin (fluoroquinolone)</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-S24, 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 or levofloxacin


Global Incidence - Typhoid Fever


Table 1. Treatment of uncomplicated typhoid fever

<table>
<thead>
<tr>
<th>Optimal Dosage</th>
<th>Antibiotic</th>
<th>Daily dose</th>
<th>Days</th>
<th>Optimal Dosage</th>
<th>Antibiotic</th>
<th>Daily dose</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolone</td>
<td>Ciprofloxacin</td>
<td>150 mg</td>
<td>5-7</td>
<td>Chloramphenicol</td>
<td>75-100</td>
<td>5-7</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>400 mg</td>
<td>2-3 per day</td>
<td>14-21</td>
<td>Ceftriaxone</td>
<td>1500</td>
<td>14-21</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1500</td>
<td>2-3 times per day</td>
<td>14-21</td>
<td>Azithromycin</td>
<td>500</td>
<td>7-10</td>
<td></td>
</tr>
</tbody>
</table>

* Three-day courses are also effective and are particularly so in epidemic situations.
* Headache or myalgias following oral parenteral fluoroquinolone therapy have been documented. All treatments, including the third generation cephalosporin, are a 5-14 day course of high dose fluoroquinolones, in effective concentrations of those seen being evaluated.

**Salmonella spp. Fluoroquinolones (FQs) Issue**

- Typhoid fever
- High morbidity/mortality if untreated or untreatable
- FQs good (inexpensive, PO route) for treatment of salmonellosis, including typhoid fever
- Emerging resistance!
- Clinical response rates to ciprofloxacin are poorer for isolates with "decreased ciprofloxacin susceptibility" (MICs of 0.12 – 1.0 µg/ml)
- Longer infection clearance times
- Relapses
- Need to test!!
- Optimal method for detection of FQ resistance not defined for *S. enterica*
- Nalidixic acid does not detect all mechanisms of fluoroquinolone resistance
- Need easy test (disk diffusion) to differentiate isolates that are "S" vs. "not S"!

**The Scoop on FQs**

- Nalidixic Acid first quinolone (1960s)
  - By product of chloroquine
  - Narrow spectrum of activity – Gram negatives in UTIs
- Fluorine atom added to quinolone molecule (1980s)
  - Fully synthetic, no pre-existing resistance genes to this class in nature
  - Next generation late 80’s, early 90’s (ciprofloxacin, ofloxacin, norfloxacin, enoxacin)
    - More readily absorbed, ↑ activity to Gram (-)
  - Newer FQs late 90’s (levofloxacin, gatifloxacin, moxifloxacin)
    - Broad spectrum with enhanced activity to many Gram (-) and Gram (+)
- Q and FQ resistance common, widespread
  - Resistance rising independently, numerous times (15 independent gyrA mutations in last decade) in fully susceptible orgs, maintained through selective pressure
  - Fitness benefit

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

**Resistance to FQs in Salmonella**

- Chromosomal mutations
  - Altered FQ binding site
  - Quinolone target protection
  - DNA gyrase, DNA gyrase, Topoisomerase IV
- Outer membrane impermeability
- Overexpression of efflux pump systems
  - AcrAB-ToIC

**FQ Resistance Mechanisms in Salmonella?**

- Several FQ resistance mechanisms described for Enterobacteriaceae
- Late 1990’s, plasmid-mediated mechanisms identified
  - Quinolone target protection
    - qnrA, qnrB, qnrS, qnrC, qnrD
  - Correlation with ESBLS?
  - Modification of quinolones by acetyltansferase
    - Aac(6’)-Ib-cr
  - Active efflux pumps
    - QepA, QpxAB

**Fluoroquinolone Resistance Mechanism Genotypes/Phenotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ciprofloxacin MIC (µg/ml)</th>
<th>Nalidixic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (No resistance)</td>
<td>0.008-0.06</td>
<td>Usually susceptible</td>
</tr>
<tr>
<td>Chromosomal gyrA (single mutation)</td>
<td>0.12 - 2.0</td>
<td>Usually resistant</td>
</tr>
<tr>
<td>Chromosomal gyrB (single mutation)</td>
<td>0.12 – 0.5</td>
<td>Usually susceptible</td>
</tr>
<tr>
<td>Chromosomal gyrA, gyrB (multiple mutations)</td>
<td>≥4.0</td>
<td>Resistant</td>
</tr>
<tr>
<td>PMQR (e.g. qnr or aac(6’)-Ib-cr)</td>
<td>0.12 - 2.0</td>
<td>Often susceptible</td>
</tr>
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PMQR, plasmid-mediated quinolone resistance - newer mechanism and less common than chromosomal gyrase mutations
Detection of FQ Resistance in Salmonella - CLSI M100 Recommendations

- Several revisions over past 3 years
- Changes driven by:
  - Recognition of several emerging FQ resistance mechanisms not detected by traditional methods
  - Reports documenting clinical therapeutic failure in patients infected with low-level FQ resistant isolates
  - Re-evaluation of FQ pharmacokinetics and pharmacodynamics

Salmonella spp. Fluoroquinolone AST and Reporting History

<table>
<thead>
<tr>
<th>CLSI Standard</th>
<th>Fluoroquinolone Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>M100-S14 (2004)</td>
<td>Screen extra-intestinal Salmonella with ciprofloxacin MIC ≤ 1 µg/mL for nalidixic acid resistance as predictor of decreased ciprofloxacin susceptibility</td>
</tr>
<tr>
<td>M100-S21 (2011)</td>
<td>One set of breakpoints for all Enterobacteriaceae including Salmonella spp. Reliability of Nalidixic acid screen for reduced ciprofloxacin susceptibility in extraintestinal isolates of Salmonella spp. questioned</td>
</tr>
<tr>
<td>M100-S22 (2012)</td>
<td>Lower ciprofloxacin breakpoints for S. Typhi and extraintestinal Salmonella spp.</td>
</tr>
<tr>
<td>M100-S23 (2013)</td>
<td>Lower ciprofloxacin, levofloxacin and ofloxacin breakpoints for use with all Salmonella spp. Establish ciprofloxacin disk breakpoints</td>
</tr>
<tr>
<td>M100-S24 (2014)</td>
<td>No changes</td>
</tr>
</tbody>
</table>

Fluoroquinolone Resistance Mechanism Genotypes/Phenotypes

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<td>Usually resistant</td>
</tr>
<tr>
<td>Chromosomal gyrB (single mutation)</td>
<td>0.12 - 0.5</td>
<td>Usually susceptible (2-16 µg/mL)</td>
</tr>
<tr>
<td>Chromosomal gyrA, gyrB (multiple mutations)</td>
<td>≥4.0</td>
<td>Resistant</td>
</tr>
<tr>
<td>PMQR (e.g. qnr or aac(6')-lb-cr)</td>
<td>0.12 - 2.0</td>
<td>Often susceptible (8-32 µg/mL)</td>
</tr>
</tbody>
</table>

PMQR, plasmid-mediated quinolone resistance - newer mechanism and less common than chromosomal gyrase mutations

What Criteria are Used to Determine if AST Results are Acceptable?

<table>
<thead>
<tr>
<th>Error Type</th>
<th>Results</th>
<th>Acceptable Error Rate</th>
<th>(cont’d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF Method</td>
<td>AST Validating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Major (VME)</td>
<td>R S</td>
<td>≤3% (min 35 R isolates)</td>
<td></td>
</tr>
<tr>
<td>Major (ME)</td>
<td>S</td>
<td>≤5%</td>
<td></td>
</tr>
<tr>
<td>Minor (mE)</td>
<td>S</td>
<td>Major + Minor ≤10%</td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>≥90%</td>
<td>Major + Minor ≤7%</td>
<td></td>
</tr>
</tbody>
</table>

Ciprofloxacin Reference MIC: Search for Surrogate FQ Disk

- Levofloxacin
- Nalidixic acid
- Ofloxacin
- Enoxacin
- Norfloxacin
- Pefloxacin
Calculating % Errors

**Very Major Error (VME)** = \( \frac{\# \text{ with VME (false R)}}{\text{Total #} \text{ "R" isolates tested}} \) \times 100

**Major Error (ME)** = \( \frac{\# \text{ with ME (false S)}}{\text{Total #} \text{ "S" isolates tested}} \) \times 100

**Minor Error (mE)** = \( \frac{\# \text{ with mE}}{\text{Total # isolates tested}} \) \times 100

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**How Many Organisms?**

- Not specified by CLIA
- Minimum of 30

**Selection Criteria**
- Represent clinical isolates tested
- Variety of susceptibility profiles
- Some around breakpoints

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**Surrogate Disk for FQ Resistance in Salmonella spp.**

- **Our definition** – A disk and zone cutoff that most reliably identifies Salmonella isolates that are not susceptible to FQs based on current ciprofloxacin susceptible or not susceptible (I + R) MIC breakpoints
  - No data to indicate monotherapy efficacious against “I” isolates
  - Assumption – ciprofloxacin MIC (using CLSI reference method) accurately differentiates FQ susceptible from FQ not susceptible isolates

- **Errors calculated:**
  - VME = total false S/total not susceptible
  - ME = total false not susceptible/total susceptible

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**Performance of Disk Diffusion (DD) and Etest for Detection of FQ-R Salmonella enterica (n=136)**

<table>
<thead>
<tr>
<th>No. of isolates (% typhoidal)</th>
<th>“R” Mechanism</th>
<th>BMD MIC range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (0)</td>
<td>aac(6’)-Ib-cr</td>
<td>1.0 32</td>
</tr>
<tr>
<td>36 (0)</td>
<td>gmr</td>
<td>0.12 - 1.0 4.0 – 32</td>
</tr>
<tr>
<td>45 (0)</td>
<td>QRDR mutation</td>
<td>0.06 - 0.5 &gt;128</td>
</tr>
<tr>
<td>29 (90)</td>
<td>Not characterized</td>
<td>0.12 – 16 128 -&gt;128</td>
</tr>
<tr>
<td>24 (25)</td>
<td>None</td>
<td>≤0.08 – 0.06 2 – 16</td>
</tr>
</tbody>
</table>

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**Distribution of Ciprofloxacin MICs (n=136)**

- R mechanism known
- R mechanism unknown

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**Ciprofloxacin MIC vs Ciprofloxacin DD (n=136)**

[Graph showing distribution of MIC values and comparison between DD and Etest results]
Ciprofloxacin MIC vs Nalidixic Acid DD (n=136)

Ciprofloxacin MIC vs Levofloxacin DD (n=136)

How well do disks identify isolates that are “S” vs. “not susceptible” to FQs?

Surrogate Agent and proposed susceptible breakpoint | VME | % (N) | MIC | % (N) | CA (%) |
--- | --- | --- | --- | --- | --- |
Ciprofloxacin ≤ 30 mm | 0.0 | 8.0 (2) | 98.5 |
Levofloxacin ≤ 27 mm | 1.0 (1) | 4.0 (1) | 98.5 |
Norfloxacin ≤ 24 mm | 0.0 | 4.0 (1) | 98.3 |
Nalidixic Acid ≤ 18 mm | 1.8 (2) | 20.0 (5) | 94.9 |
Enoxacin ≤ 17 mm | 53.2 (99) | 0.0 | 56.6 |
≤ 23 mm | 0.0 | 4.0 (1) | 90.3 |
Norfloxacin ≤ 16 mm | 91.0 (101) | 0.0 | 98.8 |
≤ 27 mm | 1.0 (1) | 8.0 (2) | 97.8 |
Pefloxacin ≤ 23 mm | 0.0 | 12.0 (3) | 98.9 |

DD generally accepted as precise to only ≤ 3 mm

Resistant zone sizes Susceptible zone sizes

Pefloxacin 5 μg Zones

1 BBL MHA II (single lot); Oxoid disks (single lot); 18 h incubation
20/47 ciprofloxacin non-susceptible isolates appeared similar
Now how can we detect Salmonella that are not susceptible to FQs?

What is pefloxacin?

- FQ introduced in early 1980s
- Used for uncomplicated gonorrhoeae, UTIs, gastroenteritis, typhoid fever
- Studies in Europe suggested pefloxacin disk superior in differentiating FQ “S” vs “not susceptible” isolates
- Neither pefloxacin drug nor disk available in USA
- CLSI added pefloxacin to address “global needs”

Current Salmonella FQ Breakpoints

Surrogate Disks Compared to Levofloxacin MICs for S. enterica (113 not-susceptible isolates)

- Levofloxacin commonly used in US hospitals as FQ
- No Salmonella levofloxacin disk breakpoints formally established
Salmonella spp. (N=135)
Etest vs. Ref MIC

<table>
<thead>
<tr>
<th>Agent</th>
<th>Performance (N(%))</th>
<th>EA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>131 (97.0)</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>125 (92.6)</td>
<td>135</td>
<td>135</td>
</tr>
</tbody>
</table>

EA, essential agreement (within +/- 1 dilution)
CA, categoric agreement (same “S” or “not S” result)


Testing for FQ Resistance in the US

- Pefloxacin disks not available in US
- No surrogate agent will detect all FQ resistance mechanisms
- $\text{aac-6}\text{-}\text{Ib-cr}$ alone will not test “R” to pefloxacin
- $\text{S. enterica}$ ser. Typhi with ORDR mutation testing “S” to ciprofloxacin by BMD
- Nalidixic acid did not detect qnr mutations in 5.9% of isolates tested
- MIC test for ciprofloxacin or levofloxacin
- Revised ciprofloxacin breakpoints by CLSI and by FDA (S. Typhi only)
- No commercial MIC susceptibility test panels with low enough drug concentrations
- Etests compared well to BMD
- Not FDA approved with current breakpoints
- Need validation study
- Is it worth it?

Ciprofloxacin disks

- No issues with reading in developed labs
- Suitable alternative
- Surrogate agent for levofloxacin susceptibility (susceptible vs not-susceptible)

Salmonella spp. - Nalidixic Acid Test

* “(39) Until laboratories can implement the current interpretive criteria for ciprofloxacin, levofloxacin, and norfloxacin, 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-S24, Table 2A.

Drug Alternatives to FQs for Salmonella

<table>
<thead>
<tr>
<th>Susceptibility</th>
<th>Optimal therapy</th>
<th>Alternative effective drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully sensitive</td>
<td>Fluoroquinolones</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>MDR resistant</td>
<td>Ceftriaxone</td>
<td>Ceftriaxone</td>
</tr>
</tbody>
</table>


Major Changes 2015

M100-S25

- Enterobacteriaceae
  - Salmonella
    - Added pefloxacin disk diffusion test to differentiate isolates “S” vs. “not susceptible” to fluoroquinolones
    - Added azithromycin disk diffusion and MIC test breakpoints for S. Typhi

Drug Alternatives to FQs for Salmonella cont’d

- No data on favorable high dose CIP monotherapy outcomes
- Ceftriaxone may be favorable
- Empiric therapy with ceftriaxone
  - ESBLs and plasmid mediated cephalosporinases reported worldwide
  - 2010 – 2.8% nontyphoidal Salmonella ceftriaxone “R” in CDC National Antimicrobial Resistance Monitoring System report
  - 2006-2007 Indian report – 6% “R” to ceftriaxone
    - Confirm susceptibility
    - Empiric therapy with azithromycin

**Salmonella spp. % Susceptible USA 2012**

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoint (µg/ml)</th>
<th>Non-typhoidal Salmonella spp. (n=2236)</th>
<th>S. Typhi (n=326)</th>
<th>S. Paratyphi A (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≤8.0</td>
<td>91.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1.0</td>
<td>97.2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.06</td>
<td>96.4</td>
<td>31.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Trimeth-sulfa</td>
<td>≤2/38</td>
<td>98.6</td>
<td>89.8</td>
<td>100</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>≤16.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*National Antimicrobial Resistance Monitoring System (NARMS) http://www.cdc.gov/narms/

**Why azithromycin for Salmonella Typhi?**

- Management of enteric fever generally includes antimicrobial agents
- Azithromycin distribution in vivo
  - Low serum concentration
  - Concentrates in PMNs, monocytes, lymphocytes, alveolar macrophages; achieves high intracellular concentrations (= 60-200 times > serum concentration)
  - Azithromycin MICs lower than intracellular concentration
- *Salmonella Typhi* is an intracellular pathogen
- Successfully used for many years; very few clinical failures
  - Found to perform significantly better than ceftriaxone


**Salmonella Typhi Azithromycin Breakpoints**

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Content (µg)</th>
<th>DD (mm)</th>
<th>MIC (µg/ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>15</td>
<td>≥13</td>
<td>≤12</td>
<td>≤16</td>
</tr>
</tbody>
</table>

EUCAST:
"Azithromycin has been used in the treatment of infections with *Salmonella typhi* (MIC ≤16 mg/L for wild type isolates) and *Shigella* spp." eucastr.org

*No dosing regimen
*FDA breakpoints needed
*Breakpoints based on epidemiological cutoff in vitro activity

**Salmonella USA, 2012 Distribution of Azithromycin MICs**

**Clinical Findings**

- EUCAST: "Azithromycin has been used in the treatment of infections with *Salmonella typhi* (MIC ≤16 mg/L for wild type isolates) and *Shigella* spp." eucastr.org
- *No dosing regimen
*FDA breakpoints needed
*Breakpoints based on epidemiological cutoff in vitro activity

**Salmonella Typhi Azithromycin**

Often observe “double” zones on Etest and DD... read inner zone

Galas et al. CLSI Agenda Book June 2014
When should we test Salmonella spp.?

What drugs?
- Extraintestinal isolates
  - Typhoidal Salmonella from all sources
  - Other when requested (select patient populations?)
  - Ampicillin, a fluoroquinolone, trimethoprim-sulfamethoxazole + 3rd generation cephalosporin for extraintestinal isolates
- Typhoidal Salmonella from all sources
- Other when requested (select patient populations?)
- Ampicillin, a fluoroquinolone, trimethoprim-sulfamethoxazole + 3rd generation cephalosporin for extraintestinal isolates

How can we test fluoroquinolones?
- Of commercial AST systems, only Etest currently encompasses new low MIC breakpoints for ciprofloxacin (not FDA cleared with Salmonella breakpoints)
- Ciprofloxacin disk diffusion, Nalidixic Acid screen
- Pefloxacin for global needs

Should we test azithromycin? If yes, how?
- On request only
- Disk diffusion and MIC breakpoints for S. Typhi only

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