

**STANDARD OPERATING PROCEDURE**

Title: Protocol for standardization of *Neisseria gonorrhoeae* antimicrobial susceptibility testing (AST) in NHLS laboratories for the detection and reporting of ceftriaxone-resistant *gonorrhoea*

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**(Changes from previous version highlighted)**

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**INTRODUCTION**

*Neisseria gonorrhoeae* has acquired resistance to all antimicrobial agents used as first-line therapy over the years, including to the extended-spectrum cephalosporins. The World Health Organisation Global Antimicrobial Resistance Surveillance System (GLASS) lists *Neisseria gonorrhoeae* as a priority pathogen and South Africa, as a participating country, monitors gonococcal antimicrobial resistance patterns annually. In 2017, ceftriaxone-resistant gonorrhoea was included on the national Notifiable Medical Conditions (NMC) list as a Category 3 condition. This necessitates notification by private and public health laboratories through written or electronic notification to the National Department of Health within 7 days of diagnosis. Additional notification should be made by laboratories to the Sexually Transmitted Infection (STI) Reference Laboratory at the Centre for HIV & STI by email of relevant laboratory form (**Appendix 1**) as soon as an isolate of ceftriaxone-resistant *Neisseria gonorrhoeae* is identified. The NMC laboratory notification system will alert the STI Reference Laboratory if a ceftriaxone-resistance *Neisseria gonorrhoeae* result is reported on the NHLS Laboratory Information System.

National antimicrobial resistance surveys of *Neisseria gonorrhoeae* have revealed that high-prevalence resistance (both intermediate- and high-level) to penicillin (99%), tetracycline (90%) and ciprofloxacin (75%) obviates the use of these agents in empiric therapy guidelines for syndromic management of genital discharge. Therefore, it is recommended that routine testing for susceptibility to these agents is no longer done by diagnostic laboratories. The currently recommended dual treatment regimen for gonorrhoea includes injectable ceftriaxone and oral azithromycin. In South Africa, extended-spectrum cephalosporin resistance in *N. gonorrhoeae* has been described, resulting in cefixime treatment failure for male urethritis.

**PRINCIPLE**

*Neisseria gonorrhoeae* AST must be standardized across NHLS laboratories that offer this service, in order to facilitate notification of ceftriaxone-resistant gonorrhoea.

**OBJECTIVES**

1. To enable NHLS laboratories to standardize AST for *Neisseria gonorrhoeae* by testing susceptibility to appropriate antimicrobials that are currently recommended for gonorrhoea treatment, in particular susceptibility to ceftriaxone.
2. To enable NHLS Diagnostic Media Products (DMP) to produce sufficient quantities of recommended media and ensure an adequate supply to the laboratories that perform AST.
3. To facilitate notification of ceftriaxone-resistant gonorrhoea for further investigation, appropriate management and relevant public health action.

**RESPONSIBILITY**

All laboratory personnel who are trained to perform these test procedures and interpret antimicrobial susceptibility results.

**PROCEDURE**

1. **Minimum Inhibitory Concentration (MIC) Determination by E-test (recommended method)**

* The European Committee on Antimicrobial Susceptibility Testing (EUCAST) document states that disc diffusion criteria for *N. gonorrhoeae* AST have not been defined, and an MIC method should be used.
* NHLS laboratories that offer *N. gonorrhoeae* AST should test MIC to **ceftriaxone** by use of ceftriaxone/ cefotaxime E-test (bioMérieux, Marcy-l’Étoile, France). This is an essential component of AST.
* NHLS laboratories may additionally consider AST for azithromycin by E-test. Cefixime or Ciprofloxacin E-test may also be performed, if clinically relevant (e.g. if IV to oral conversion desirable in treatment of disseminated gonococcal infection). Routine AST for additional antimicrobials is unnecessary.
* The recommended agar medium for E-test is **GC agar base and 1% Isovitalex** (supplied by NHLS DMP).
* Make a 0.5 McFarland inoculum in sterile saline or Mueller-Hinton broth, ideally using pure colonies from a chocolate agar plate incubated for 18-24 hours in 5% CO2. If organism is isolated on a New York City agar plate, subculture onto chocolate agar for purity and AST.
* Incubate AST plates at 36°C + 1°C in 5% CO2 for 20-24 hours.
* Refer to **SOP NIC0597** on QPulse for information on testing procedure and interpretation of results.

**Table 1. EUCAST MIC Clinical Interpretive breakpoints for *N. gonorrhoeae* (µg/ml)**

|  |  |  |
| --- | --- | --- |
| Antimicrobial | Susceptible | Resistant |
| Ceftriaxone/ Cefotaxime (for susceptibility to ceftriaxone) | </= 0.125 | >/= 0.25 |
| Cefixime | </= 0.125 | >/= 0.25 |
| Azithromycin | No clinical breakpoint interpretive criteria (EUCAST 2021). For testing purposes with the aim of detecting acquired resistance, the ECOFF is 1 µg/ml. Alert MIC > 1µg/ml | |

1. **Disc diffusion testing and zone diameter reading (optional method, if MIC testing cannot be performed)**

* The Clinical and Laboratory Standards Institute (CLSI) guidelines give clinical interpretive criteria for *Neisseria gonorrhoeae* disc diffusion AST.
* The recommended agar medium for E-test is **GC agar base and 1% Isovitalex** (supplied by NHLS DMP).
* NHLS laboratories should use the **30 µg ceftriaxone disc or 30 µg cefotaxime disc** to test for ceftriaxone susceptibility.
* NHLS laboratories may additionally consider use of 5 µg cefixime disc to report cefixime susceptibility. There are no CLSI zone diameter interpretive criteria for azithromycin.
* Routine AST for additional antimicrobials is unnecessary.
* Make a 0.5 McFarland inoculum in sterile saline or Mueller-Hinton broth, ideally using pure colonies from a chocolate agar plate incubated for 18-24 hours in 5% CO2. If organism is isolated on a New York City agar plate, subculture onto chocolate agar for purity and AST.
* Incubate AST plates at 36°C + 1°C in 5% CO2 for 20-24 hours.
* Measure the diameter of the zones of complete inhibition (as judged by the unaided eye). An intermediately-resistant result may be indicative of a technical problem that will need to be resolved by repeat testing. Strains with intermediately-resistant results have documented lower clinical cure rates.
* ***If using a disc diffusion method, it is recommended that the finding of new or rare antimicrobial resistance (e.g. resistance to extended-spectrum cephalosporins) is confirmed by MIC determination.***

**Table 2. CLSI zone diameter interpretive criteria for disc diffusion susceptibility testing (mm)**

|  |  |  |  |
| --- | --- | --- | --- |
| Antimicrobial | Susceptible | Intermediate | Resistant |
| Ceftriaxone | >/= 35 | - | - |
| Cefotaxime (to infer ceftriaxone susceptibility) | >/= 31 | - | - |
| Cefixime | >/= 31 | - | - |

**COMMENTS FOR EXTENDED-SPECTRUM CEPHALOSPORIN (ESC) RESISTANT *NEISSERIA GONORRHOEAE* ON LABORATORY INFORMATION SYSTEM**

One of the following codes should be inserted manually on TRAK LIS, once ESC resistance has been identified:

1. **NGTR**

Comment label: N. gonorrhoeae Ceftriaxone R MIC > 0.25 (EUCAST)

This isolate is resistant to ceftriaxone. Please use high dose ceftriaxone (1g stat IM) and azithromycin (2g stat PO) therapy for urogenital gonorrhoea. The isolate will be referred to the NICD STI Reference laboratory for further molecular testing and confirmation of ceftriaxone resistance.  We recommend follow-up of patient in two weeks for clinical assessment and test-of-cure using culture and/or PCR.  Please discuss this result with a microbiologist.

1. **NGIRTS**

Comment label: N. gonorrhoeae Cefixime R MIC > 0.25 and Ceftriaxone S MIC < 0.125 (EUCAST)

This isolate is resistant to cefixime and susceptible to ceftriaxone. The recommended therapy for urogenital gonorrhoea is ceftriaxone 250mg IM and azithromycin 1g PO stat.  The isolate will be referred to the NICD STI Reference laboratory for further molecular testing and confirmation of cefixime resistance.  We recommend follow-up of patient in two weeks for clinical assessment and test-of-cure using culture and/or PCR.  Please discuss these results with a microbiologist.

**QUALITY CONTROL**

QC should be performed using a selection of 2016 WHO reference strains for new lots of media and E-tests. The recommended strains are WHO G, W, X, Y, Z and may be ordered fromNICD-CHARM Antimicrobial Resistance Laboratory and Culture Collection (AMRL-CC). Any queries regarding orders may be communicated to the following address: [nscc@nicd.ac.za](mailto:nscc@nicd.ac.za) Orders may be placed by accessing the NSCC tab on the NHLS intranet (refer SOP NIC0799)

**Table 3. Phenotypic characteristics of antimicrobial susceptibility patterns displayed by the 2016 WHO *N. gonorrhoeae* reference strains (MIC µg/ml)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antimicrobial | **WHO G** | **WHO W** | **WHO X** | **WHO Y** | **WHO Z** |
| Ceftriaxone | 0.008 | 0.064 | 2 | 1 | 0.5 |
| Cefixime | < 0.016 | 0.25 | 4 | 2 | 2 |
| Azithromycin | 0.25 | 0.5 | 0.5 | 1 | 1 |

**PRESERVATION OF CEFTRIAXONE-RESISTANT GONOCOCCAL ISOLATES FOR TRANSPORT AND STORAGE**

To maintain the viability of *N. gonorrhoeae* strains on gonococcal agar media, it is necessary to subculture at least every 48 hours.

1. Conservation on Chocolate agar slopes (supplied by NHLS DMP)

* Strains keep viable during transportation up to 5 days
* Inoculate an 18-24 hour pure culture (**heavy inoculum**) of a ceftriaxone-resistant isolate onto a chocolate agar slope in a screw-capped Bijou bottle.
* Incubate with the screw-cap loosened for a minimum of 24 hours at 36 + 1°C for a minimum 24 hours in CO2 incubator.
* Completely overlay the agar slope with sterile liquid paraffin and store at 37C in CO2 for a further 24 hours; then transport at room temperature.
* *NB: These slopes are prone to contamination, so careful handling when inoculating, and flaming of lid prior to closing bottle is required.*

1. Conservation by freezing prior to transport

* Inoculate all growth from an 18-24 hour pure culture of a ceftriaxone-resistant isolate into a vial containing trypticase soy broth and 10% glycerol (supplied by DMP). Immediately store at -20°C (for transport and storage for up to 3 months). Transport on ice to reference laboratory.

***Use both of the above methods of conservation for a ceftriaxone or cefixime resistant isolate***

Additional (optional) method:

* Inoculate all growth from an 18-24 hour pure culture of a ceftriaxone-resistant isolate in a small vial containing microbank fluid with cryobeads (Microbank vials supplied by Davies Diagnostics), suspend by inverting 5 times, then remove as much liquid as possible before freezing at **-70°C** (for transport and longterm storage)

**MEDIA ORDER (NHLS DMP)**

|  |  |  |  |
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| **Agar** | **Oracle Order Number** | **Lead time to Supply** | **Shelf-life** |
| GC agar + 1% isovitalex | DMPC0311 | 10 days | 2 months |
| Chocolate agar slope | DMPW0046 | 3-5 days (contact Valentino Horne, DMP Green Point: valentino.horne@nhls.ac.za) | 3 months |
| Trypticase soy broth + 10% glycerol | DMPC0876 | 10 days | 1 year |

**TRANSPORT OF CEFTRIAXONE-RESISTANT GONOCOCCAL ISOLATES TO NICD STI REFERENCE LABORATORY**

Transport isolates that have been conserved appropriately to the NICD STI Reference laboratory in Johannesburg via NHLS transport or courier services. Ship isolates from Monday to Thursdays only. Send isolates together with the completed laboratory information form (**Appendix 1**).

The completed laboratory information form should also be scanned and e-mailed to the following persons at the NICD STI Reference laboratory:

Venessa Maseko (Laboratory Manager): [venessam@nicd.ac.za](mailto:venessam@nicd.ac.za)

Etienne Muller (Senior Scientist): [etiennem@nicd.ac.za](mailto:etiennem@nicd.ac.za)

**CONFIRMATION OF CEFTRIAXONE-RESISTANT GONORRHOEA AND PUBLIC HEALTH ACTION**

The NICD STI Reference laboratory will perform additional identification and confirmatory tests for ceftriaxone-resistance in clinical isolates of *N. gonorrhoeae*. Once confirmed, appropriate patient management advice will be conveyed. Laboratories will be asked to contact the attending healthcare provider or infection control personnel for completion of the Clinical Case Investigation Form (**Appendix 2**).

The completed Clinical Case Investigation Form should also be scanned and e-mailed to the following persons at the NICD STI Reference laboratory:

Venessa Maseko (Laboratory Manager): [venessam@nicd.ac.za](mailto:venessam@nicd.ac.za)

Etienne Muller (Senior Scientist): [etiennem@nicd.ac.za](mailto:etiennem@nicd.ac.za)

The NICD will facilitate partner contact tracing and relevant public health action based on the information provided on the Clinical Case Investigation Form.

**References**

1. Goire N, Lahra MM, Chen M, Donovan B, Fairley CK, Guy R, et al. Molecular approaches to enhance surveillance of gonococcal antimicrobial resistance. Nature reviews Microbiology. 2014;12(3):223-9
2. National Health Act, 2003 (Act No 61 of 2003): Regulations relating to the surveillance and the control of notifiable medical conditions
3. Kularatne R, Maseko V, Gumede L et al. *Neisseria gonorrhoeae* antimicrobial resistance surveillance in Gauteng Province, South Africa. NICD Communicable Diseases Surveillance Bulletin 2016; 14 (3): 57-65
4. Lewis DA, Sriruttan C, Muller EE, et al. Phenotypic and genetic characterization of the first two cases of extended-spectrum-cephalosporin-resistant *Neisseria gonorrhoeae* infection in South Africa and association with cefixime treatment failure. J Antimcrob Chemother. 2013;68(6):1267-70.
5. NHLS SOP NIC 0597: Etest minimum inhibitory concentration (MIC) determination (STIRC)
6. European committee on antimcrobial susceptibility testing breakpoint tables for interpretation of MICs and zone diameters, version 11.0, 2021
7. Clinical and Laboratory Standards Institute M100, 29th Ed 2019. Performance standards for antimicrobial susceptibility testing
8. WHO 2013. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus.

**Appendix 1:** Laboratory Notification Form to be sent with ceftriaxone-resistant *N. gonorrhoeae* isolate to STI Reference Laboratory, NICD

**Appendix 2:** Clinical Case Investigation Form to be completed by clinician for management advice and public health action

**Notifier details**

**(Laboratory staff)**

Name and Surname:



Designation:



Hospital/Clinic:



Address:



Province: **Choose an item.**

Tel:



Cell:



Email:



Alternative contact details:



**Patient details**

Patient name:



Date of birth:



Age:



Sex:



Hospital number:



Referring laboratory:



**Specimen and testing details**

Specimen type:



Date specimen collected:

**Click or tap to enter a date.**

Specimen laboratory number:



Assays/tests used for ID confirmation

(please list all):



Assay/test used for AST:

**Choose an item.**

**AST results**

**EUCAST MIC by E-test (µg/ml)**

Antibiotic / Breakpoint

**Choose an item. Choose an item.**

**Choose an item. Choose an item.**

**Choose an item. Choose an item.**

**Disc diffusion (mm)**

Antibiotic / Zone diameter (mm)

**Choose an item.**



**Choose an item.**



**Choose an item.**



**Notifying health care provider’s details**

Name and surname:



Health Care Facility:



Designation:



Contact number (landline):



Mobile number:



SANC/HPCSA number:



**Patient demographics**

Full name



Surname



SA ID number



Passport/other ID number



Citizenship



Date of birth



Age



Gender



Sexual orientation



Hospital number



Is patient pregnant?



Residential address



Patient contact number



Alternative contact details



**Medical condition details**

Date of symptom onset

**Click or tap to enter a date.**

Date of diagnosis

**Click or tap to enter a date.**

Patient admission status



Clinical symptoms relating to the condition



Treatment given for condition



**Past medical history**

Was patient previously treated for this condition in past 3 months?



Previous treatment given, if applicable



Did condition persist (not resolve) despite treatment?



Were sexual partners notified/treated for this condition?



**Sexual history**

Number of regular sexual partners in past 3 months



Number of casual sexual partners in past 3 months



**Travel history**

Did the patient travel outside of usual place of residence?



Place travelled from: Place travelled to:



Date patient left residence: Date patient returned to residence:

**Click or tap to enter a date.** **Click or tap to enter a date.**

**Specimen details**

Was a specimen collected?



Specimen type collected, if applicable



Date of specimen collection, if applicable

**Click or tap to enter a date.**

Specimen barcode/lab number



**Acknowledgement of Reading Form**

***My signature confirms that I have read and understood the content of this document and relevant kit insert (where applicable).***

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| **Name** | **Signature** | **Date** |
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**Note to the Quality Rep: -** This form must be filed for 5 years to provide audit traceability.