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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMR</td>
<td>antimicrobial resistance</td>
</tr>
<tr>
<td>AST</td>
<td>antimicrobial susceptibility testing</td>
</tr>
<tr>
<td>CC</td>
<td>clonal complexes</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention (United States)</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical Laboratory and Standards Institute</td>
</tr>
<tr>
<td>DTA</td>
<td>Data Transfer Agreement</td>
</tr>
<tr>
<td>ECOFF</td>
<td>epidemiological cut-off</td>
</tr>
<tr>
<td>ENA</td>
<td>European Nucleotide Archive</td>
</tr>
<tr>
<td>EG</td>
<td>extragenital</td>
</tr>
<tr>
<td>EGASP</td>
<td>WHO Enhanced Gonococcal Antimicrobial Surveillance Programme</td>
</tr>
<tr>
<td>ETEST</td>
<td>Epsilometer-Test</td>
</tr>
<tr>
<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
</tr>
<tr>
<td>GARLRN</td>
<td>Global Antimicrobial Resistance Laboratory and Research Network</td>
</tr>
<tr>
<td>GASP</td>
<td>WHO Gonococcal Antimicrobial Surveillance Programme</td>
</tr>
<tr>
<td>GLASS</td>
<td>Global Antimicrobial Resistance and Use Surveillance System</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>ID</td>
<td>identifiers</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular route</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>IT</td>
<td>information technology</td>
</tr>
<tr>
<td>MDR</td>
<td>multidrug-resistant</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MLST</td>
<td>multilocus sequence typing</td>
</tr>
<tr>
<td>MSM</td>
<td>men who have sex with men</td>
</tr>
<tr>
<td>MTA</td>
<td>Material Transfer Agreement</td>
</tr>
<tr>
<td>NG</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td>PO</td>
<td>per os (oral route)</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>NAAT</td>
<td>nucleic acid amplification test</td>
</tr>
<tr>
<td>NG-STAR</td>
<td>N. gonorrhoeae Sequence Typing for Antimicrobial Resistance</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SOP</td>
<td>standard operational procedure</td>
</tr>
<tr>
<td>ST</td>
<td>sequence typing</td>
</tr>
<tr>
<td>STD</td>
<td>sexually transmitted disease</td>
</tr>
<tr>
<td>STI</td>
<td>sexually transmitted infection</td>
</tr>
<tr>
<td>TOC</td>
<td>test of cure</td>
</tr>
<tr>
<td>WGS</td>
<td>whole genome sequencing</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHOCC</td>
<td>WHO collaborating centre</td>
</tr>
<tr>
<td>XDR</td>
<td>extensively drug-resistant</td>
</tr>
</tbody>
</table>
1. Introduction
1. Introduction

Widespread antimicrobial resistance (AMR) in highly variable strains of Neisseria gonorrhoeae continues to cause significant public health concerns and compromise the management and control of gonorrhoea. The Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) aims to strengthen sentinel surveillance for gonococcal AMR in selected countries. EGASP is a collaboration between World Health Organization (WHO), the United States Centers for Disease Control and Prevention (CDC) and other WHO collaborating centres (WHOCC). This standardized enhanced surveillance monitors trends in antimicrobial susceptibility in N. gonorrhoeae by using sampling and laboratory protocols in selected countries. This surveillance approach allows collaborators to improve the quality, comparability and timeliness of gonococcal AMR data across multiple countries. It also aims at enhancing the capacity of early detection and reporting of N. gonorrhoeae strains with elevated minimum inhibitory concentrations (MICs) to the internationally recommended treatment for gonorrhoea, and of emerging or novel mechanisms of resistance to new antibiotics for gonorrhoea at the national and global levels.

Data and country experiences from EGASP implementation have shown a need to develop three essential supplementary protocols that play pivotal roles in the management of N. gonorrhoeae infections. These protocols are designed to address distinct aspects of the infection control process, each with its unique significance. They are part of the enhanced gonococcal surveillance and are annexed to the general protocol:

- **Treatment failure protocol**: The rising incidence of antibiotic-resistant strains of N. gonorrhoeae has necessitated a proactive approach to managing treatment failures. This protocol will outline strategies for identifying and managing cases where initial treatment proves ineffective, emphasizing the importance of surveillance, antimicrobial susceptibility testing (AST) and alternative treatment options.

- **Extragenital sampling protocol**: The emergence of extragenital infections with N. gonorrhoeae has heightened the need for accurate sampling techniques beyond traditional genital sites. This protocol will provide guidance on proper specimen collection from extragenital sites such as the rectum and pharynx, enabling health workers to effectively diagnose and monitor infections that may otherwise go undetected. This complementary sampling tool will allow a complete surveillance of pathogen resistance, amid reports of decreased susceptibility in the pharyngeal and/or rectal anatomic sites.

- **Whole genome sequencing (WGS) framework**: WGS has emerged as a powerful tool in understanding the genetic diversity and mechanisms of AMR in N. gonorrhoeae. This protocol will guide health workers through the process of conducting WGS, data analysis, and interpretation, thereby facilitating a deeper understanding of the pathogen’s evolution and informing targeted intervention strategies.

By mastering these protocols, health workers, researchers and public health officials can contribute to a more effective and informed approach to combat N. gonorrhoeae infections. In the following sections of this manual, each protocol will be described in detail, accompanied by concise instructions based on best practices and relevant countries’ experiences. Together, these protocols serve as invaluable tools in the ongoing battle against this STI, helping to safeguard public health and advance our knowledge of N. gonorrhoeae. Implementing effective strategies for diagnosis, treatment and monitoring of N. gonorrhoeae is crucial to combat the ever-evolving challenges posed by this pathogen.

---

2. Best practices in the management of suspected treatment failure for gonorrhoea: general protocol
2. Best practices in the management of suspected treatment failure for gonorrhoea: general protocol

2.1 Assessment after treatment

Assessment after treatment is useful to detect treatment failure and emerging antimicrobial resistance (AMR), ensure resolution of signs and symptoms, confirm compliance with treatment, enquire about adverse events, take note of sexual history and explore the possibility of re-infection, and offer partner notification and health promotion (1, 2).

2.2 Test of cure (TOC)

TOC is an effective strategy to prevent further transmission and, in the case of an AMR strain of N. gonorrhoeae, further transmission of AMR. TOC involves follow-up retesting to ensure the initial infection has been treated and cured successfully. In the absence of risk of reinfection, all patients with a positive TOC should be considered as treatment failure.

Although the strategy remains unrealistic in most settings, ideally, all patients diagnosed with gonorrhoea (urogenital and extragenital) should be advised to return for TOC (especially if they have persistent signs and/or symptoms) (1).

2.3 When should I perform a TOC?

Extra emphasis (3) should be given to patients who:

- have persistent signs and/or symptoms;
- are treated for oropharyngeal infection;
- are treated with any medication different from the first-line regimen recommended in evidence-based guidelines by WHO or guidelines by regional or national authorities.

TOC should be considered in the following additional circumstances (4, 5):

- infection with N. gonorrhoeae with decreased susceptibility to extended-spectrum cephalosporins (or sexual contact with a person infected with N. gonorrhoeae with decreased susceptibility to extended-spectrum cephalosporins);
- in a patient with prior treatment failure;
- after treatment, when a patient has been exposed to an untreated partner.
2.4 Testing strategies for TOC

**Symptomatic patients:** When signs and/or symptoms persist after treatment, culture is recommended **three to seven days after completion of therapy**, possibly supplemented two to three weeks after completion of therapy with a nucleic acid amplification test (NAAT) if culture is negative. Antimicrobial susceptibility should be assessed for all *N. gonorrhoeae* positive-culture patients.

**Asymptomatic patients:** TOC should be performed with NAAT two to three weeks after completion of treatment (so residual nucleic acid from non-viable gonococci have been eliminated). Ideally, all TOC-positive patients should have swabs for culture and antimicrobial susceptibility testing (AST) should be assessed in all *N. gonorrhoeae* culture-positive patients.

Treatment failure is suspected in patients who have a positive test result (true positive TOC), but positive results may also be due to reinfection or residual nucleic acid from non-viable gonococci when NAAT has been performed (false positive TOC). Thus, a TOC should always be interpreted in the clinical context (1, 5-7).

2.5 Treatment failure case definition

**Possible treatment failure:**

1. A patient who returns for a TOC or who has persistent or recurrent signs and/or symptoms (e.g. 3–5 days (5, 6)) after having received treatment for laboratory-confirmed gonorrhoea with a first-line regimen, including an extended-spectrum cephalosporin antibiotic

**AND**

2. Remains positive for one of the following tests for *N. gonorrhoeae*:
   - isolation of *N. gonorrhoeae* by culture taken at least 72 hours after completion of treatment, OR
   - positive NAAT from specimens collected two to three weeks after completion of treatment

**AND**

3. No history of unprotected sexual contact at the anatomic site of infection of the suspected treatment failure during the post-treatment follow-up period.

**Confirmed treatment failure:**

1. A patient fulfilling the possible treatment failure case definition (all 3 criteria proposed above) **AND**

2. Decreased susceptibility to cephalosporin used for treatment:
   - cefixime: minimum inhibitory concentration (MIC) > 0.250 mg/L
   - ceftriaxone: MIC > 0.125 mg/L.

For confirmed treatment failure, when possible, pre- and post-treatment isolates should be examined by whole genome sequencing (WGS) to confirm an indistinguishable genome sequence and the presence of AMR determinants for the antimicrobial used for treatment (11-13).

2.6 Management of Treatment Failure

Treatment failures should be managed according to WHO guidelines; therapy should be guided by the results of the AST when available (14, 15). The management includes patient counselling, partner notification and treatment of contacts. TOC in the follow-up of re-treatment is imperative.

**Patient counselling:** Patients should be given a detailed explanation of their condition with particular emphasis on the health implications for themselves and their sexual partners. Although gonococci are eliminated from the genital tract soon after the completion of treatment (16), patients with gonorrhoea are generally counselled to avoid sexual activity for 14 days when using dual therapy including azithromycin or doxycycline (17). This is to limit possible re-exposure in the presence of residual azithromycin (1). Patients should only resume having sex after completion of therapy and, ideally, sex partners have been treated (5). As infections with one STI increases the risk of infection with others, an individual diagnosed with gonorrhoea (and their sexual partners) should be offered testing for other STIs, including HIV, chlamydia, and syphilis (18).

**Partner notification:** Where feasible, the following partners should be notified (3):

- all sexual contacts within the preceding two weeks for patients with symptomatic urethral gonococcal infection;
- all sexual contact within the preceding three months for patients with infection at other anatomic sites (e.g. pharynx or rectum) or asymptomatic infection.

**Treatment of contacts:** All notified sexual contacts should be offered testing and receive counselling for gonorrhoea and other STIs. Ideally, treatment should only be given to those partners with positive test results for *N. gonorrhoeae* or when the clinician considers that contact will not return for treatment when testing results are available (3, 19, 20).
2.7 Reporting

All cases of treatment failure with the recommended first-line treatment regimen should be accurately recorded and reported to the relevant local or national district level as soon as possible. These reports will form part of the early warning system and should alert the authorities to the possibility of AMR in the community. A more systematic investigation should be initiated in collaboration with a national reference laboratory and/or the WHO regional or international network of the Gonococcal Antimicrobial Surveillance Programme (GASP) or WHO Enhanced GASP (EGASP). This reporting will allow clinicians and public health authorities to (i) monitor the spread of AMR gonococcal strains in a timely way, (ii) recognize factors leading to AMR, (iii) identify populations associated with increased AMR and (iv) facilitate further control interventions (9).

When a case of confirmed infection with *N. gonorrhoeae* with decreased susceptibility to extended-spectrum cephalosporin is identified at a clinic, the clinician and local health authority should collect additional clinical and epidemiological information from the index patient and their sexual partners, using a standard data-collection form. Based on existing standard (8), a proposition of notification form is presented in Box 2.1.

**Box 2.1 Proposed structure for the notification form for treatment failure cases**

<table>
<thead>
<tr>
<th>Treatment classification and reporting details</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Confirmed or possible treatment failure</td>
</tr>
<tr>
<td>• Date of notification</td>
</tr>
<tr>
<td>• Name and contact of reporting body</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case details</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Age</td>
</tr>
<tr>
<td>• Sex at birth and gender identity</td>
</tr>
<tr>
<td>• Antibiotic use in the past two weeks</td>
</tr>
<tr>
<td>• Travel history</td>
</tr>
<tr>
<td>• Gender of sex partner(s) (last 30 days)</td>
</tr>
<tr>
<td>• Number of sex partners (last 30 days)</td>
</tr>
<tr>
<td>• Sexual behaviour characteristics (last 30 days)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnostic and treatment at the first visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Date of first visit</td>
</tr>
<tr>
<td>• Signs and symptoms</td>
</tr>
<tr>
<td>• Site(s) of infection</td>
</tr>
<tr>
<td>• Anatomic site(s) being sampled</td>
</tr>
<tr>
<td>• AST results (MIC for EGASP targeted antibiotics)</td>
</tr>
<tr>
<td>• Treatment prescribed (drug, dosage, route, duration of treatment)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnostic and treatment at the follow-up visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Date of follow-up visit</td>
</tr>
<tr>
<td>• Anatomic site(s) being sampled</td>
</tr>
<tr>
<td>• Signs and symptoms at the follow-up visit</td>
</tr>
<tr>
<td>• If culture performed: available MIC</td>
</tr>
<tr>
<td>• Treatment prescribed (drug, dosage, route, duration of treatment)</td>
</tr>
<tr>
<td>• TOC performed after retreatment: specific test used, result</td>
</tr>
</tbody>
</table>
Box 2.2 International guidelines and protocols


References for section 2


3. Extragenital specimen collection for EGASP: general protocol
3. Extragenital specimen collection for EGASP: general protocol

3.1 Introduction

- Unlike urethral gonococcal infections, most anorectal and oropharyngeal gonococcal infections are asymptomatic (1,2,3). When a symptomatic infection in the anorectum occurs, symptoms include tenesmus, anorectal pain, rectal fullness, constipation, anorectal bleeding and mucopurulent discharge (4). The infection is usually acquired through anal receptive intercourse.
- The prevalence of rectal gonococcal infections usually ranges between 0.2% to 24% (median 5.9%) among men who have sex with men (MSM), and from 0.6% to 35.8% (median 1.9%) among women (5). These findings are particularly concerning since rectal gonococcal infection in MSM is associated with a threefold increase in the risk of contracting an HIV infection and an increased risk of circulating antibiotic-resistant \( N. \text{ gonorrhoeae} \) (6).
- Although most oropharyngeal gonococcal infections are asymptomatic, sore throat or pharyngeal exudates are present in some cases (3, 7). The infection is usually acquired through receptive oral intercourse. The prevalence usually ranges between 0.5% and 17% (median 5%) among MSM, and from 0% to 29.6% (median 2.1%) among women (5). Cross-reactivity when using molecular diagnostics for gonorrhoea may occur with commensal Neisseria species present in the pharynx, and the exchange of material between Neisseria species can lead to circulating drug-resistant strains of gonorrhoea. This underscores the importance of identifying and treating these infections in high-risk groups likely to transmit to a large number of sexual contacts (8, 9).

3.2 Objectives

At the country level, the objectives of extragenital specimen collection for EGASP are:

- to monitor trends in antimicrobial susceptibility in \( N. \text{ gonorrhoeae} \) using standardized protocols for the systematic inclusion of patients and quality-assured laboratory testing;
- to characterize epidemiological, clinical and laboratory characteristics of men with extragenital gonorrhoea at selected sentinel sites, especially those with \( N. \text{ gonorrhoeae} \) with reduced susceptibility to recommended antimicrobial agents;
- to strengthen the surveillance of antimicrobial-resistant strains of \( N. \text{ gonorrhoeae} \).

3.3 Surveillance setting

3.3.1 Eligibility criteria and informed consent

- EGASP general protocol systematically includes symptomatic men attending participating clinics with a suspected urogenital gonorrhoea episode (such as the presence of urethral discharge). Because of the significant prevalence of extragenital infections in MSM and the frequency of asymptomatic infections, routine screening for oropharyngeal and rectal gonorrhoea is warranted in MSM (3, 10-13). Where feasible, all adult (aged > 18 years) MSM (with or without signs or symptoms of urogenital and/or extragenital infection) are eligible for surveillance.
3.3.2 Sentinel sites

- Sentinel sites are selected health-care facilities with a known higher volume of extragenital gonorrhoea cases compared to other health-care facilities. Usually, these are outpatient facilities or STI clinics that provide care, sexual health, and STI prevention and testing services to MSM.

- Sentinel sites should be able to: (i) collect specimens from extragenital sites of infection (for culture and molecular assays); (ii) transport, store and maintain the viability of these specimens until reception at the designated reference laboratory (which will perform the AST); and (iii) collect, enter and maintain the core demographic, behavioural and clinical data elements (14).

3.3.3 Sample size

- The usual target of EGASP surveillance is ideally to include at least 100 urogenital gonococcal cases, per year and clinic. As gonorrhoea usually accounts for up to 50% of cases of acute urethritis, it has been suggested to enrol at least 200 urethritis cases yearly to achieve the key objectives of EGASP.

- The prevalence of extragenital gonorrhoea in MSM typically falls within the range of 10 to 15% (5, 15-19). It is estimated that the inclusion of 200 MSM in extragenital surveillance would allow the enrolment of 20–30 gonococcal cases per year, based on NAAT results. However, it is important to note that only approximately half of those will yield a positive culture result, which is the sole method that allows for AST. This consideration takes into account the relatively lower sensitivity of bacterial culture when performed on extragenital samples.

- The results of the tests performed on extragenital samples will be combined with those performed on urogenital samples, to have a higher total number of gonorrhoea samples to be used for the estimation of \( N. \ gonorrhoeae \) susceptibility to antimicrobials.

The prime objective of EGASP will then be achieved, despite the relatively limited number of extragenital gonococcal cases.2

3.3.4 Data collection

See detailed procedures in pages 8–9 of the EGASP general protocol (14).

3.4 Diagnostic techniques

Diagnosis is established by the detection of \( N. \ gonorrhoeae \) at an infected site, either by a nucleic acid amplification test or by culture. Because of its low sensitivity and specificity, Gram stain is not recommended for the testing of extragenital specimens since other Gram-negative diplococci can be present at anorectal and pharyngeal testing sites (20, 21).

3.4.1 Culture

Methods

Culture specimens should be obtained using appropriate swabs such as plastic or wire shafts with rayon, Dacron, or calcium alginate tips. Other materials (e.g. wood shafts and cotton-tipped swabs) may be toxic to \( N. \ gonorrhoeae \). Rectal specimens may be collected by inserting the swab 3–4 cm into the rectal vault and rotating it clockwise for 5–10 seconds to sample the anal crypts. Pharyngeal specimens are taken by extending the swab between the tonsillar pillars and the posterior pharyngeal wall, and sweeping it back and forth across the posterior pharynx, both tonsillar areas (including the crypts) and any inflamed or ulcerated areas (22).

The sample swab should be plated and streaked onto a selective growth medium3 that supports \( N. \ gonorrhoeae \) growth (e.g. Thayer-Martin agar). Within two hours of inoculation, gonococcal cultured plates should be incubated at conditions conducive to gonococcal growth. The plates should be then transported within 18–24 hours of inoculation to the appointed reference laboratory for isolation, identification and AST. For EGASP, AST is mandatory for cefixime, ceftriaxone and azithromycin4 (14, 23).

---

2 The achievement of the secondary objectives (risk factor analysis, estimation of gonorrhoea prevalence per anatomical site, epidemiological characterization of cases, etc.) will require disaggregated analyses conducted within subgroups. Larger samples of MSM (up to 1000) will be needed. Such a sample size remains difficult to achieve for most sites, and it is proposed to conduct such sub-analyses on aggregated data (from several sites or countries, at the regional level).

3 Alternatively, depending on the setting and resources, a non-growth promoting transport medium can also be used.

4 Detailed procedures related to specimen collection and handling, isolation, and identification of \( N. \ gonorrhoeae \) and antimicrobial susceptibility testing can be found in pages 5–7 of the EGASP protocol.
3. Extranegital specimen collection for EGASP: general protocol

### Performance

The bacterial culture of *N. gonorrhoeae*, including appropriate species confirmation tests, has a test specificity of more than 99%, the highest of all testing methods, and is the only diagnostic method that enables AST. The reported sensitivity ranges from 50% up to 92%; however, the sensitivity is also lower in asymptomatic and rectal and pharyngeal infections, and is reduced with suboptimal transport time (i.e. exceeding 24–48 hours) (24–26).

The major disadvantage to culture is that the specimen needs to be inoculated immediately onto the appropriate medium and/or transported rapidly to the microbiology laboratory; the results are not available to the clinician until 48 hours later. The costs are quite moderate in comparison with more expensive molecular techniques. However, this must be weighed against the strict requirements for specimen transportation, time constraints, and the fastidious nature of the organism that can result in false negative results (3).

#### 3.4.2 Nucleic acid amplification testing (NAAT)

#### Methods

The use of several commercial NAAT for the detection of *N. gonorrhoeae* at rectal and pharyngeal sites has now been cleared by the U.S. Food and Drug Administration. NAAT methodology consists of amplifying *N. gonorrhoeae* DNA or RNA sequences using various techniques, such as polymerase chain reaction, transcription-mediated amplification, or strand displacement amplification. Specific procedures and specimen collection kits should be used according to the manufacturer’s instructions. NAAT cannot be used for AST, and is not ideal for test of cure (TOC), as it can result in false positive results from samples that contain DNA or RNA from non-viable bacteria, sometimes more than two weeks post-treatment (23,27-28)

#### Performance

Several studies have demonstrated that NAAT has a significantly higher sensitivity (usually between 92% and 97.2%) than culture for symptomatic and asymptomatic infections, especially for rectal and pharyngeal infections (29-32). This high sensitivity of NAAT for *N. gonorrhoeae* is less affected by suboptimal transport times and other conditions that affect organism viability for culture. NAAT specificity (usually 96.1–99.8%) is slightly lower than culture, leading to a higher risk of false positive results (24).

In Europe and some other settings, it is estimated that if the positive predictive value (PPV) is less than 90% in the target population or for rectal and pharyngeal specimens, it is essential to add a supplementary molecular test to the care pathway (12), This is especially important for oropharyngeal infections. Cross-reactivity may occur with non-gonococcal Neisseria species present in the pharynx. When feasible, a minimally cross-reactive initial pharyngeal NAAT should be used (22). Otherwise, all initial NAAT positive test results must be confirmed with an alternative molecular assay (33).

#### Proposed testing strategies

For the diagnosis of extragenital gonorrhoea, as the infection is mostly asymptomatic, collection of specimens from each extragenital site of infection (rectum and pharynx) is required. Several strategies are possible, depending on the context and the resources available in each country:

- **Strategy 1:** Collection of four specimens (two from the rectum and two from the pharynx) at first contact with the patient; two will be used for NAAT analysis, and two for culture (and AST if positive). Under this strategy, NAAT and culture will be performed simultaneously in all eligible patients.

- **Strategy 2:** Collection of four specimens (two from the rectum and two from the pharynx) at first contact with the patient; two will be used for NAAT analysis, and two to be collected on the swab-based transport system and kept in case there is a need to perform culture. Under this strategy, culture (and AST if positive) will be performed only when there is an NAAT positive result. This strategy requires NAAT to be quickly performed and reported (so that culture can be performed within a maximum of 24 hours).

- **Strategy 3:** Collection of two specimens (one from the rectum and one from the pharynx) to be used for NAAT analysis. Patients with positive NAAT results will be recalled for the collection of two additional specimens to be used for culture (and AST if positive).

Due to its higher sensitivity, NAAT analysis will be conducted for all eligible patients. Positive NAAT results will be confirmed by culture and processed for AST.

The final approach used for *N. gonorrhoeae* testing will be influenced by the clinical setting, storage and transport system to the laboratory, the local prevalence of infection and the range of tests available in the laboratory.

---

5 If a population with a true prevalence of 1% were tested with a hypothetical NAAT screening test with a sensitivity and specificity of 99%, this would result in a PPV of 50% (meaning detection of 50 unconfirmed diagnoses [false positive] for every 100 initial positive NAAT results). In this example, gonorrhoea prevalence would need to be at 8% or above before the PPV reaches the “acceptable” value of 90% when using single unconfirmed NAAT testing (34).
3.5 Treating and managing patients

Participation in EGASP does not imply any difference in patients’ care. Regardless of participation in EGASP, all men with a complaint and/or laboratory confirmation of gonococcal infection should receive appropriate antimicrobial treatment and should be managed according to national STI management guidelines and by WHO recommendations. All patients should be counselled that their recent sex partners should be evaluated and treated. The patients should also be advised to return to the clinic for a follow-up for TOC, especially if symptoms are not resolved. Based on the TOC result, potential treatment failure should be investigated (see detailed procedures in the treatment failure section of the general protocol (14)).

3.6 EGASP data

EGASP collects patient-based surveillance data as part of routine clinical and laboratory activities. EGASP variables are categorized as core and optional. Core variables are required to be collected by each participating EGASP country; these data ensure that the objectives of surveillance can be met. If a country is able and has the resources to allow it, collecting optional variables, which provide additional information and are insightful in identifying risk behaviour and groups at the highest risk for antimicrobial-resistant gonorrhoea, is recommended (see detailed procedures in pages 8–12 of the EGASP general protocol). A proposal for the data collection form is provided in Box 3.1.

Box 3.1 EGASP extragenital surveillance data collection form

* variable is mandatory

<table>
<thead>
<tr>
<th>General information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country* ______________________</td>
</tr>
<tr>
<td>Sentinel clinic (code)*__________________________</td>
</tr>
<tr>
<td>EGASP extragenital (EG) surveillance enrolment date*: dd/mm/yyyy</td>
</tr>
<tr>
<td>EGASP-ID (episode identification number) *: ________________</td>
</tr>
</tbody>
</table>

Eligibility

- Male patient (MSM or not) presenting with urethral discharge ➔ eligible for EGASP surveillance
- MSM patient presenting without urethral discharge ➔ eligible for EGASP EG surveillance

*(MSM is defined as men who had sex with another with men during the past 12 months)*

<table>
<thead>
<tr>
<th>Patient information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient ID number: ____________________ *</td>
</tr>
</tbody>
</table>

Type of patient*:
- O New EGASP patient (the patient is included in EGASP for the first time)
- O Established EGASP patient (the patient has been previously included in EGASP)

Type of specimen(s) collected*:
- Urethral: □ No □ Yes
- Oropharyngeal: □ No □ Yes
- Anorectal: □ No □ Yes

Sex at birth*: Male

Gender identity*:
- □ Male
- □ Female
- □ Other (transgender, non-binary, gender fluid, etc.)
- □ Unknown

Age (please enter 999 if the age is unknown)*: ________________
**Box 3.1 (continued) EGASP extragenital surveillance data collection form**

*variable is mandatory*

<table>
<thead>
<tr>
<th>Place of birth of the patient:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born in country</td>
</tr>
<tr>
<td>Born outside the country</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Clinical information**

<table>
<thead>
<tr>
<th>Symptoms of gonorrhoea (select all the options that apply)*</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethral discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorectal discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discharge not specified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysuria <em>(painful urination)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspareunia <em>(painful intercourse)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower abdominal pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorectal pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenesmus <em>(feeling like you need to pass stool, even when no stools are present)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorectal bleeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If other, please specify: …………………………………………………………………………………………………

**Diagnosis at current EGASP visit***:  
- Screening of asymptomatic patient  
- Suspected gonococcal urethritis  
- Suspected non-gonococcal urethritis  
- Suspected anorectal gonococcal infection  
- Suspected oropharyngeal gonococcal infection  
- Other  
- Unknown  

If other, please specify: __________________________________

**Primary treatment for gonorrhoea***:  
- None/not given  
- Ceftriaxone intramuscular route (IM), 250 mg  
- Ceftriaxone IM, 500 mg  
- Ceftriaxone IM, 1 g  
- Cefixime per os (PO), 400 mg  
- Cefixime PO, 800 mg  
- Azithromycin PO, 1 g  
- Azithromycin PO, 2 g  
- Other  
- Unknown/not documented
Box 3.1 (continued) EGASP extragenital surveillance data collection form

* variable is mandatory

If other, please specify the name and dosage of other drugs: _____________________________

Second antibiotic used as part of dual therapy for gonorrhoea (and treatment of chlamydia or/and non-gonococcal infection)*:

- None/not given
- Azithromycin IM, 1 g
- Azithromycin IM, 2 g
- Doxycycline PO, 100 mg, two times daily for 7 days
- Doxycycline PO, 200 mg (Doxy PEP)
- Tetracycline PO, 500 mg, four times daily for 7 to 10 days
- Other antibiotics or posology

Specify the name and dosage of other drugs: _____________________________

- Unknown/not documented

Outcome of treatment for patient*:

- Treatment completed
- Partial treatment completed
- Not treated – patient refused treatment
- Not treated – patient never came back for treatment

Outcome of follow-up visit*:

- Returned to the clinic with symptoms
- Returned to the clinic without symptoms
- No follow-up visit

A first test of cure (TOC) was performed after the completion of the appropriate treatment*:

- Yes, based on culture
- Yes, based on NAAT
- Not performed

Result of the first TOC*:

- Positive
- Negative

A second TOC was performed (only if the first TOC had a positive result)*:

- Yes, based on culture
- Yes, based on NAAT
- Not performed

Result of the second TOC*:

- Positive
- Negative

Comment on positive TOC:
-----------------------------------------------------------------------------------------
-----------------------------------------------------------------------------------------
**Box 3.1 (continued) EGASP extragenital surveillance data collection form**

*variable is mandatory*

### Epidemiological information

#### Antibiotic use in the past 2 weeks:
- ○ Yes
- ○ No
- ○ Unknown

*If yes, please specify the name of the antibiotic(s): __________________________*

#### History of travel (within the last 30 days):
- ○ Within country
- ○ Outside the country
- ○ Both, within and outside
- ○ No travel
- ○ Unknown

#### History of sex partner(s) in the last 30 days:
- ○ Women only
- ○ Men only
- ○ Women and men
- ○ Unknown

*Number of sexual partners in the past 30 days (please enter 999 if unknown): ___________

#### Country of residency of sexual partners from the last 30 days:
- ○ Living in the country
- ○ Living in another country
- ○ Both (has partner(s) living in the country and other(s) living in other country(ies))
- ○ Unknown

### Sexual behaviour characteristics in the last 30 days

*(patient as the reference, select all the options that apply)*

<table>
<thead>
<tr>
<th>Sexual behaviour</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal/urethral insertive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal receptive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal insertive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal not specified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral receptive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral insertive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral not specified</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Presence of other STIs:
- ○ Yes
- ○ No
- ○ Unknown

*If yes, please select all that apply*

<table>
<thead>
<tr>
<th>Presence of other STIs</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital ulcer disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anogenital warts</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Box 3.1 (continued) EGASP extragenital surveillance data collection form**

* variable is mandatory

<table>
<thead>
<tr>
<th>Pathogen/Infection</th>
<th>Lab positive</th>
<th>Lab negative</th>
<th>Not tested/unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma genitalium</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genital HSV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lymphogranuloma venereum</em> (LGV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syphilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If other, please specify: ____________________________________________________________

**Laboratory results**

Laboratory (code)*: ______________________

**URETHRAL SPECIMEN**

Date specimen received in the reference laboratory*: mm/dd/yyyy

Date of GRAM stain performed*: mm/dd/yyyy

Gram stain result*:
- □ Gram-negative diplococcus identified
- □ No gram-negative diplococcus identified
- □ Not performed

Specimen quality before culture*:
- □ Acceptable
- □ Contaminated
- □ Non-viable
- □ Improperly transported

Date of culture performed*: mm/dd/yyyy

Culture result*:
- □ *Neisseria gonorrhoeae* positive
- □ *Neisseria gonorrhoeae* negative

Specimen quality before susceptibility testing*:
- □ Acceptable
- □ Contaminated
- □ Non-viable

Date of susceptibility test result*: mm/dd/yyyy

Was the isolate retested because of an alert value*?: □ yes □ no

Date of retest AST result*: mm/dd/yyyy
<table>
<thead>
<tr>
<th>First test</th>
<th>Confirmatory test</th>
<th>Final AST results</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC value*</td>
<td>Alert*</td>
<td>MIC value*</td>
</tr>
<tr>
<td>Cefixime*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Azithromycin*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>□ A □ No</td>
<td>□ A □ No</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>□ A □ No</td>
<td>□ A □ No</td>
</tr>
</tbody>
</table>

* A = Alert Value; HA = High alert value

Date of NAAT performed*: mm/dd/yyyy

Specimen quality before NAAT:
- Acceptable
- Not acceptable

Result of NAAT for NG (Neisseria gonorrhoeae)*:
- NG detected
- NG not detected
- Invalid
- NAAT not performed
- Tick here if the specimen has been included in the WGS analysis (only for WHO to add retrospectively in the analysis)

ANORECTAL SPECIMEN

Date specimen received in the reference laboratory*: mm/dd/yyyy

Date of NAAT performed*: mm/dd/yyyy

Specimen quality before NAAT:
- Acceptable
- Not acceptable

Result of NAAT for NG*:
- NG detected
- NG not detected
- Invalid
- NAAT not performed

Specimen quality before culture*
- Acceptable
- Contaminated
- Non-viable
- Improperly transported

Date of culture performed*: mm/dd/yyyy
**Culture result**:  
- Neisseria gonorrhoeae positive  
- Neisseria gonorrhoeae negative

Specimen quality before susceptibility testing:  
- Acceptable  
- Contaminated  
- Non-viable

Date of susceptibility test result*: mm/dd/yyyy

Was the isolate retested because of an alert value*:  
- yes  
- no

Date of retest AST result*: mm/dd/yyyy

### First test

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC value*</th>
<th>Alerta</th>
<th>MIC value*</th>
<th>Alerta</th>
<th>MIC value*</th>
<th>Alerta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefixime*</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Azithromycin*</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Gentamicin</td>
<td></td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: ________</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Oropharyngeal specimen**

Date specimen received in the reference laboratory*: mm/dd/yyyy

Date of NAAT performed*: mm/dd/yyyy

Specimen quality before NAAT:
- Acceptable  
- Not acceptable

Result of NAAT for NG*:  
- NG detected  
- NG not detected  
- Invalid  
- NAAT not performed

Specimen quality before culture:
- Acceptable  
- Contaminated  
- Non-viable  
- Improperly transported

Date of culture performed*: mm/dd/yyyy

---

* A = Alert Value; HA = High alert value  

[ ] Tick here if the specimen has been included in the WGS analysis  
(only for WHO to add retrospectively in the analysis)
**Culture result**:  
- Neisseria gonorrhoeae positive  
- Neisseria gonorrhoeae negative

**Specimen quality before susceptibility testing**:  
- Acceptable  
- Contaminated  
- Non-viable

**Date of susceptibility test result**: mm/dd/yyyy

**Was the isolate retested because of an alert value**:  
- yes  
- no

**Date of retest AST result**: mm/dd/yyyy

<table>
<thead>
<tr>
<th>First test</th>
<th>Confirmatory test</th>
<th>Final AST results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC value*</td>
<td>Alert*</td>
</tr>
<tr>
<td>Cefixime*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Azithromycin*</td>
<td>□ A □ HA □ No</td>
<td>□ A □ HA □ No</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>□ A □ No</td>
<td>□ A □ No</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: ________</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A = Alert Value; HA = High alert value

Tick here if the specimen has been included in the WGS analysis  
(only for WHO to add retrospectively in the analysis)
Box 3.2 International guidelines and protocols


References for section 3


4. Whole genome sequencing (WGS) framework for EGASP: general protocol
4. Whole genome sequencing (WGS) framework for EGASP: general protocol

4.1 Introduction

International surveillance of the sexually transmitted infection (STI) gonorrhoea and antimicrobial resistance (AMR) in the etiological agent Neisseria gonorrhoeae is imperative. The WHO Gonococcal Antimicrobial Surveillance Programme (GASP) was revitalized in 2009 and has since then provided global gonococcal AMR data (1–3). However, the WHO GASP has limitations that include a low number of isolates in several countries; limited standardization, representativeness and quality assurance; and a lack of clinical and epidemiological data. To improve the surveillance of gonococcal AMR, WHO, in collaboration with the U.S. CDC, developed the WHO Enhanced GASP (EGASP). This EGASP includes a standardized EGASP general protocol and standard operating procedures (SOPs) for collection of clinical and epidemiological information of gonorrhoea patients, laboratory procedures and data reporting (4). The standardized and quality-assured EGASP has also recently developed protocols for inclusion of extragenital samples (oropharyngeal and anorectal samples) and performance of test of cure (TOC), where feasible. The EGASP started in Thailand in 2015 (5); the programme has subsequently enrolled the Philippines, Cambodia, Uganda, South Africa and Malawi. Several additional countries are in the process of enrolment in 2023–2024 (Argentina, Brazil, Côte d’Ivoire, India, Indonesia, Qatar and Zimbabwe).

For improved monitoring of the molecular epidemiology and AMR of *N. gonorrhoeae* strains circulating in the EGASP countries, whole genome sequencing (WGS) has been introduced in the EGASP in 2023. WGS has previously shown to be exceedingly valuable in determining epidemiology and fluctuations in *N. gonorrhoeae* AMR over time in international GASPs such as the European GASP (Euro-GASP) (6–8). WGS has also been successfully implemented in several national GASPs (9-19), including studies performed in many of the current and candidate EGASP countries in collaboration with the WHO Collaborating Centre (WHOCC) for Gonorrhoea and other STIs, Örebro, Sweden and the WHO headquarters (9-13,17-19). As in other international *N. gonorrhoeae* GASPs (6-8), the EGASP will use joint global analysis of quality-assured WGS, AMR and epidemiological data from all the EGASP countries combined, which will help to further understand both the international and national transmission of gonococci and gonococcal AMR in the EGASP countries. This strategy has previously been shown to substantially enhance the understanding of the distribution and replacement of AMR, and antimicrobial susceptible clades/clones in several risk groups nationally and internationally (6–8). WGS of *N. gonorrhoeae* provides a high resolution to describe the population dynamics and to predict and infer transmission of and changes in AMR. The EGASP WGS component will support the elucidation of the global and national spread of gonorrhoea, AMR, and antimicrobial-susceptible gonococcal clones, which can inform gonorrhoea epidemiology, preventative measures, AMR prediction, diagnostics, and development of new antimicrobials and gonococcal vaccines.

The present protocol describes the genomic surveillance of *N. gonorrhoeae* within the WHO EGASP through standardized sampling and the use of quality-assured WGS, followed by a collective (joint) global analysis of WGS with linkage of AMR and EGASP metadata (demographic, behavioural and clinical) for each isolate/patient from all the EGASP countries.
4.2 Objectives

- To provide an up-to-date baseline in regard to genomic diversity of *N. gonorrhoeae* isolates in EGASP countries, including identification of novel WGS-based genotypes, in order to inform outbreak investigations and identify circulating AMR gonococcal strains;
- To link WGS-based molecular epidemiological and AMR data with characteristics of patients infected with gonorrhoea in EGASP countries over time, to further assess (temporally and geographically) the spread of AMR gonococcal clones in the EGASP countries (and compared to gonococcal WGS data from other GASPs);
- To examine trends and stability of associations over time of resistance profiles for therapeutically relevant antimicrobials, risk groups and *N. gonorrhoeae* WGS-based genotypes within and between EGASP countries;
- To detail changes in AMR and genotype to support prediction of AMR from WGS data, which will inform future molecular surveillance activities.

4.3 Material and methods

4.3.1 *N. gonorrhoeae* isolates

A minimum of 100 gonococcal isolates per EGASP country and year will be examined in the EGASP WGS component. Isolates collected during the current year should be selected based on their proximity to the present time, with a focus on obtaining the most up-to-date samples. Minimum inhibitory concentration (MIC) alert isolates and additionally consecutive isolates with full AMR/susceptibility profiles, in conjunction to metadata from the EGASP participants, will be sequenced.

4.3.2 Antimicrobial susceptibility testing (AST)

AST will be performed through the EGASP in each country to determine the MIC with Epsilometer-Test ([ETESTs] such as bioMérieux; Marcy-l’Etoile, France) or agar dilution for ceftriaxone, cefixime and azithromycin (ciprofloxacin and gentamicin are optional to additionally test). EGASP MIC alert values and additionally consecutive isolates with full AMR/susceptibility profiles, in conjunction to metadata from the EGASP participants, will be sequenced.

4.3.3 Whole genome sequencing (WGS)

A single colony of each isolate (ideally from the culture used to prepare the ASTs) should be sub-cultured onto a non-selective media. Following high-quality DNA extraction, sequencing should be performed using an Illumina platform with an average genome coverage of >50-fold. Prior to decentralized EGASP WGS roll-out and the fulfilment of set quality criteria by EGASP countries for performing decentralized EGASP WGS, sequencing will be performed at the WHOCC in Sweden, in close collaboration with WHO headquarters, participating EGASP countries and partner WHOCCs. All isolates shipped for centralized WGS should be labelled with the EGASP ID, which has been used in EGASP data reporting on the WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) IT platform. Raw sequences data (fastq files) will be requested from sites performing decentralized EGASP WGS in the EGASP country or in other WHOCCs. These data will also be included in the EGASP WGS reports, provided they meet the set WGS quality criteria (see below).

4.3.4 Current EGASP whole genome sequencing landscape

EGASP isolates collected from South Africa (2022), Thailand (2022) and Uganda (2022–2023) have already been sequenced at the WHOCC in Sweden. For additional years and EGASP countries, agreements are in place or in final processing. The overall aim is that WGS capacity will be decentralized and built up in the EGASP countries through appropriate training and upskilling of EGASP representatives as funding within EGASP and/or countries allows. It should be noted that EGASP representatives from Uganda and Thailand have received WGS training at the WHOCC in Sweden in 2022–2023, and training of EGASP representatives from South Africa, Malawi, Viet Nam and, according to plans, at least one additional EGASP country, is booked for early 2024.

4.3.5 Data linkage

The WGS data will be linked to antimicrobial susceptibility/resistance data and to EGASP participant metadata for each isolate/patient that has been collected through the EGASP module in the WHO GLASS IT platform. The EGASP participant metadata include (i) the age of the EGASP participant, (ii) symptoms of gonorrhoea, treatment received and any antibiotic use within the previous two weeks, (iii) treatment outcomes determined at follow-up visits, (iv) results of any test of cure, (v) history of travel within the recent 30 days, (vi) sexual preferences in the recent 30 days (sex with men, women or both), (vii) number of sexual partners in the last 30 days, (viii) sexual intercourse in the last 30 days and (ix) coinfections with other STIs, including HIV. Data linkage will be performed using the EGASP ID.

If any EGASP country requests a Material Transfer Agreement (MTA) or Data Transfer Agreement (DTA) for sharing gonococcal isolates, fastq files, or metadata for linkage, this will be developed between the WHOCC in Sweden and/or WHO headquarters and the specific EGASP country.
4.4 Data analysis and reporting

Raw sequences data (fastq files) will be requested from sites performing decentralized EGASP WGS in country or in other WHOCCs. These data will be added to those sequenced at the WHOCC in Sweden. Sequencing reads will be filtered against the following quality metrics: (i) median base quality > 30, (ii) low levels of adapter contamination, (iii) consistent base content along the length of the reads, (iv) *N. gonorrhoeae* content consistent with an *N. gonorrhoeae* reference genome (approximately 52–53% mean), (v) mapping to > 90% of the bases of the closest 2016 *N. gonorrhoeae* reference genome sequence (based on k-mer analysis) and (vi) consistent mapping to parts of the reference genome with different *N. gonorrhoeae* contents.

Assemblies and analysis will be generated using a previously published and applied in-house WHOCC Sweden pipeline. Prior to analysis, contigs will be filtered to remove contaminating sequences (inter- and intra-species) and flag potential mixed samples. Core gene families will also be identified and genes annotated. Validation of antimicrobial susceptibility/resistance phenotype and WGS-based genotype (AMR determinants) will also be performed, and EGASP isolates with discrepant results between phenotypic AST and AMR determinants will be re-cultured and recharacterized (antimicrobial susceptibility testing and/or WGS), as required.

In accordance with the deliverables of the current WHO EGASP grant (from the CDC Global Antimicrobial Resistance Laboratory Response Network [GARLRN]), in-depth WGS analysis will be reported in an EGASP global collective, validated and quality-assured analysis report in 2024 (incorporating EGASP WGS data from 2022 and 2023) and 2026 (EGASP WGS data from 2022-2025). These reports will be written in the format of detailed peer-reviewed papers.

4.4.1 Main analysis and content of reports

- An overview of the antimicrobial susceptibility/resistance phenotypes.
- The cumulative baseline in regard to genomic diversity associated with AMR in the EGASP countries.
- *N. gonorrhoeae* Sequence Typing (ST) for Antimicrobial Resistance (NG-STAR) types (22), NG-STAR clonal complexes (CCs) (23), and multilocus sequence typing (MLST) STs (24) in silico derived from the WGS data, as previously described (6-8).
- The frequency and proportions of different NG-STAR STs, NG-STAR CCs and MLST STs within each EGASP country and in the entire EGASP WGS project will be included in the report.
- Presence or absence of known relevant AMR determinants (and their association with the MICs of relevant antimicrobials) will be described. These include AMR determinants for antimicrobials currently used for treatment of gonorrhoea (such as ceftriaxone, cefixime, azithromycin, spectinomycin and ciprofloxacin), obsolete therapeutic antimicrobials (penicillins and tetracyclines) and novel antimicrobials (zoliflodacin and gepotidacin). Accordingly, as a minimum, alleles or AMR-associated mutations in the following genes will be described:
  - penA
  - 23S rRNA
  - mtrR
  - mtrRCDE operon mosaics
  - gyrA
  - gyrB
  - parC
  - parB1b
  - ponA
  - 16S rRNA
  - rpsJ
  - tetM
  - blaTEM
- Characteristics of EGASP participants infected with the most frequently observed NG-STAR types, NG-STAR CCs and/or MLST STs.
- Potential associations of different NG-STAR types, NG-STAR CCs and/or MLST STs and:
  - geographical distribution;
  - antimicrobial susceptibility/resistance;
  - EGASP participant characteristics (age, sexual orientation, etc.).
- A phylogenomic tree including (i) the comparison of WGS, (ii) NG-STAR types, NG-STAR CCs and MLST STs (will also be longitudinally compared over the years), (iii) clades of main importance (especially clades that are associated with relevant AMR and clades with the highest prevalence), (iv) resistance/susceptibility to ceftriaxone, cefixime, azithromycin and ciprofloxacin and (v) the AMR determinants in penA, 23S rRNA gene, mtr mosaics and gyrA (as a minimum).
4.5 Data storage, ownership and sharing

The generated EGASP WGS data will be analysed, in accordance with the objectives listed in Section 4.2, by the WHOCC in Sweden in collaboration with the WHO headquarters, participating EGASP countries and partner WHOCCs. All data will belong to WHO, WHOCCs, participating EGASP countries and the United States CDC (taking into account any MTAs and DTAs with the EGASP countries, as required). All contributing EGASP partners will also be involved in the reports of the EGASP global collective, validated, and quality-assured WGS analysis in 2024 and in 2026. Furthermore, in accordance with scientific practice, all of the fastq files from the EGASP WGS will be uploaded to the European Nucleotide Archive (ENA) and linked to metadata approved by each participating EGASP country (which should at a minimum include EGASP country and year of isolation) prior to any publication.

For feasibility and analysis, the WGS data will be stored in a secure database at the WHOCC in Sweden (with full access for the WHO headquarters and taking into account any MTAs and DTAs with the EGASP countries, as required). The WGS data will be linked to the antimicrobial susceptibility/resistance phenotypic data and EGASP participant metadata (extracted from the EGASP module in the WHO GLASS IT platform) by the WHOCC in Sweden and WHO headquarters (taking into account any MTAs and DTAs with the EGASP countries, as required). After initial analysis, the WHOCC in Sweden will also share, upon request, all “national” fastq files from centralized WGS. These files will be linked to antimicrobial susceptibility/resistance phenotypic data and EGASP participant metadata. EGASP countries may also write national WGS reports, in collaboration with WHO.
References for section 4


