



TECHNICAL REPORT

Euro-GASP external quality assessment (EQA) scheme for *Neisseria gonorrhoeae* antimicrobial susceptibility testing

2016

ECDC TECHNICAL REPORT

**Euro-GASP external quality assessment
(EQA) scheme for *Neisseria gonorrhoeae*
antimicrobial susceptibility testing**

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Abbreviations

AMR	Antimicrobial Resistance
BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards Institute
DSN	Dedicated surveillance network
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EQA	External Quality Assessment
ESSTI	European Surveillance of Sexually Transmitted Infections Project
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
Euro-GASP	European Gonococcal Antimicrobial Surveillance Programme
GC	Gonococcal
GRASP	Gonococcal Resistance to Antimicrobials Surveillance Programme
MIC	Minimum Inhibitory Concentration
NG-MAST	<i>Neisseria gonorrhoeae</i> multi-antigen sequence typing
PHE	Public Health England
SFM	Société Française de Microbiologie
STI	Sexually Transmitted Infection
UKAS	United Kingdom Accreditation Service
UK-NEQAS	United Kingdom National External Quality Assessment Service
QMS	Quality management systems

Executive Summary

Introduction

External quality assessment (EQA) is an essential part of any laboratory-based surveillance system, allowing for the monitoring of performance and comparability of results from participating laboratories, the identification of potential issues and deployment of resources and training where necessary. An EQA scheme for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* has been available to laboratories participating in ECDC's European Sexually Transmitted Infections (STI) surveillance network since 2010. This EQA scheme has so far shown high levels of inter-laboratory comparability in the presence of differing methodologies. Problems identified previously included reduced comparability of results determined using discs, compared with those determined by agar dilution and E-tests, media not suitably supporting gonococcal growth, and the use of gradient strips from one manufacturer. This is the second report to be published on the European *N. gonorrhoeae* EQA.

Materials and methods

The EQA specimen panel was selected by Public Health England (PHE) and distributed by the United Kingdom National External Quality Assessment Service (UK-NEQAS). In February 2016, 27 laboratories in 25 participating countries received 10 gonococcal isolates for susceptibility testing. Of the 10 gonococcal isolates provided, one was in triplicate and two were in duplicate to test intra-laboratory concordance. The remaining isolates were all provided singularly meaning that the *N. gonorrhoeae* antimicrobial susceptibility EQA panel comprised of six different strains in total. The isolates chosen by PHE were representative of a range of different antimicrobial susceptibility profiles and consisted of the six new WHO reference strains, WHO U, V, W, X, Y and Z. Participating laboratories were requested to test the EQA panel using local methodology (i.e. E-test, agar dilution or disc diffusion) and relevant international breakpoints (i.e. CLSI, EUCAST etc.) against a range of antimicrobial agents. Results were submitted directly to UK-NEQAS who issued individual laboratory reports. The results were then supplied to PHE who decoded and analysed the results based on the categories of susceptibility assigned.

Results

Twenty-seven laboratories returned EQA results to UK-NEQAS. Most laboratories used E-tests and EUCAST breakpoints. The highest level of susceptibility category concordance was seen with ciprofloxacin (99.8%), whilst the lowest was seen with azithromycin (68.1%).

Overall concordance increased for most antimicrobials in comparison with the previous distribution, except for azithromycin where the overall concordance decreased to the lowest level yet recorded (68.1%). Overall, 93.1% and 97.5% of the reported minimum inhibitory concentrations (MICs) were within one and two doubling dilutions of the modal MIC, respectively.

Discussion and conclusion

There has been further harmonisation of susceptibility testing methodologies and breakpoints used by participating laboratories; most laboratories used gradient MIC strips and applied EUCAST breakpoints for interpretation of MIC results. Overall, the laboratories participating in EQA scheme QA16 performed very well and showed good levels of competency in testing *N. gonorrhoeae* strains of unknown phenotype. The susceptibility category concordances increased slightly in this distribution when compared to 2015, with the exception of azithromycin. The inter-laboratory concordance was high in most cases (91-100%, lower for azithromycin at 68%), demonstrating comparability between different testing methodologies and allowing confidence in decentralised testing for surveillance purposes. Most susceptibility category discrepancies were attributable to strains with MICs on or close to a breakpoint, such as for azithromycin, for example, where four strains had MICs close to a breakpoint. This highlights the need to consider the actual MIC as well as susceptibility category when interpreting susceptibility results. Analysis of the individual results submitted by the participating laboratories highlighted six centres in need of further support to help bring them into line with the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) recommended target of 95% of MICs within two doubling-dilutions (four-fold) of the modal MICs and beta-lactamase assessment.

1 Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to operate the dedicated surveillance networks (DSNs) and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC shall 'foster the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes' (Article 5.3, EC 851/2004¹).

As part of its mandate, ECDC commissions and supports external quality assessment (EQA) exercises across public health microbiology laboratories in the EU Member States with the objective to:

- verify the quality and comparability of surveillance data reported at European level;
- ensure threat detection capability for emerging and epidemic disease or drug resistance.

EQA is part of quality management systems (QMS) and evaluates performance of laboratories by an outside agency on material that is supplied specially for the purpose. ECDC's disease specific networks organize a series of EQA for EU/European Economic Area (EEA) countries. In some specific networks, non-EU/EEA countries are also involved in the EQA activities organized by ECDC. The aim of the EQA is to identify needs of improvement in laboratory diagnostic capacities relevant to surveillance of diseases listed in Decision No 2119/98/EC and to ensure comparability of results in laboratories from all EU/EEA countries. The main purposes of EQA schemes include:

- Assessment of the general standard of performance ('state of the art')
- Assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration)
- Evaluation of individual laboratory performance
- Identification and justification of vulnerabilities
- Providing continuing education for participating laboratories
- Identification of needs for training activities

A major aim of the European Sexually Transmitted Infections (STI) surveillance network is to strengthen the surveillance of *Neisseria gonorrhoeae* antimicrobial susceptibility in EU/EEA Member States. An EQA scheme for *N. gonorrhoeae* antimicrobial susceptibility testing was established in 2007 as part of the European Surveillance of STIs (ESSTI) programme funded by the Directorate-General for Health and Food Safety, and has been part of the ECDC STI microbiology project since 2009, with the first ECDC EQA distributed in 2010.

The EQA scheme is available to all laboratories in the STI surveillance network, which are mainly national reference laboratories or provide expert services (national or regional) in respect to *N. gonorrhoeae* diagnostics and antimicrobial susceptibility testing. An EQA scheme is an essential component of the laboratory-based surveillance programme; ensuring comparability of data between and within testing centres, and successful performance in EQA is a requirement for laboratories participating in decentralised testing as part of antimicrobial resistance (AMR) surveillance across Europe [1,2].

Between 2010 and 2015, the number of participating laboratories ranged from 18 to 24 and in general the EQA revealed high levels of inter-laboratory comparability even in the presence of different antimicrobial susceptibility testing methodologies. Problems identified previously included reduced comparability of results determined using discs compared with those determined by agar dilution and E-tests, media not suitably supporting gonococcal growth, and the use of gradient strips from a particular manufacturer.

The United Kingdom National External Quality Assessment Service (UK-NEQAS) collaborated with Public Health England (PHE) for the EQA described in this report. UK-NEQAS are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17043 (Conformity Assessment – General Requirements for Proficiency Testing). Participation in this EQA scheme for *N. gonorrhoeae* antimicrobial susceptibility provides a mechanism for laboratories in the network to meet the requirements of these standards.

¹ Regulation (EC) no 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control

2 Materials and methods

2.1 Antimicrobial susceptibility testing external quality assessment panel

In February 2016, 27 laboratories within 25 countries received ten gonococcal isolates (QA16) for susceptibility testing from UK-NEQAS. The isolates included in the panel were selected by PHE to demonstrate a range of susceptibility profiles to relevant therapeutic antimicrobial agents and consisted of the six new WHO reference gonococcal strains, WHO U, V, W, X, Y and Z [3]. To measure intra-laboratory reproducibility, one of these isolates was supplied in triplicate (Strain 1 (WHO U) = 3118/3124/3127) and two were supplied in duplicate (Strain 2 (WHO V) = 3119/3125 and Strain 6 (WHO Z) = 3123/3126). The remaining three isolates were supplied as individual different strains (Strain 3 (WHO W) = 3120; Strain 4 (WHO X) = 3121 and Strain 5 (WHO Y) = 3122). Therefore six different strains were included in the distribution.

Participating laboratories tested the EQA panel of isolates using their own routine methodologies against the following therapeutic antimicrobials, where possible:

- Azithromycin
- Cefixime
- Ceftriaxone
- Ciprofloxacin
- Gentamicin
- Spectinomycin

Participating laboratories also tested the EQA panel of isolates for beta-lactamase production, where possible.

The antimicrobials listed are those detailed in the ECDC Instructions, External Quality Assessment v5 [4].

The EQA strains had also been typed by the *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) method by PHE to offer a NG-MAST EQA to European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) laboratories using this method. NG-MAST typing was required formally for the EQA scheme.

2.2 Susceptibility testing methods

The methodology and the clinical breakpoints or guidelines used for determining the category of susceptibility for each antimicrobial tested was requested. Examples of breakpoints and guidelines used include the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints² (Table 1) and the Clinical Laboratory Standards Institute (CLSI) guidelines³ (Table 2). Antimicrobial susceptibility testing results for each isolate were reported as both the category of susceptibility (resistant (R), intermediate (I) or susceptible (S)), and either the minimum inhibitory concentration (MIC) for the gradient strip and agar dilution methods, or the diameters of any zones of inhibition for the disc diffusion method.

² http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf

³ <http://clsi.org>

Table 1. European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, 2016

	MIC breakpoint (mg/L)		
	S ≤	I	R >
Azithromycin	0.25	0.5	0.5
Cefixime	0.125		0.125
Ceftriaxone	0.125		0.125
Ciprofloxacin	0.03	0.06	0.06

Note: Currently there are no EUCAST interpretive criteria for gentamicin

Table 2. Clinical and Laboratory Standards Institute (CLSI) breakpoints, 2016

	MIC breakpoint (mg/L)		
	S ≤	I	R >
Cefixime	0.25	-	-
Ceftriaxone	0.25	-	-
Ciprofloxacin	0.06	0.12 – 0.5	0.5
Spectinomycin	32	64	64

Note: Currently there are no CLSI interpretive criteria for azithromycin and gentamicin

2.3 Analysis and interpretation of the results

Raw results for the EQA were submitted by each participating laboratory directly to UK-NEQAS for the production of individual laboratory reports. The results were also forwarded to PHE for further collated analysis.

For the analysis, all MIC results that lay within the E-test full-dilution scale were rounded up to the next full E-test dilution. The E-test dilution scale was used as E-tests were the most frequently used testing method. The minimum, maximum and modal MIC for each strain was established. The number of MICs within two MIC dilutions of the modal MIC and the number of MICs above or below two MIC dilutions of the modal MIC for each strain was established.

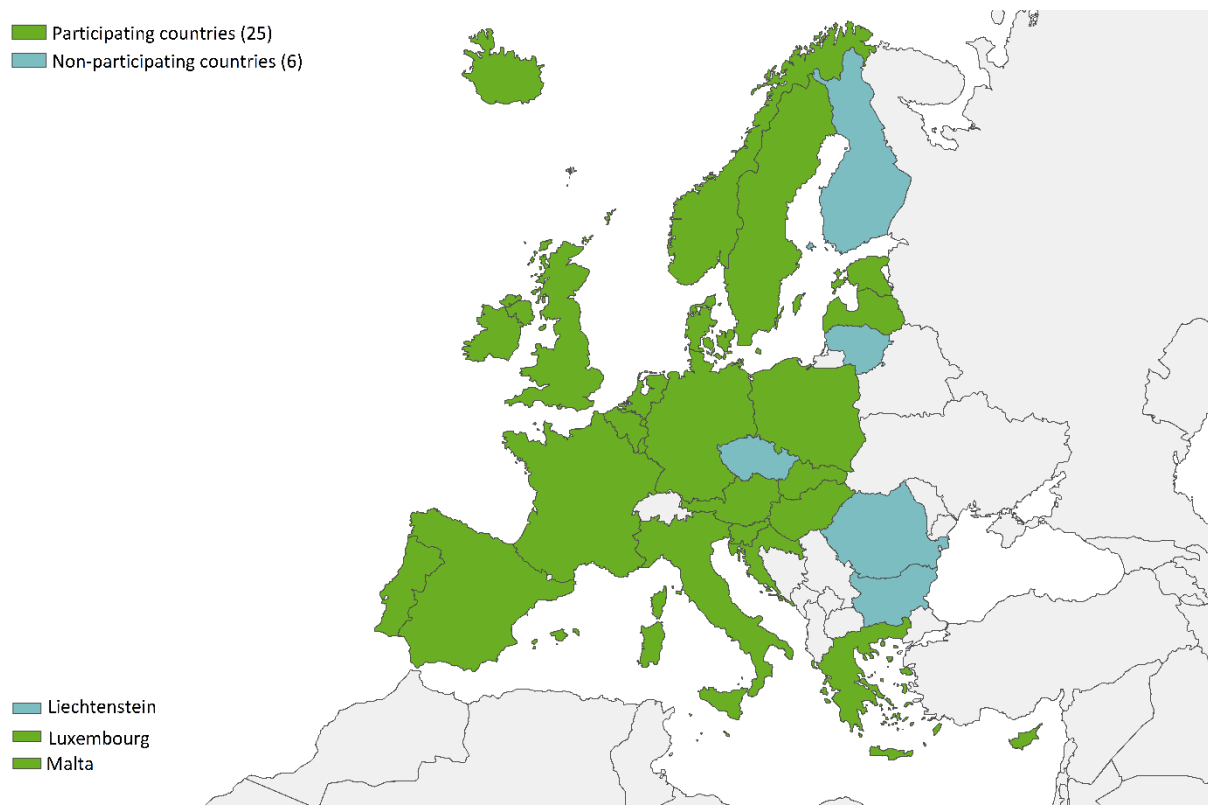
To allow for the differences in local methods and breakpoints used, analysis of blind testing results was performed using the susceptibility categories only. For this report, consensus categories of susceptibility for each strain tested (six in total in this distribution; consensus calculated from all isolates in the triplicate or duplicate sets) were calculated once all participating laboratories had reported results back. The 'consensus' was assigned to the category reported most often irrespective of breakpoint criteria used. The overall concordance for each antimicrobial was established by taking the average of each strain percentage concordance.

3 Results

3.1 Susceptibility testing methods

Twenty-seven laboratories in 25 countries returned results to UK-NEQAS (Figure 1). All laboratories provided details on the methodology and breakpoints or guidelines (Table 3) used to test the isolates in the EQA. E-test (88.9%) and gonococcal (GC) agar (51.9%) were the most common testing methodology and medium used.

Figure 1. Country of laboratories participating in the 2016 *N. gonorrhoeae* susceptibility testing EQA scheme



Note: 27 laboratories participated in the 2016 EQA scheme; the United Kingdom and Austria each had two participating laboratories.

3.2 Interpretation of MICs

Twenty-three laboratories reported adherence to the EUCAST breakpoints⁴ (Table 1). One participating laboratory reported that it performed susceptibility testing in accordance with the CLSI guidelines⁵ (Table 2). One laboratory used a combination of British Society for Antimicrobial Chemotherapy (BSAC) (for the azithromycin disc) and EUCAST (for gradient MIC strips), and the remaining two laboratories used other guidelines (Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) and Société Française de Microbiologie (SFM); Table 1).

Most laboratories that tested gentamicin did not interpret categories of susceptibility as there are currently no internationally-defined interpretive criteria for this antimicrobial. However, three laboratories did submit categories of susceptibility for gentamicin, using local interpretive criteria; these data were not analysed in this report.

⁴ http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf

⁵ <http://clsi.org>

Table 3. Susceptibility methods used by laboratories participating, February 2016 EQA

	Number of participating laboratories (27)*
Type of susceptibility test used	
Gradient MIC strips	24
Agar dilution	2
Disc diffusion**	1
Testing guidelines used	
EUCAST ⁶	23 (E-test) 1 (Disc diffusion)
CLSI ⁷	1 (Agar dilution)
BSAC ⁸	1 (Disc diffusion)
SFM ⁹	1 (E-test)
GRASP ¹⁰	1 (Agar dilution)
Agar base used	
GC agar base	14
Chocolatised blood agar	7
Thayer-Martin/Mueller-Hinton	3
Diagnostic sensitivity agar	2
No information provided	1

* One laboratory reported two different testing methods and guidelines

**Used by one laboratory only for azithromycin (15 µg). One laboratory (92784) originally submitted disc diffusion results for isolates 3118-3120, however gradient MIC results were subsequently re-submitted

3.3 Susceptibility categories concordance

Eight laboratories submitted incomplete susceptibility category results. Incomplete data were submitted for spectinomycin (laboratories 874, 90984, 92613, 92629, 93997, 94602 [isolates 3125 and 3127 only]), azithromycin (laboratory 92623 [isolate 3125]), cefixime (laboratory 94602 [all except isolate 3118]), ceftriaxone (laboratory 90984 [isolate 3123]), ciprofloxacin (laboratory 90984 [isolate 3125]). Sixteen laboratories submitted complete gentamicin data (Table A1.11) and one submitted incomplete data (laboratory 92784 [isolates 3118, 3119, 3120]). One laboratory (92629) did not test for the production of beta-lactamase.

The highest levels of susceptibility category concordance were seen for ciprofloxacin, with 99.8% concordance, and the lowest level was seen for azithromycin, with 68.1% concordance (Figure 2 and Tables A1.1, A1.3, A1.5, A1.7, A1.9 and A1.12). Consensus susceptibility categories were not assigned for gentamicin as there are currently no published breakpoints for interpretation of results. Two centres incorrectly identified beta-lactamase production (false-positive tests) (Table A1.12).

When susceptibility category concordance data is compared with previous EQA distributions from both ESSTI (QA2007, QA2008 and QA2009) [5] and ECDC Euro-GASP (QA2010–15) [6–10], there is a slight increase for most antimicrobials tested (Figure 2) with the exception of azithromycin which displayed the lowest concordance measured (68.1%). Ciprofloxacin concordance increased in 2016 to 99.8%, after the fall to 89.2% in 2015. Beta-lactamase result concordance remains high at 99% (Figure 2). It should be noted that the methods used for the susceptibility testing and the breakpoints used have changed over time, although there has been greater consistency in later years. A full analysis of the different methods and breakpoints used in this EQA over the years is currently underway.

⁶ http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf

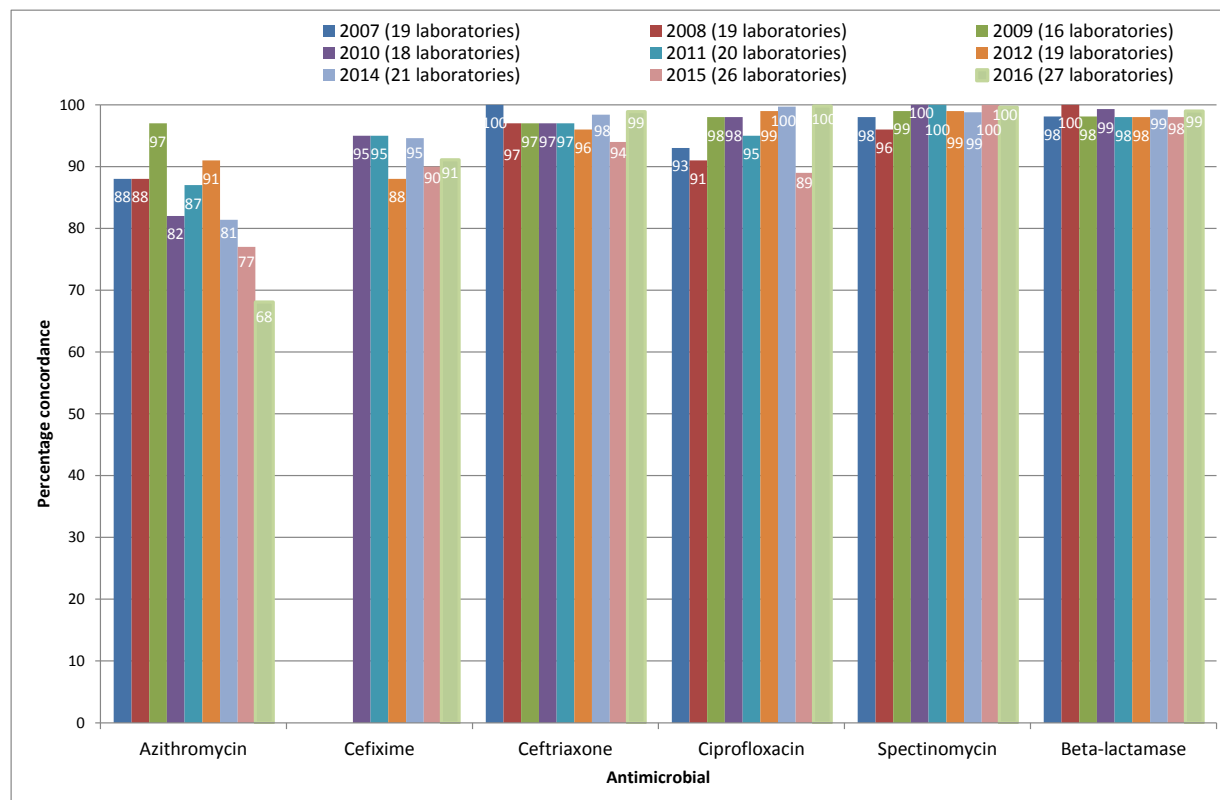
⁷ <http://clsi.org/>

⁸ http://bsac.org.uk/wp-content/uploads/2012/02/Version-12-Apr-2013_final1.pdf

⁹ <http://www.sfm-microbiologie.org/>

¹⁰ https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/476582/GRASP_2014_report_final_111115.pdf

Figure 2. Longitudinal comparison of EQA inter-laboratory concordance, 2007-2016, EU/EEA



Note: Cefixime became part of the EQA scheme from 2010.

ESSTI EQA distributions (2007 – 2009) constituted 30 isolates (10 strains in triplicate)

3.5 MIC concordance

Overall, 93.1% of the MIC results were within one doubling dilution (two-fold variation) of the modal MIC recorded (Table 4) for all antimicrobials tested, showing an increase in concordance from the previous EQA panel distribution (84.6%) (10). Highest MIC concordances were seen for ciprofloxacin and gentamicin (97.0%), whilst the lowest was seen for azithromycin (84.6%) (Table 4). For all MICs combined, 97.5% were within two doubling dilutions of the modal MIC and a further 2.5% differed from the modal MICs by more than two doubling dilutions. Azithromycin had the highest number of isolates with an MIC greater than two dilutions of the modal MIC (5.8%) and spectinomycin had the lowest (0.5%).

Table 4. Variation from modal MIC for EQA QA16

QA16	Azithromycin		Cefixime		Ceftriaxone		Ciprofloxacin		Gentamicin		Spectinomycin		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Within +/- 1 doubling dilution	220	84.6	257	95.2	244	90.4	262	97.0	162	97.0	211	95.9	1357	93.1
Within +/- 2 doubling dilutions	25	9.6	11	4.1	16	5.9	0	0.0	4	2.4	8	3.6	64	4.4
+/- >2 doubling dilutions	15	5.8	2	0.7	10	3.7	8	3.0	1	0.6	1	0.5	36	2.5
Total no. of isolates with MIC data	260		270		270		270		167		220		1457	

No. - Number of isolates with MIC data

3.6 QA16 panel strain characteristics

Table 5 shows the overall consensus category, the modal/range MIC for all tests, and the percentage concordance for each strain in the EQA panel. Consensus phenotypes for each strain tested are also shown. The strains tested demonstrated a range of phenotypes and no strain was fully susceptible to all antimicrobials tested:

- One strain was only resistant to azithromycin (Strain 1; WHO U).
- One strain had high-level resistance to azithromycin and ciprofloxacin (Strain 2, WHO V).
- One strain was intermediate to azithromycin, highly-resistant to ciprofloxacin and had decreased susceptibility to cefixime (Strain 3, WHO W).
- The remaining three strains were multi-resistant against ciprofloxacin, cefixime and ceftriaxone with different combinations of high- and low-level resistance, and different azithromycin susceptibility categories (Strain 4 (WHO X), Strain 5 (WHO Y) and Strain 6 (WHO Z)).
- A range of gentamicin and spectinomycin MICs were present throughout the panel.

3.7 Coded country breakdown of concordance

Due to the confidential nature of the EQA scheme, coded laboratory breakdowns for beta-lactamase assessment concordance, category of susceptibility concordance and MIC values for E-test and agar dilution method are shown in Annex 1 (Tables A1.6 – A1.12). Analysis of the breakdown of results has highlighted that 13 laboratories reported isolates with MICs greater than two doubling dilutions different from the mode MIC or submitted a beta-lactamase result different from the consensus. Six laboratories reported more than 5% variation, calculated from the total number of antimicrobials including beta-lactamase from each laboratory, from the modal MIC and beta-lactamase assessment. Four of the laboratories provide data directly to Euro-GASP from their own decentralised testing. For three centres, the MICs were lower than expected for some antimicrobials, particularly azithromycin, suggesting that the media was not supporting the growth of the isolates sufficiently. Two centres used GC agar base (Oxoid and Becton Dickinson) with supplements, and one centre used Thayer-Martin media, which is a non-recommended selective media. All three centres are currently identifying different media and supplement options. Other identified problems included unusually high MICs with a batch of ceftriaxone gradient MIC strips and contamination or strain mix-ups; expected MICs were achieved upon repeat testing for all three centres.

3.8 NG-MAST EQA

Only one of the 27 participating laboratories reported NG-MAST sequence types, and these gave 100% concordance with results determined by PHE. NG-MAST typing was not formally required for the EQA scheme.

Table 5. Consensus category, modal (range) MIC for E-test and agar dilution (mg/L) and the percentage concordance of susceptibility category for the 2016 EQA panel

Strain		Azithromycin consensus	Cefixime consensus	Ceftriaxone consensus	Ciprofloxacin consensus	Gentamicin consensus	Spectinomycin consensus	Beta-lactamase consensus
Strain 1: 3118/3124/3127 (WHO U) (3) AzR, PorA mutant	Consensus category	R	S	S	S	N/A	S	NEG
	Modal MIC (range)	2 (0.064-16)	<0.016 (0.008-1)	<0.002 (<0.002-0.5)	0.004 (<0.002->0.032)	4 (0.5-8)	8 (2-16)	-
	Susceptibility category concordance (%)	90	98.7	98.8	98.8	N/A	100	100
	Reference MIC (3)	4	<0.016	0.002	0.004	4	8	NEG
Strain 2: 3119/3125 (WHO V) (3) AzR, CipR	Consensus category	R	S	S	R	N/A	S	POS
	Modal MIC (range)	>256 (>2->256)	<0.016 (<0.016-0.064)	0.016 (0.008-0.064)	>32 (16->32)	4 (2-16)	8 (4-16)	-
	Susceptibility category concordance (%)	100	100	100	100	N/A	100	100
	Reference MIC (3)	>256	<0.016	0.064	>32	8	16	POS
Strain 3: 3120 (WHO W) (3) CipR, CfmR, AzI, cefixime DS	Consensus category	S*	S/R	S	R	N/A	S	NEG
	Modal MIC (range)	0.25/0.5 (0.125-2)	0.125 (0.032-0.5)	0.032 (0.016-0.125)	>32 (32->32)	4 (2-8)	16 (4-16)	-
	Susceptibility category concordance (%)	63	50	100	100	N/A	100	100
	Reference MIC (3)	0.5	0.25	0.064	>32	4	16	NEG
Strain 4: 3121 (WHO X) (3) CipR, CfmR, CroR, AzI	Consensus category	S*	R	R	R	N/A	S	NEG
	Modal MIC (range)	0.5 (0.064-2)	4 (2-8)	1 (0.5->32)	>32 (32->32)	4 (2-8)	8 (2-32)	-
	Susceptibility category concordance (%)	63	100	100	100	N/A	100	96
	Reference MIC (3)	0.5	4	2	>32	4	16	NEG
Strain 5: 3122 (WHO Y) (3) CipR, CfmR, CroR, AzS/I	Consensus category	S/I	R	R	R	N/A	S	NEG
	Modal MIC (range)	0.5 (0.064-2)	2 (1-8)	1 (0.25-32)	>32 (4->32)	4 (4-8)	8 (4-32)	-
	Susceptibility category concordance (%)	40.7	100	100	100	N/A	100	100
	Reference MIC (3)	1	2	1	>32	8	16	NEG
Strain 6: 3123/3126 (WHO Z) (3) CipR, CfmR, CroR, AzR	Consensus category	R	R	R	R	N/A	S	NEG
	Modal MIC (range)	1 (0.125->256)	1 (0.016-4)	0.25 (0.032-8)	>32 (32->32)	4 (1-16)	8 (1-16)	-
	Susceptibility category concordance (%)	52	98	94.3	100	N/A	97.7	98
	Reference MIC (3)	1	2	0.5	>32	4	16	NEG

Note: No consensus category of susceptibility was assigned to gentamicin as there are currently no published breakpoints for this antimicrobial.

Disc diffusion zones not shown as only one laboratory performed this technique

N/A – not available

DS- Decreased susceptibility

*Intermediate according to EUCAST breakpoints and the modal MIC

4 Discussion

The 2016 Euro-GASP EQA distribution was sent out to 27 laboratories in 25 participating countries, and all laboratories reported results for all or most of the requested tests. Most laboratories (88.9%) used gradient MIC strips to perform antimicrobial susceptibility testing of *Neisseria gonorrhoeae*, which is the same as the previous year. EUCAST guidelines were used by the majority (85.2%) of the participating laboratories to interpret MIC results, which again was the same as the previous year. Where gradient MIC strips were used, all but one laboratory used the EUCAST guidelines for interpretation of MICs, which shows the continuing harmonisation of the EUCAST guidelines and of gradient MIC strips across the Euro-GASP participating laboratories.

In general, susceptibility category concordance levels increased for most antimicrobials in comparison with the previous distribution; the exception was azithromycin, for which concordance decreased to the lowest level yet recorded (68.1%). For azithromycin, four of the strains - 3, 4, 5 and 6 had MICs close to a breakpoint so the lower concordance is not unexpected. The slight increase for most other antimicrobials may be due to a higher proportion of strains in this distribution that were either susceptible or had high-level resistance, with fewer strains 'having' MICs close to breakpoints. It is important that reference and expert laboratories for *N. gonorrhoeae*, and ideally all primary diagnostic laboratories have access to appropriate internal quality control strains such as the new WHO control panel [3] to ensure their own quality assurance in a variety of diagnostic and antimicrobial susceptibility testing. The choice of strains with MICs close to breakpoints will have an impact on category of susceptibility concordance; this highlights the need to consider the actual MIC of the isolates as well as susceptibility category when interpreting susceptibility results. Category of susceptibility agreed with the consensus (overall) assigned for each antimicrobial testing method in most cases and any discordant susceptibility category consensus results were because the MICs for few isolates were on or near breakpoints. For example, the modal azithromycin MIC for strains 3, 4 and 5 (3120, 3121 and 3122) were on the azithromycin intermediate breakpoint (MIC=0.5 mg/L) which resulted in discordant susceptibility category results. Concordance of beta-lactamase detection also slightly increased and remained at high levels as for previous years.

Concordance of MIC results was high, with 93.1% of results being within one doubling dilution of the modal MIC reported which is an increase from the previous distribution where concordance was 84.6%. Ciprofloxacin and gentamicin gave the highest levels of concordance whilst azithromycin gave the lowest levels of concordance.

EQA susceptibility testing results were broken down by laboratory. This allowed for detailed analysis of individual laboratory performance. On the whole laboratories performed well, with a good level of inter-laboratory and intra-laboratory concordance of results. However, six laboratories reported more than 5% variation from the modal MIC or beta-lactamase assessment. Investigations identified the root causes to be GC base not supporting the growth of the isolates sufficiently to produce the expected MICs, particularly for azithromycin, the use of selective media, a particular batch of ceftriaxone gradient MIC strips and strain mix-ups or contamination.

The gonococcal strains were also typed by the *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) method by PHE to ensure a NG-MAST EQA is available to Euro-GASP laboratories. One laboratory reported back NG-MAST sequence types and achieved 100% concordance with PHE.

5 Conclusion

The laboratories participating in the QA16 EQA scheme for susceptibility testing of *N. gonorrhoeae* showed very good levels of competency and capability in recovering and testing strains of unknown phenotype. Inter- and intra-laboratory concordance of categories of susceptibility for the different strains were again very good, allowing confidence in de-centralised susceptibility testing and comparison of surveillance data from the members of the STI network, and indicate that the Euro-GASP antimicrobial surveillance quality is of a good standard. This EQA scheme allows the performance of laboratories, with respect to antimicrobial susceptibility testing, to be monitored. The identification of results which are out of range can trigger appropriate troubleshooting to ensure the methodology being implemented is appropriate. In turn, quality standards should improve. It is encouraging that laboratories continue to adhere to the EUCAST breakpoints.

This Euro-GASP EQA is important to ensure (i) that results from different submitting laboratories are comparable and (ii) that significant over- and under-reporting of resistance does not occur. Confidence in antimicrobial susceptibility results is essential as Euro-GASP contributes to the evidence-base of gonorrhoea treatment guidelines, and in ensuring the use of local susceptibility testing for individual patient management.

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Table A1.11 Country coded MIC values (mg/L) – GENTAMICIN

Strain	Laboratory codes																	Modal MIC	Min MIC	Max MIC	2 MIC dilutions different	>2 MIC dilutions different	
	91431	92621	92622	92623	92624	92625	92626	92627	92630	92631	92632	92784	93994	93995	93996	93997	94602						
1	3118	2	8	4	4	4	2	4	4	4	4	2	N	4	0.5	4	4	2	4	0.5	8	1	1
	3124	1	8	4	4	4	4	2	4	8	4	2	4	4	4	4	4						
	3127	2	8	4	4	4	4	4	4	8	4	2	8	4	4	4	4						
2	3119	2	8	4	8	8	4	4	8	8	4	2	N	4	4	4	4	4	4	2	16	1	0
	3125	2	8	4	8	8	4	4	8	16	4	4	8	4	8	8	8						
3	3120	2	8	4	4	4	4	8	4	8	4	2	N	4	4	4	2	4	4	2	8	0	0
4	3121	2	8	4	4	4	4	8	4	8	4	4	8	4	8	4	4	4	4	2	8	0	0
5	3122	4	8	4	8	8	4	4	4	8	4	4	8	4	8	8	8	4	4	4	8	0	0
6	3123	1	8	4	4	4	4	8	4	8	4	4	4	4	16	4	2	2	4	1	16	2	0
	3126	2	8	4	4	4	2	4	4	8	4	4	8	4	4	4	2						

Note: Laboratories 582, 874, 90984, 92613, 92628, 92629, 92634, 92636 and 92945 did not submit gentamicin data

Table A1.12 Country coded concordance – BETA-LACTAMASE

Strain	Laboratory codes																								Total	No. sensitive	No. inter-mediate	No. resistant	Consensus	Concordance %			
	582	874	90984	91431	92613	92621	92622	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93994	93995	93996							93997	94602	94603
1	3118	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	78	78	0	0	S	100
	3124	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S							
	3127	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S							
2	3119	R	R	R	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	R	R	R	R	R	R	R	52	0	0	52	R	100
	3125	R	R	R	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	R	R	R	R	R	R							
3	3120	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	26	26	0	0	S	100	
4	3121	S	S	S	R	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	26	25	0	1	S	96	
5	3122	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	26	26	0	0	S	100	
6	3123	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	52	51	0	1	S	98	
	3126	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	R	S	S	S	S	S	S								
		Total																													99.0		

N – No result; not retrieved or beta-lactamase result not supplied. Laboratory 92629 did not submit any beta-lactamase testing results

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