Identification of Multi-Drug Non-Susceptible Gonorrhea in Massachusetts
A Public Health Laboratory Perspective

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Bureau of Infectious Disease and Laboratory Sciences
Massachusetts Department of Public Health
Overview

Gonorrhea Introduction
  • Disease & Public Health Importance

MA Sexually Transmitted Disease (STD) Program
  • Focus on Gonorrhea

Gonorrhea Testing
  • MA Clinical Microbiology Laboratory Test Algorithm

Index Case
  • MA results → Collaborator results
  • Interpretation

Emergent Situation
  • Response
  • Routine surveillance

Partnerships
  • Internal/External
Gonorrhea Overview

Sexually Transmitted Infection (STI) caused by bacterium *Neisseria gonorrhoeae* (also referred to as GC or gonococcus)

- Urethra, rectum, throat

Public Health Importance:

- Infected are often asymptomatic = not seeking treatment
- Untreated infection can lead to serious health problems:
  - Increased pregnancy complications
  - Infertility
  - Unknowing transmission to others > accelerated spread of infection > increased risk of eventual treatment failure
  - Disseminated gonococcal infection
- Currently there is *one* recommended treatment option remaining
  - Ceftriaxone 500mg IM
## MA STD Program

### Prevention Mission
- Detect/respond to STI in MA
- Reduce morbidity
- Address barriers to health using health equity lens

### Regulatory Requirements
- Reporting of all GC infections
- Submission of GC isolates to MA SPHL for surveillance testing

### Outreach
- Partner with community health centers providing specific care for at-risk groups
- Provide laboratory support to the clinics
- Specific care

### Improve Local/State Capacity to Respond to Antimicrobial Resistant GC (ARGC)
## GC Testing Overview

### Nucleic Acid Amplification Test (NAAT)
- **Pros**
  - Sensitive and specific
  - Ease of handling samples
  - Fast turn around time (TAT)
- **Cons**
  - Detects genetic material, does not indicate an active infection
  - Does not yield a viable organism for further testing such as antimicrobial susceptibility testing (AST)

### Culture
- **Pros**
  - Positive cultures yield a viable isolate available for downstream testing and characterization
- **Cons**
  - Transport: sensitive to the cold
  - Special growth conditions: humidity and increased CO₂
MA SPHL Clinical Microbiology Laboratory (CM LAB) Test Algorithm Overview

Specimen Receipt → Culture → Identification → Antimicrobial Susceptibility Testing
Sample Receipt

MA SPHL Surveillance Testing

ISOLATES
- Hospitals
- Reference Labs
- Local Micro Labs

CULTURES
- Partner Clinical Sites
MA SPHL
CM Lab
Test Algorithm

Specimen Receipt
- Isolates
- Primary cultures/collections

Culture
- Growth
  - CHOC Agar
  - MTM Agar
  - Note: BAP or TSA
    - Not optimal for GC, not used in test protocol
- Environment
  - Humidity
  - CO2
Identification

- Bench Tests
  - Gram Stain
  - Oxidase
- Definitive ID
  - Primary: Bruker Maldi-ToF
    - Mass Spectometry
  - Back-up: Remel RapID NH
    - Biochemical
MA SPHL
CM Lab
Test Algorithm

AST

• Etest AST method
  • Inoculate Remel GC Agar Base with 0.5 MacFarland dilution of test organism
  • Apply E strip > incubate 20-24 hours > read
  • Generates a quantitative value, a minimum inhibitory concentration (MIC)
• Antimicrobials: bioMerieux Etest strips
  • Ciprofloxacin
  • Ceftriaxone
  • Spectinomycin
  • Azithromycin
  • Cefoxitin
  • Cefixime
Interpretive Categories and MIC Breakpoints ug/mL (CLSI M100 32nd Ed.)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>= 1</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Cefixime</td>
<td>= 0.25</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>= 2</td>
<td>4</td>
<td>= 8</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>= 0.25</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>= 0.06</td>
<td>0.12-0.5</td>
<td>= 1</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>= 32</td>
<td>64</td>
<td>= 128</td>
</tr>
</tbody>
</table>
Specimen Receipt

- GC isolate: 2 distinct morphologies received for surveillance testing
- Original source was urine
  - More commonly isolated from urethra, rectum, throat
- Isolated on a bi-plate
  - GC was growing confluently on the blood agar

Index Case
Index Case

Culture
- 2 unique morphologies
- Submitted isolates were pure

Identification
- Gram Stain: GNDC
- Oxidase: Positive
- Both identified by Maldi-ToF as *Neisseria gonorrhoeae*
Index Case

- AST Etest performed

<table>
<thead>
<tr>
<th>Antimicrobial Etest Strip</th>
<th>Isolate #1 MIC (ug/mL)</th>
<th>Isolate #2 MIC (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Cefixime</td>
<td>1.5*</td>
<td>1.5*</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>0.5*</td>
<td>0.38*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

*Observation of Cefixime or Ceftriazone MIC > 0.25 ug/mL is rare and requires notification of Supervisor and repeat testing to confirm.
Index Case

**AST Repeat Etest Performed**

<table>
<thead>
<tr>
<th>Antimicrobial Etest Strip</th>
<th>Isolate #1 MIC (ug/mL)</th>
<th>Isolate #1 Repeat MIC (ug/mL)</th>
<th>Isolate #2 MIC (ug/mL)</th>
<th>Isolate #2 Repeat MIC (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Cefixime</td>
<td>1.5*</td>
<td>1.5*</td>
<td>1.5*</td>
<td>1.5*</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2</td>
<td>2</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.5*</td>
<td>0.5*</td>
<td>0.38*</td>
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<td>Ciprofloxacin</td>
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<td>Spectinomycin</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

*Remember: Observation of Cefixime or Ceftriaxone MIC > 0.25 ug/mL is rare*
Index Case

AST Interpretation: *N. gonorrhoeae*

<table>
<thead>
<tr>
<th>Antimicrobial Etest Strip</th>
<th>Isolate #1 MIC (ug/mL)</th>
<th>Interpretive Category</th>
<th>Isolate #2 MIC (ug/mL)</th>
<th>Interpretative Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>3</td>
<td>NS</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Cefixime</td>
<td>1.5*</td>
<td>NS</td>
<td>1.5*</td>
<td>NS</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>2</td>
<td>S</td>
<td>1.0</td>
<td>S</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.5*</td>
<td>NS</td>
<td>0.38*</td>
<td>NS</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>32</td>
<td>R</td>
<td>6</td>
<td>R</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>16</td>
<td>S</td>
<td>12</td>
<td>S</td>
</tr>
</tbody>
</table>

S=Susceptible, NS=Non-susceptible, I=Intermediate, R=Resistant

Remember: “Non-Susceptible” is a category used for isolates where only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains.
Collaboration with Centers for Disease Control (CDC)

CDC STD Laboratory and Research Branch

Confirmatory Testing

- Culture
- ID
- AST: 2 methods performed
  - Etest
  - Agar Dilution
    - Gold standard in AST protocols
### CDC Confirmatory Testing

**ID**
- Confirmed as *Neisseria gonorrhoeae* (Maldi-ToF)

**AST Etest and Agar Dilution**
- Confirmed MA SPHL results
  - Non-susceptible to azithromycin, ceftriaxone and cefixime
  - Resistant to ciprofloxacin
  - Additional antimicrobial testing
    - Also resistant to penicillin and tetracycline
AST Summary

*First Case of Multi-Drug Non-Susceptible GC
Isolated in United States

<table>
<thead>
<tr>
<th></th>
<th>Resistant</th>
<th>Non-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Cefixime</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Genetic Analysis Method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Downstream Research Use Only (RUO) Genetic Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Real-time PCR to Detect AMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanger Sequencing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Genome Sequencing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis identified penA-60.001 allele associated with ceftriaxone NS and also MLST 8123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia-Pacific lineage</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Monitoring Emerging Pathogen

**Expand Surveillance**
- Additional enhanced surveillance identified a second isolate
- Molecular testing of NG-positive remnant diagnostic specimens
- Analysis supports this organism likely has similar reduced susceptibility to cefixime and ceftriaxone
- Expanded surveillance at health center
- No additional cases of concern

**MA Clinical Alert Sent Out 1/19/2023**
- In direct response to identification of 2nd case
- Recommending *culture* on symptomatic patients in addition to routine NAAT testing
- Old school culture will enhance surveillance efforts to give us a better picture of antimicrobial susceptibility
Routine Surveillance

Framework of mandated reporting and the additional mandated submission of isolates in MA was critical to identifying this strain.

Routine AST on all GC

Supports goal of improving local/state capacity to respond to ARGC
Partners: Importance of Relationships

Internal Partners
Lab/EPI
- Routine outreach
- Outbreak response

Community Partners
Submission of culture/isolates

National Partners
CDC
- Confirmatory downstream testing
- Collaborate on outbreak response
APHL
- Public forum to share knowledge and discuss important public health issues
ARGC is here.

It is our relationships and partnerships that most support response to emerging pathogens.

For PHL teams: your work has real world impact in real time!
Connect with DPH

@MassDPH

Massachusetts Department of Public Health

mass.gov/dph