August 28, 2017

CDC AR LABORATORY NETWORK:
Antimicrobial Susceptibility Testing of *Neisseria gonorrhoeae* Isolates

PURPOSE.

As part of the Antibiotic Resistance (AR) Regional Lab Network, four ELC-funded Laboratories will perform bacterial identification and antimicrobial susceptibility determination of confirmed *N. gonorrhoeae* isolates. Urethral discharge specimens and specimens other anatomic sites (e.g., vaginal, endocervical, pharyngeal, and/or rectal specimen-derived isolates) will be collected from those presenting at CDC-selected sexually transmitted disease clinics (sentinel sites) using a strategy that allows for conducting surveillance for emerging antimicrobial resistance of *N. gonorrhoeae*. The Guidance document will identify expectations for ELC-funded AR Labs regarding testing and reporting methods, hand-off, storage, and lab training and proficiency.

GENERAL CONSIDERATIONS.

When using this guidance, ELC-funded *N. gonorrhoeae* laboratories should consider:

- This Guidance recommends options for bacterial identification and specific requirements for antimicrobial susceptibility testing. Bacterial identification methods used by laboratories are not limited to these, but alternative choices in testing should yield comparable results, turn-around-time and accuracy. Antimicrobial susceptibility testing must be performed using agar dilution.
- All testing should be implemented in accordance with current Clinical and Laboratory Standards Institute (CLSI) standards and in complains with Clinical Laboratory Improvement Amendments (CLIA) regulations
- Laboratories will designate SME(s) to support coordination of laboratory and epidemiology functions, and cooperation with CDC and state STD prevention programs. Up-to-date SME(s) contact information should be provided to CDC and relevant ARLN partners.
- Each laboratory maintains a database of testing results, which the laboratory can easily access, and from which the laboratory can query results and generate summary and line-listed reports.

ISOLATE COLLECTION.

Sentinel Site Laboratory Collection, Handling, and Shipping of Isolates

- Isolates derived from urethral discharge specimens and specimens other anatomic sites (e.g., vaginal, endocervical, pharyngeal, and/or rectal specimen-derived isolates) will be collected from those presenting at CDC-selected sexually transmitted disease clinics (sentinel sites). The *N. gonorrhoeae* isolates will be initially processed at CDC-selected sentinel site laboratories and those presumptively identified as *N. gonorrhoeae* by Gram stain and oxidase reaction will be shipped to an AR Regional Lab for bacterial identification and antimicrobial susceptibility testing.
- Laboratories are expected to test 400-500 isolates per month.

LABORATORY TESTING & REPORTING.

Methods

Expected methods are more fully described by CDC's Division of STD Prevention at http://www.cdc.gov/std/gisp/gisp-protocol-feb-2015_v3.pdf. In brief:

1. **Bacterial identification:** The identity of all isolates presumed to be *N. gonorrhoeae* will be confirmed using either carbohydrate utilization and enzyme reactivity or possibly MALDI-TOF.
   1.1. The utilization of glucose, maltose, lactose, sucrose and fructose will be performed on fresh cultures and the results compared to bacterial identification tables for Gram-negative diplococci.
   1.2. The presence of o-nitrophenyl-β-galactosidase, γ-glutamyl aminopeptodase and prolyliminopeptidase will be assessed and the results compared to bacterial identification tables for Gram-negative diplococci.
   1.3. The mass spectra generated and analyzed with a MADI-TOF may be used in place of the biochemical activity listed in subsections 1.1 and 1.2. CDC will provide assistance with validation panels.
2. **Antimicrobial susceptibility testing (AST):** Agar dilution will be the only method acceptable for susceptibility testing of *N. gonorrhoeae* at the AR Lab.
   2.1. GC medium base supplemented with 1% IsoVitaleX will be used for all antimicrobial agent dilutions. The GC base medium will be reconstituted, stream sterilized in an autoclave and, when cooled, inoculated with the appropriate antimicrobial agent dilution before being dispensed into plastic petri plates.
3. **Drugs for AST:** All *N. gonorrhoeae* isolates will be tested for susceptibility to azithromycin, ceftriaxone, cefixime, ciprofloxacin, gentamicin, penicillin and tetracycline by agar dilution. It is recommended that all isolates should be tested against the following drug dilutions:
   3.1. Azithromycin: 0.008 - 16.0 µg/ml
   3.2. Ceftriaxone: 0.001 - 1.0 µg/ml
   3.3. Cefixime: 0.002 - 1.0 µg/ml
   3.4. Ciprofloxacin: 0.01 - 32.0 µg/ml
   3.5. Gentamicin: 1.0 - 32.0 µg/ml
   3.6. Penicillin: 0.008 - 64.0 µg/ml
   3.7. Tetracycline: 0.06 - 64.0 µg/ml
4. **B-Lactamase activity:** Nitrocefin will be used to assess the isolates for β-lactamase. Two test options are listed below:
   4.1. A drop of nitrocefin can be added directly to an isolated colony on a plate containing an overnight culture.
   4.2. A slide, broth or filter paper can also be used to mix an isolated colony with nitrocefin to determine the presence of β-lactamase.
5. **Quality control**: Three quality control strains will be tested with each AST run. One strain, ATCC 49226, must be within acceptable ranges as published in the CLSI M-100 document for the data to be considered valid and reportable to CDC. Tests with out of range MIC values must be repeated until the quality control strain is within range. Two additional quality control strains with undisclosed ranges will be supplied by CDC each year. The data from these two strains will be analyzed by CDC quarterly to further assess laboratory quality.

Required Reporting

Antimicrobial Susceptibility Testing Result Reporting

It is expected that susceptibility testing, including results of quality control strains, will be completed within two weeks of receipt of isolates from a sentinel site and reported to CDC on a monthly basis.

The AR Laboratory should notify CDC DTSDP **within one working day** by e-mail (ARLN_alert@cdc.gov) of any isolate(s) identified to demonstrate an alert MIC (currently defined as a cefixime MIC ≥0.25µg/ml, ceftriaxone MIC ≥0.125µg/ml, or azithromycin MIC ≥2µg/ml). All susceptibility testing should be reported along with relevant IDs in the following template:

SURRG ID	GISP ID	B-Lac	PEN	TET	CFX	CRO	CIP	AZI	GEN	Date Tested	Date Retested

Note: Include both SURRG and GISP ID's if applicable. Send the date tested and resend the result when the isolate is retested and the alert confirmed. Do not resubmit the result if the alert MIC is not reproduced upon retesting.

Acronyms used:
B-Lac – beta lactamase
PEN – Penicillin MIC (µg/ml)
TET – Tetracycline MIC (µg/ml)
CFX – Cefixime MIC (µg/ml)
CRO – Ceftriaxone MIC (µg/ml)
CIP – Ciprofloxacin MIC (µg/ml)
AZI – Azithromycin MIC (µg/ml)
GEN – Gentamicin MIC (µg/ml)

If the isolate with the alert MIC result is confirmed, CDC, the sentinel site, and the pertinent local and/or state STD programs should be notified of the confirmed alert value(s) **within one working day.**

Storage and Handover of Certain Isolates

1. **Isolates selected for preservation and shipping to CDC:** *N. gonorrhoeae* isolates with certain MIC values will be selected for preservation and shipping to CDC. These will include ceftriaxone MIC values ≥0.125µg/ml, cefixime MIC values ≥0.125µg/ml and/or azithromycin MIC values ≥2.0µg/ml. Additional isolates may be requested at the discretion of CDC.
2. **Preservation:** An overnight growth of a pure culture will be suspended into at least four cryotubes with Mueller Hinton broth containing 20% glycerol and labeled with the isolate's unique identifier. These tubes will be frozen at -70°C and three tubes will be batched shipped monthly to CDC using dry ice for archival storage.
3. **Storage:** AR Labs should store isolates (one sub-cultured copy of each isolate per patient) for a minimum of two years.

Proficiency Testing & Laboratory Training

1. **Training:** CDC will conduct necessary training of AR Network labs. Training will be coordinated either by APHL or through direct communication of technical assistance needs with CDC SMEs.
2. **Proficiency testing:** CDC will supply AR Labs a proficiency test panel of 15 isolates twice a year. These isolates are to be tested in the same method as those for AST within one month of receipt. Results will be reported to CDC for scoring. A proficiency test report will be compiled by CDC after each challenge. Labs are expected to score at least 80% to maintain testing. Labs with proficiency test scores below 80% will be required to perform another proficiency test within two weeks and achieve a passing grade before resuming AST of unknown isolates. A second failure will require additional training and review of technical competency.

CONTACT INFORMATION.

For questions or further information, please contact Dr. John Papp (jwp6@cdc.gov), Dr. Cau Pham (whi4@cdc.gov) Dr. Ellen Kersh (ekersh@cdc.gov), or ARLN@cdc.gov.