

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 pharynx and nasopharynx of >90% of healthy individuals.

Haemophilus influenzae

“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.”

Haemophilus influenzae: Treatment recommendations

Infection	Primary	Alternative	Prevalence of Resistance
Meningitis and invasive infection	Ceftriaxone and Cefotaxime		0% ¹
Non-life threatening infections	Trim-sulfa, amox/clav, amp/sulbactam, oral 2 nd or 3 rd generation cephalosporin	Azithromycin, clarithromycin, fluoroquinolone, amp or penicillin	Variable (next slide)
Prophylaxis	Rifampin		

H. Influenzae Resistance

Antibiotic	% Resistance
Ampicillin	~33%
Amox/Clav	~0%
Azithromycin	0%
Trim-Sulfa	~20%
Levofloxacin	0%

Haemophilus influenzae

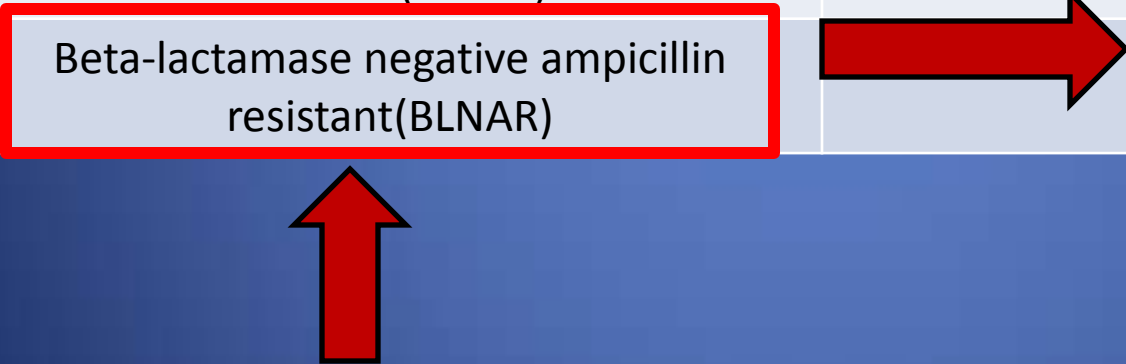
- Beta-lactamase testing

(7) The result of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. The majority of isolates of *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

Phenotype	Prevalence (n=1,434)
Beta-lactamase negative ampicillin susceptible (BLNAS)	1019 (71.1%)
Beta-lactamase positive ampicillin resistant(BLPAR)	406 (28.3%)
Beta-lactamase negative ampicillin resistant(BLNAR)	9 (0.6%)



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

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

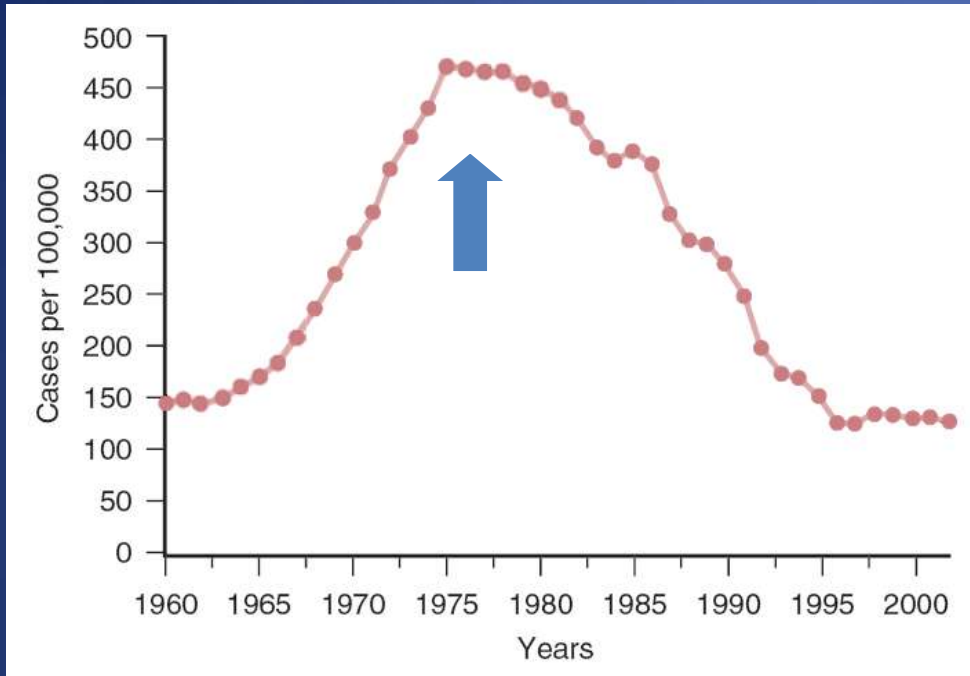
Haemophilus influenzae Test Method

Inoculation: Direct colony suspension
0.5 McFarland

Medium: DD – *Haemophilus* test medium(HTM)
Broth – HTM broth

Incubation: 35 +/- 2°C
DD – 5% CO₂ 16-18 hrs
Broth – Ambient air; 20-24 hrs

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



SEARCH

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Sexually Transmitted Diseases (STDs)

Sexually Transmitted Diseases

- Diseases & Related Conditions
- Pregnancy & Infertility
- Publications & Products
- Program Tools
- Projects & Initiatives
- ▶ **Gonococcal Isolate Surveillance Project (GISP)**
- Infertility Prevention Project (IPP)
- STD Awareness Month
- Syphilis Elimination Effort (SEE)
- Data & Statistics
- Training
- Treatment
- About the Division of STD Prevention

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Gonococcal Isolate Surveillance Project (GISP)

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).

On this Page

- [Protocol](#)
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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](#)
- [GISP Profiles \(2008-2009\) and Annual Reports \(1998-2007\)](#)

Sentinel Sites and Regional Laboratories



Click thumbnail for larger map

* indicates Regional Laboratories

Albuquerque, NM

Atlanta, GA *

Miami, FL

Minneapolis, MN

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Contact Us:

Centers for Disease Control and Prevention
1600 Clifton Rd
Atlanta, GA 30333

800-CDC-INFO
(800-232-4636)
TTY: (888) 232-6348
24 Hours/Every Day

cdcinfo@cdc.gov

Current Neisseria gonorrhoeae

Treatment recommendations

Infection	Primary	Alternative
Urethritis, cervicitis and proctitis	Ceftriaxone or cefixime PLUS doxycycline or azithromycin	
Conjunctivitis	Ceftriaxone IM	
Disseminated gonococcal infection (DGI)	IM or IV Ceftriaxone	IV Cefotaxime or IV ceftizoxime
Pharyngitis	Ceftriaxone IM PLUS doxycycline or azithromycin	

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

Centers for Disease Control and Prevention

MMWR

Morbidity and Mortality Weekly Report

Weekly / Vol. 60 / No. 26

July 8, 2011

Morbidity and Mortality Weekly Report

**Cephalosporin Susceptibility Among *Neisseria gonorrhoeae* Isolates —
United States, 2000–2010**

Centers for Disease Control and Prevention

MMWR

Morbidity and Mortality Weekly Report

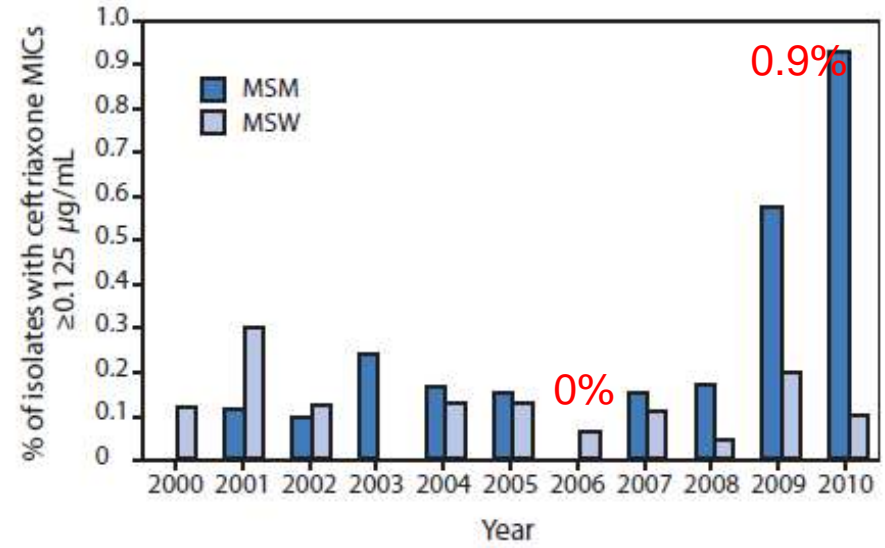
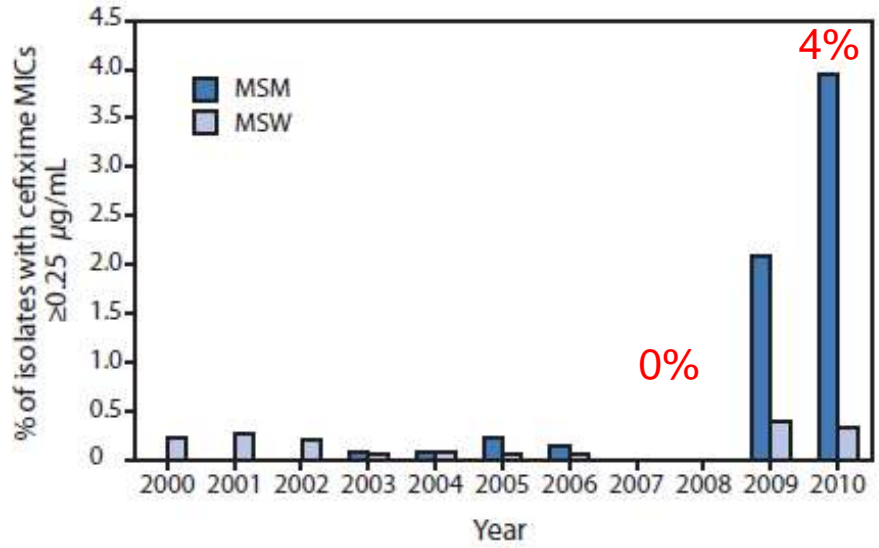
Weekly / Vol. 60 / No. 18

May 13, 2011

Morbidity and Mortality Weekly Report

***Neisseria gonorrhoeae* with Reduced Susceptibility to Azithromycin —
San Diego County, California, 2009**

FIGURE 2. Percentage of gonorrhea isolates with cefixime MICs $\geq 0.25 \mu\text{g/mL}$ and ceftriaxone MICs $\geq 0.125 \mu\text{g/mL}$, by sex of sex partner — Gonococcal Isolate Surveillance Project, United States, 2000–2010



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

Drug	Susceptible (MIC ($\mu\text{g/mL}$))	Susceptible (Disk (mm))
Cefotaxime	≤ 0.5	≥ 31
Ceftriaxone	≤ 0.25	≥ 35
Cefixime	≤ 0.25	≥ 29
Azithromycin	Eucast ≤ 0.25 GISP ≤ 1	No interpretation

Neisseria gonorrhoeae: Regional Resistance

Oklahoma City, OK

Figure J. Distribution of Minimum Inhibitory Concentrations (MICs) to azithromycin among GISP isolates, 2005-2009.

Azithromycin

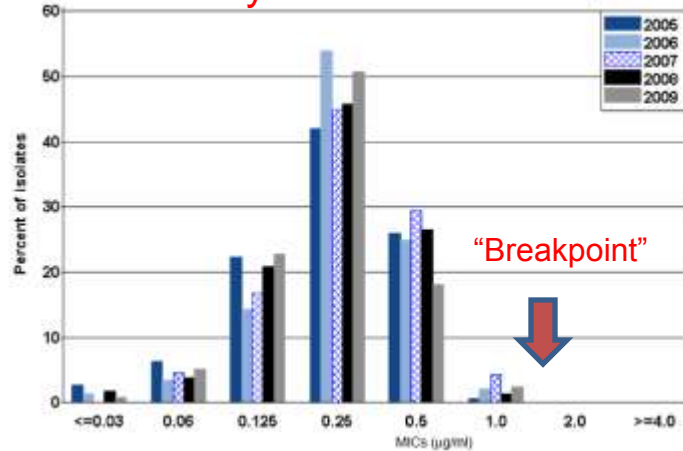


Figure G. Distribution of Minimum Inhibitory Concentrations (MICs) to ceftriaxone among GISP isolates, 2005-2009.

Ceftriaxone

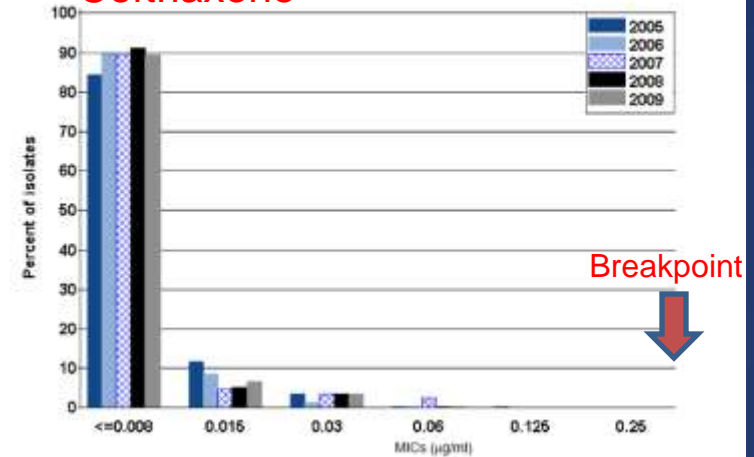


Figure I. Intermediate resistance and resistance to ciprofloxacin among GISP isolates, 1990-2009.

Ciprofloxacin

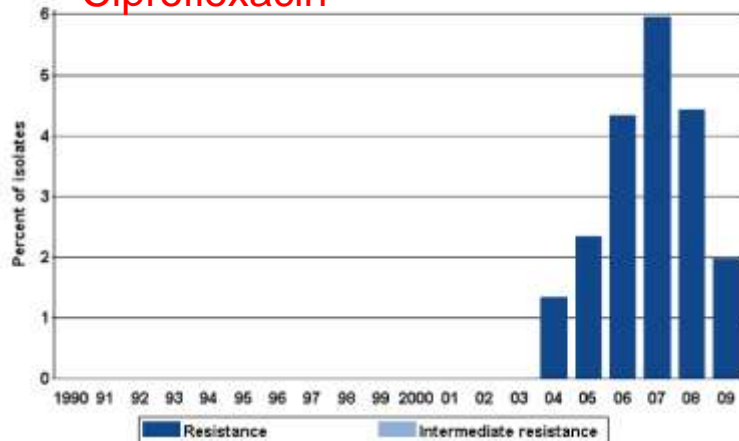
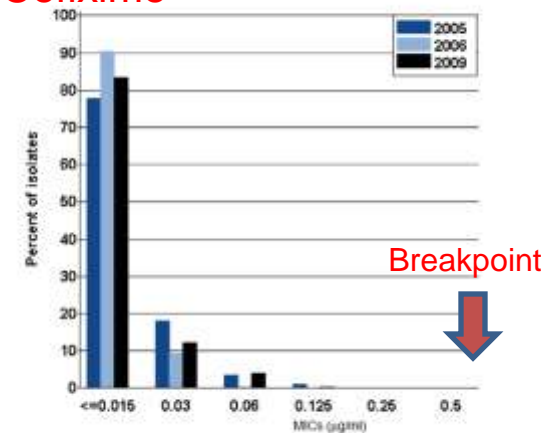


Figure H. Distribution of Minimum Inhibitory Concentrations (MICs) to cefixime among GISP isolates, 2005-2006 and 2009.

Cefixime



NOTE: Isolates were not tested for cefixime susceptibility in 2007 and 2008.

Neisseria gonorrhoeae: Regional Treatment

Oklahoma City, OK

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

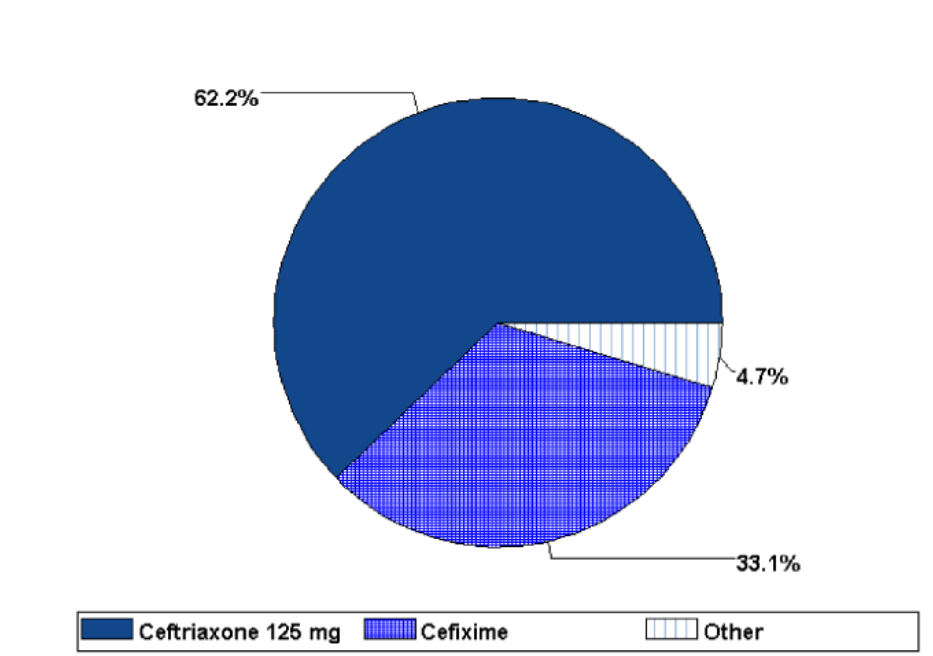
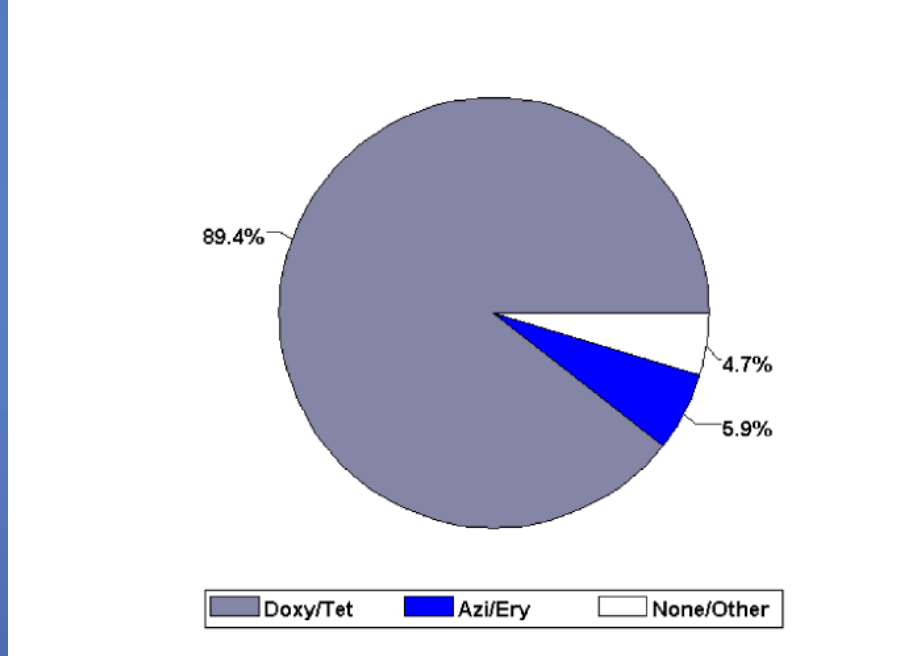


Figure E. Drugs used to treat Chlamydia trachomatis infection among GISP participants, 2009



Neisseria gonorrhoeae: SWACM Region

Different in Dallas...

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

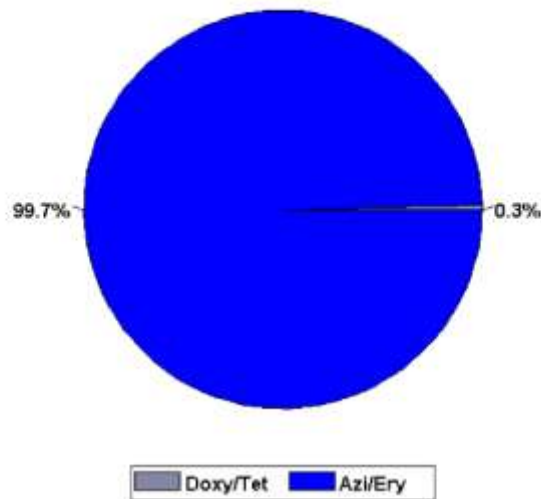
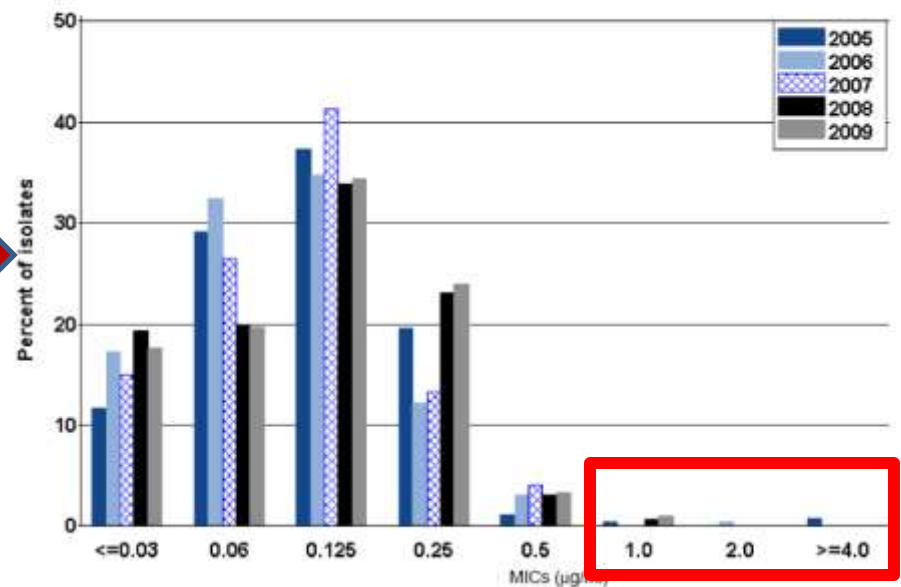


Figure J. Distribution of Minimum Inhibitory Concentrations (MICs) to azithromycin among GISP isolates, 2005-2009



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

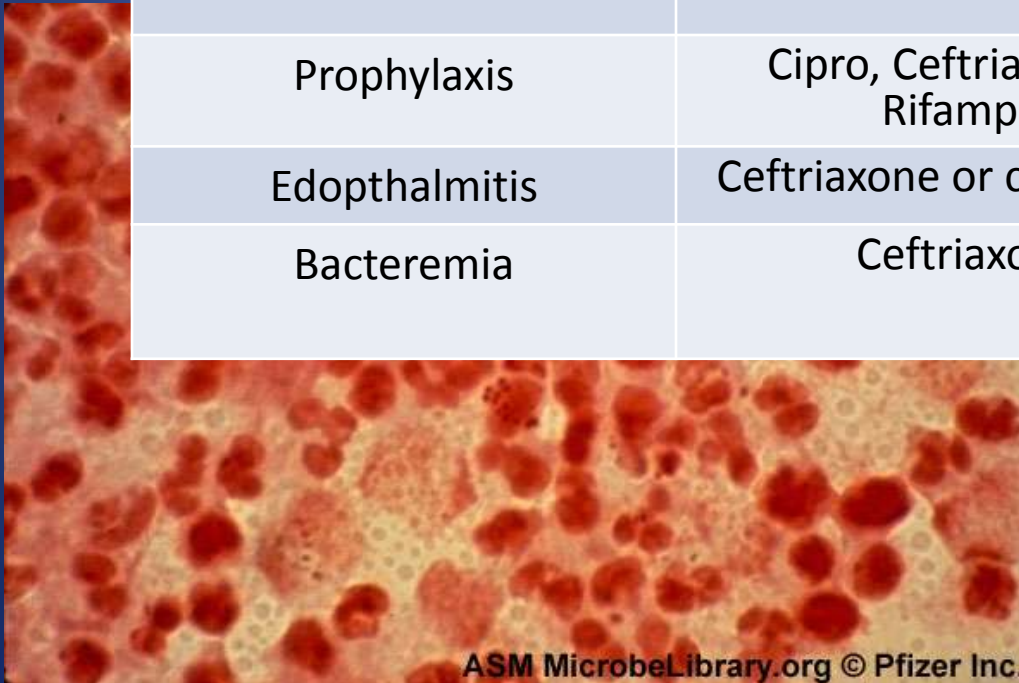
Neisseria gonorrhoeae:

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

Indication	Primary	Alternative
Central nervous system	Ceftriaxone	Chloramphenicol, Meropenem or Moxifloxacin
Prophylaxis	Cipro, Ceftriaxone or Rifampin	
Edophthalmitis	Ceftriaxone or cefotaxime	
Bacteremia	Ceftriaxone	Levofloxacin or Moxifloxacin



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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:

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Table 2I
Neisseria meningitidis
M02 and M07

Table 2I. Zone Diameter and MIC Interpretive Standards for *Neisseria meningitidis*

Testing Conditions		Minimal QC Recommendations (See Tables 3A, 3B, 4A, and 4B for acceptable QC ranges.)
Medium:	Disk diffusion: MHA with 5% sheep's blood Broth microdilution: CAMHB supplemented with LHB (2.5% to 5% v/v) (see M07-A8 for preparation of LHB) Agar dilution: MHA supplemented with sheep blood (5% v/v)	<i>Streptococcus pneumoniae</i> ATCC® 49619: Disk diffusion: incubate in 5% CO ₂ . Broth microdilution: incubate in ambient air or CO ₂ (except azithromycin QC tests that must be incubated in ambient air).
Inoculum:	Direct colony suspension from 20 to 24 hours growth from chocolate agar incubated at 35 °C; 5% CO ₂ ; equivalent to a 0.5 McFarland standard. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep's blood agar will contain approximately 50% fewer CFU/mL. This must be taken into account when preparing the final dilution before panel inoculation, as guided by colony counts.	<i>E. coli</i> ATCC® 25922 Disk diffusion, broth microdilution or agar dilution for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole: incubate in ambient air or CO₂.
Incubation:	35 ± 2 °C; 5% CO ₂ ; 20 to 24 hours	

General Comments

MIC and Disk diffusion methods

For more information, see *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. Washington, DC: US Government Printing Office, 2009. <http://www.fda.gov/bmbll5/bmbll5toc.htm>.

- (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) Laboratorians 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.

January 2011

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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 β -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)