Susceptibility Testing of *Neisseria* and *Haemophilus influenzae* and *parainfluenzae*
Types of Infection

*Haemophilus influenzae*

- Invasive
  - Meningitis, epiglottitis and bacteremia (subacute endocarditis)
    - Typically caused by type B (HIB)
- Otitis media, acute conjunctivitis, acute sinusitis, bronchitis and pneumonia.

*Haemophilus parainfluenzae*

- Otitis media, acute conjunctivitis, acute sinusitis, bronchitis and pneumonia
- Rare cause of subacute endocarditis

Non-typeable *H. influenzae* and *parainfluenzae* together colonize the pharynges and nasopharynges of >90% of healthy individuals.
"Amox-clav, azithromycin, clarithromycin, cefaclor, loracabef, cefdinir, cefixime, cefpodoxime, cefuroxime axetil and telithromycin are oral agents that may be used as empiric therapy for respiratory tract infections due to Haemophilus spp. The results of susceptibility tests with these antimicrobial agents are often not useful for management of individual patients. However, susceptibility testing of Haemophilus spp. with these compounds may be appropriate for surveillance or epidemiologic studies."

CLSI M100-S21 Table 2E
# Haemophilus influenzae: Treatment recommendations

<table>
<thead>
<tr>
<th>Infection</th>
<th>Primary</th>
<th>Alternative</th>
<th>Prevalence of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis and invasive infection</td>
<td>Ceftriaxone and Cefotaxime</td>
<td></td>
<td>0(^1)</td>
</tr>
<tr>
<td>Non-life threatening infections</td>
<td>Trim-sulfa, amox/clav, amp/sulbactam, oral 2(^{nd}) or 3(^{rd}) generation cephalosporin</td>
<td>Azithromycin, clarithromycin, fluoroquinolone, amp or penicillin</td>
<td>Variable (next slide)</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>Rifampin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Karlowsky et al. IJAA. 2002
# H. Influenzae Resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>~33%</td>
</tr>
<tr>
<td>Amox/Clav</td>
<td>~0%</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0%</td>
</tr>
<tr>
<td>Trim-Sulfa</td>
<td>~20%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0%</td>
</tr>
</tbody>
</table>

Karlowsky et al. AAC. 2003
Haemophilus influenzae

• Beta-lactamase testing

(7) The result of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of isolates or *H. Influenzae* that are resistant to ampicillin and amoxicillin produce a TEM-type beta-lactamase. In most cases, a direct beta-lactamase test can provide a rapid means of detecting resistance to ampicillin and amoxicillin.

Beta-lactamase negative ampicillin resistant strains (BLNAR) of *H. influenzae* exist and should be considered resistant to amox/clav, amp/sulbactam, cefaclor, cefetamet, cefonicid, cefprozil, cefuroxime, loracarbef and pip/tazo despite apparent in vitro susceptibility of some BLNAR strains to these agents.
Haemophilus influenzae in the United States 2001-2002

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Prevalence (n=1,434)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-lactamase negative ampicillin susceptible (BLNAS)</td>
<td>1019 (71.1%)</td>
</tr>
<tr>
<td>Beta-lactamase positive ampicillin resistant (BLPAR)</td>
<td>406 (28.3%)</td>
</tr>
<tr>
<td>Beta-lactamase negative ampicillin resistant (BLNAR)</td>
<td>9 (0.6%)</td>
</tr>
</tbody>
</table>

This is the population that is missed by beta-lactamase testing.

Karlowsky et al. 2001. JCM
Haemophilus influenzae: Strategy

• Use Beta-lactamase test clinically significant isolates.
  – If positive report as resistant to amoxicillin and ampicillin

• Additional susceptibility testing...
  – Consult with MD
  – Send to reference lab
  – Perform using Haemophilus test medium
### Haemophilus influenzae Test Method

<table>
<thead>
<tr>
<th>Inoculation:</th>
<th>Direct colony suspension 0.5 McFarland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium:</td>
<td>DD – <em>Haemophilus</em> test medium (HTM)</td>
</tr>
<tr>
<td></td>
<td>Broth – HTM broth</td>
</tr>
<tr>
<td>Incubation:</td>
<td>35 +/- 2°C</td>
</tr>
<tr>
<td></td>
<td><em>DD</em> – 5% CO₂ 16-18 hrs</td>
</tr>
<tr>
<td></td>
<td><em>Broth</em> – Ambient air; 20-24 hrs</td>
</tr>
</tbody>
</table>
Neisseria gonorrhoeae - Epidemiology

…but as long as people are still having promiscuous sex with many anonymous partners without protection while at the same time experimenting with mind-expanding drugs in a consequence-free environment, I'll be sound as a pound!

CDC implementation of GC control program in the mid 70's.

- Decreased incidence of GC in the US by 74%
- However, 5.5% increase from 2005-2006
The Gonococcal Isolate Surveillance Project (GISP) was established in 1986 to monitor trends in antimicrobial susceptibilities of strains of *N. gonorrhoeae* in the United States in order to establish a rational basis for the selection of gonococcal therapies. GISP is a collaborative project among selected sexually transmitted diseases (STD) clinics, five regional laboratories, and the Centers for Disease Control and Prevention (CDC).

In GISP, *N. gonorrhoeae* isolates are collected from the first 25 men with urethral gonorrhea attending STD clinics each month in approximately 28 cities in the United States. At regional laboratories, the susceptibilities of these isolates to penicillin, tetracycline, spectinomycin, ciprofloxacin, ceftriaxone, cefixime, and azithromycin are determined by agar dilution. Minimum inhibitory concentrations (MICs) are measured, and values are interpreted according to criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

**Protocol**
- GISP Protocol

**Annual Reports and Profiles**
- 2009 GISP Profiles

**Sentinel Sites and Regional Laboratories**

Click thumbnail for larger map

* indicates Regional Laboratories

Albuquerque, NM
Atlanta, GA
Miami, FL
Minneapolis, MN
**Current Neisseria gonorrhoeae Treatment recommendations**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Primary</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethritis, cervicitis and proctitis</td>
<td>Ceftriaxone or cefixime PLUS doxycycline or azithromycin</td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Ceftriaxone IM</td>
<td></td>
</tr>
<tr>
<td>Disseminated gonococcal infection (DGI)</td>
<td>IM or IV Ceftriaxone</td>
<td>IV Cefotaxime or IV ceftizoxime</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>Ceftriaxone IM PLUS doxycycline or azithromycin</td>
<td></td>
</tr>
</tbody>
</table>

As of 2007, fluoroquinolones no longer recommended due to widespread emergence of resistance.

MMWR 2010 – Dec 17, 2010 – STD Treatment Guidelines
Cephalosporin Susceptibility Among Neisseria gonorrhoeae Isolates — United States, 2000–2010

Neisseria gonorrhoeae with Reduced Susceptibility to Azithromycin — San Diego County, California, 2009
### Drug Susceptibility Table

<table>
<thead>
<tr>
<th>Drug</th>
<th>Susceptible (MIC (µg/mL))</th>
<th>Susceptible (Disk (mm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>&lt;= 0.5</td>
<td>&gt;= 31</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&lt;= 0.25</td>
<td>&gt;= 35</td>
</tr>
<tr>
<td>Cefixime</td>
<td>&lt;= 0.25</td>
<td>&gt;= 29</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Eucast &lt;= 0.25 GISP &lt;= 1</td>
<td>No interpretation</td>
</tr>
</tbody>
</table>

**Abbreviations:** MICs = minimum inhibitory concentrations; MSM = men who have sex with men; MSW = men who have sex exclusively with women.

**EUCAST Version 1.1, April 2010**
**CLSI – M100-S21**
**MMWR 2011 – July 8, 2011**
Neisseria gonorrhoeae: Regional Resistance

Oklahoma City, OK

Azithromycin

“Breakpoint”

Ceftriaxone

Breakpoint

Ciprofloxacin

Cefixime

Breakpoint

Neisseria gonorrhoeae: Regional Treatment
Oklahoma City, OK

Figure D. Drugs used to treat gonorrhea among GISP participants, 2009

Figure E. Drugs used to treat Chlamydia trachomatis infection among GISP participants, 2009

Neisseria gonorrhoeae: SWACM Region

Different in Dallas...

Same treatment pattern in MO and LA but with AZT susceptibility patterns resembling that of OKC

Neisseria gonorrhoeae: Susceptibility testing

- Isolation of organism rare in era of NAAT
- Not performed by most laboratories
  - Requires specialized media which includes GC agar base and growth supplement.
- Generally performed by state and public health laboratories for epidemiological purposes.
- Not usually performed by reference labs.
  - Not Mayo, Focus or ARUP
Current *Neisseria meningitidis*: Treatment recommendations

<table>
<thead>
<tr>
<th>Indication</th>
<th>Primary</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>Ceftriaxone</td>
<td>Chloramphenicol, Meropenem or Moxifloxacin</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>Cipro, Ceftriaxone or Rifampin</td>
<td></td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>Ceftriaxone or cefotaxime</td>
<td></td>
</tr>
<tr>
<td>Bacteremia</td>
<td>Ceftriaxone</td>
<td>Levofloxacin or Moxifloxacin</td>
</tr>
</tbody>
</table>
Important points for testing of fastidious GNRs

• *N. meningitidis* testing should be performed in a biological safety cabinet (BSC).
  
  – *Laboratory acquired disease is associated with a case fatality rate of 50%.*
    
    • *Substantially higher than that of the general population (12-15%)*
  
• *Even if you’ve been vaccinated...*
  
  – Vaccine not 100% effective
  
  – Does not protect against serogroup B which caused 50% of lab acquired disease in 2000.

**Neisseria meningitidis:**

**Table 21: Zone Diameter and MIC Interpretive Standards for Neisseria meningitidis**

<table>
<thead>
<tr>
<th>Testing Conditions</th>
<th>MIC and Disk diffusion methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium:</strong> Disk diffusion: MHA with 5% sheep's blood</td>
<td></td>
</tr>
<tr>
<td>Agar dilution: MHA supplemented with sheep blood (5% v/v)</td>
<td></td>
</tr>
<tr>
<td>Incubation: 35 ± 2 °C, 5% CO₂</td>
<td>20 to 24 hours</td>
</tr>
</tbody>
</table>

**Minimal QC Recommendations** (See Tables 3A, 3B, 4A, and 4B for acceptable QC ranges.)

- **Streptococcus pneumoniae ATCC®:** 49619
  - Disk diffusion: incubate in 5% CO₂.
  - Broth microdilution: incubate in ambient air or CO₂ (except azithromycin QC tests that must be incubated in ambient air).
- **E. coli ATCC®:** 25922
  - Disk diffusion, broth microdilution or agar dilution for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole: incubate in ambient air or CO₂.

**General Comments**

1. Caution: Perform all antimicrobial susceptibility testing (AST) of *N. meningitidis* in a biological safety cabinet (BSC). Manipulating suspensions of *N. meningitidis* outside a BSC is associated with a high risk for contracting meningococcal disease. Laboratory-acquired meningococcal disease is associated with a case fatality rate of 50%. Exposure to droplets or aerosols of *N. meningitidis* is the most likely risk for laboratory-acquired infection. Rigorous protection from droplets or aerosols is mandated when microbiological procedures (including AST) are performed on all *N. meningitidis* isolates.

2. Recommended precautions: Specimens for *N. meningitidis* analysis and cultures of *N. meningitidis* not associated with invasive disease may be handled in Biosafety Level 2 (BSL-2) facilities, with rigorous application of BSL-2 standard practices, special practices, and safety equipment. All sterile-site isolates of *N. meningitidis* should be manipulated within a BSC. If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogroup identification using phenolized saline solution while wearing a laboratory coat and gloves, and working behind a full face splash shield. Use Biosafety Level 3 (BSL-3) practices, procedures, and containment equipment for activities with a high potential for droplet or aerosol production and for activities involving production quantities or high concentrations of infectious materials. If BSL-2 or BSL-3 facilities are not available, forward isolates to a reference or public health laboratory with a minimum of BSL-2 facilities.

3. Laboratories who are exposed routinely to potential aerosols of *N. meningitidis* should consider vaccination according to the current recommendations of the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (www.cdc.gov). Vaccination will decrease, but not eliminate the risk of infection, because it is less than 100% effective and does not provide protection against serogroup B, a frequent cause of laboratory-acquired cases.

4. For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
Neisseria meningitidis: Therapeutic Agents

* Penicillin
* Ampicillin
Cefotaxime
Ceftriaxone
Meropenem
Chloramphenicol

All drugs listed are in Test/Report Group C
“Supplemental, Report Selectively”..implies routine testing not necessary
*No disk diffusion breakpoints; must do MIC test
Neisseria meningitidis: Agents for Prophylaxis

- Azithromycin
- Ciprofloxacin
- Minocycline
- Nalidixic acid  
  (for surveillance only; may detect diminished fluoroquinolone susceptibility)
- Rifampin
- Trimethoprim-sulfamethoxazole  
  (predicts susceptibility to sulfonamides also)

• (9) May be appropriate only for prophylaxis of meningococcal case contacts. These interpretive criteria do not apply to therapy of patients with invasive meningococcal disease.

All drugs listed are in Test/Report Group C “Supplemental, Report Selectively”..implies routine testing not necessary
Neisseria meningitidis: Resistance Issues

- Rare $\beta$-lactamase producing isolates
  - 6 to date; most recent 1996 (Spain)
  - None isolated of 442 collected from ABCs network
    - Between 1917 and 2004

- Penicillin “I” or “R” isolates
  - Mechanism – altered PBP
  - MICs to extended-spectrum cephalosporins remain low

- Resistance to prophylactic agents:
  - Sulfonamides - (common)
  - Ciprofloxacin - (rare)
  - Rifampin – (uncommon)

Jorgensen et al. 2005. JCM