

TECHNICAL REPORT

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

2020

#### **ECDC** TECHNICAL REPORT

# Euro-GASP external quality assessment scheme for *Neisseria gonorrhoeae* antimicrobial susceptibility testing

2020



This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Gianfranco Spiteri, Benjamin Bluemel, Andrew J Amato-Gauci and Marieke J. van der Werf and produced by Michaela Day and Michelle Cole, Public Health England (now the UK Health Security Agency), London, and Susanne Jacobsson and Magnus Unemo, Örebro University Hospital, on behalf of the Euro-GASP network participants.
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# **Abbreviations**

CLSI Clinical and Laboratory Standards Institute

ECDC European Centre for Disease Prevention and Control

ECOFF Epidemiological cut-off
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

I Susceptible, increased exposure MIC Minimum inhibitory concentration

PHE Public Health England

R Resistant
S Susceptible

STI Sexually transmitted infection

UK United Kingdom

UK NEQAS United Kingdom National External Quality Assessment Service

WHO World Health Organization

# **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, identification of potential issues and deployment of resources and training where necessary. An EQA scheme for antimicrobial susceptibility testing in *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.

#### **Materials and methods**

The EQA specimen panel of 10 gonococcal isolates was selected by Public Health England (PHE, now UK Health Security Agency (UKHSA)) and distributed by the United Kingdom National External Quality Assessment Service (UK NEQAS). Of the 10 gonococcal isolates provided, one strain was in triplicate and two strains were in duplicate to test intralaboratory 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 were representative of a range of different antimicrobial susceptibility profiles and consisted of the six candidate WHO reference strains, 316/-11, G7944 (WHO Q), VNM11-4, 500461, VNM11-65 and EST\_11\_14. Participating laboratories were requested to test the EQA panel using local methodology (i.e. MIC gradient strip test, agar dilution or disc diffusion) and relevant international breakpoints (i.e. EUCAST, CLSI, 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. Susceptibility category concordance (categorical agreement) was assessed using the consensus category (most often reported category) of susceptibility for each tested strain. MIC concordance was assessed by examining MIC results within one (essential agreement) and two doubling dilutions of the modal MIC. Intralaboratory concordance was examined using the triplicate and the two duplicate strains.

#### Results

In September 2020, 27 laboratories in 26 participating countries received 10 gonococcal isolates for antimicrobial susceptibility testing. Only 25 of the 27 participating laboratories returned EQA results to UK NEQAS. Two laboratories were unable to obtain sufficient growth from the freezer-dried vials to perform any susceptibility testing. All laboratories used EUCAST v10.0 guidelines except for one which used the 2019 guidelines and most used MIC gradient strip tests for at least some of the antimicrobials tested.

The highest level of categorical agreement was seen with spectinomycin (100%) and ciprofloxacin (99.3%), while the lowest was seen with azithromycin (90.4%). Compared to the previous distribution there was a slight decrease in concordance for azithromycin (90.4% vs. 92.5%), ceftriaxone (95.1% vs. 99.1%) and beta-lactamase production (96.4% vs. 100%) whereas concordance for cefixime (97.3% vs. 96.3%) and ciprofloxacin (99.3% vs. 91.1%) increased.

Overall, 93.8% and 98.9% of the reported minimum inhibitory concentrations (MICs) were within one (essential agreement) and two doubling dilutions of the modal MIC, respectively demonstrating essential agreement has remained consistent after the increase observed in 2018 (94.7% in 2019, 95.4% in 2018, 87.7% in 2017). No relevant changes in the essential agreement for any one antimicrobial were observed between QA19 and QA20. Of the 25 laboratories, 19 (76%) reached an intralaboratory MIC concordance percentage score of 95% or higher with four laboratories obtaining a score of 100%.

#### **Discussion and conclusion**

There has been further harmonisation of susceptibility testing methodologies and breakpoints used by participating laboratories; most laboratories used MIC gradient strip tests and all applied EUCAST breakpoints for interpretation of MIC results. Overall, the laboratories participating in the EQA scheme QA20 performed well and showed good levels of competency in testing *N. gonorrhoeae* isolates of unknown phenotype. Categorical agreement remained comparable to the level recorded for the previous distribution. The inter- and intralaboratory concordance was high in most cases, 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, which 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 demonstrated all participants were in line with the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) recommended target of 95% of MICs within two doubling-dilutions 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/European Economic Area (EEA) Member States with the objective of:

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

EQAs are conducted within a quality management system and evaluate performance of laboratories. They are carried out by an outside agency and with materials supplied specially for this purpose. ECDC's disease-specific networks organize a series of EQA for EU/EEA countries. In some networks, ECDC also includes non-EU/EEA countries in its EQA activities. The aim of these EQAs is to identify weak points in the diagnostic capacities of EU/EEA laboratories that are relevant to the surveillance of diseases listed in Commission Implementing Decision (EU) 2018/945; another aim is to ensure comparability of laboratory results 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 of vulnerabilities;
- Provision of continuing education for participating laboratories; and
- 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 Directorate-General for Health and Consumers (DG-SANCO), 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. The 2020 EQA scheme was carried out under an ECDC framework contract running from 2017 to 2022 including EU/EEA countries and the UK. The UK participated in the EQA activities during the transition period (after the withdrawal of the UK from the EU) which ended on 31 December 2020.

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 surveillance across Europe [1, 2].

Between 2010 and 2019, the number of Euro-GASP collaborating laboratories participating in the *N. gonorrhoeae* antimicrobial susceptibility testing EQA increased from 18 to 28. In 2020, the number of laboratories decreased to 27, as one laboratory could no longer collect sufficient *N. gonorrhoeae* cultures to participate in Euro-GASP. In general, the EQA revealed high levels of inter-laboratory comparability even in the presence of different antimicrobial susceptibility testing methodologies. Problems identified in previous EQA distributions included reduced comparability of results determined using discs compared with those determined by agar dilution and MIC gradient strip tests, media not suitably supporting gonococcal growth, and reduced comparability of results among laboratories using MIC gradient strip tests 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 is accredited by the United Kingdom Accreditation Service 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.

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<sup>&</sup>lt;sup>1</sup> 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

Members of the STI network and Euro-GASP contact points were invited by ECDC to participate in the EQA scheme. All laboratories that expressed interest in the EQA received 10 gonococcal isolates from UK NEQAS. The isolates included in the panel were selected by PHE to demonstrate a range of susceptibility profiles for relevant therapeutic antimicrobial agents and consisted of six candidate WHO reference gonococcal strains, 316/-11, G7944 (WHO Q) [3], VNM11-4, 500461, VNM11-65 and EST\_11\_14. To measure intralaboratory reproducibility, one of these strains was supplied in triplicate (Strain 4 (500461), coded in the EQA as 6346/6347/6348), and two strains were supplied in duplicate (Strain 2 (G7944 (WHO Q)), EQA codes 6343/6344 and Strain 6 (EST\_11\_14), EQA codes 6350/6351). The remaining three strains were supplied as individual isolates (Strain 1 (316/-11), EQA code 6342; Strain 3 (VNM11-4), EQA code 6345 and Strain 5 (VNM11-65), EQA code 6349). 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 v6 [4].

## 2.2 Susceptibility testing methods

The methodology and the clinical breakpoints/guidelines (e.g. European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (Table 1) [5]) used for determining the category of susceptibility for each antimicrobial tested was requested. Antimicrobial susceptibility testing results for each isolate were reported as both the category of susceptibility (resistant (R), susceptible, increased exposure (I), susceptible (S)) or ECOFF (above ECOFF (R), below ECOFF (S), and the minimum inhibitory concentration (MIC) for the gradient strip and agar dilution methods.

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

Antimicrobial	MIC	breakpoint (m	ıg/L)
	S≤	I	R >
Azithromycin	*		*
Cefixime	0.125		0.125
Ceftriaxone	0.125		0.125
Ciprofloxacin	0.03	0.06	0.06
Spectinomycin	64		64

<sup>\*:</sup> Since the 2019 version of the EUCAST guidelines, the SIR categories have been removed for azithromycin and replaced with an epidemiological cut-off (ECOFF) value of 1 mg/L. Please note currently there are no EUCAST interpretive criteria for gentamicin [5].

#### 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 fell between the MIC gradient strip full-dilution scale were rounded up to the next full MIC gradient strip dilution, as this was the most commonly used testing method. The minimum, maximum, and modal MIC for each strain was established. The number of MIC measurements within two MIC

dilutions of the modal MIC and the number of MIC measurements above or below two MIC dilutions of the modal MIC for each strain were established.

A percentage of overall MIC concordance for each laboratory was calculated for the number of isolates within two doubling dilutions of the modal MIC from the total number of antimicrobials including beta-lactamase from each laboratory. Essential agreement (MICs within one doubling dilution of the modal) was also examined and used as the basis for an overall MIC score for each participating laboratory. The overall MIC score for each laboratory was calculated based on minor and major faults in the MIC for ceftriaxone, azithromycin, and ciprofloxacin. Where the MIC result matched the modal result, a score of five was assigned; a one MIC doubling dilution difference from the modal was considered a minor fault and a score of four was given; a difference of two doubling dilutions from the modal MIC was classed as a major fault and given a score of one. An MIC greater than two doubling dilutions from the modal was classed as a very major fault and a score of zero was given. The total score was then converted into a percentage of the maximum score achievable (150 = (10x5) + (10x5)).

Consensus categories of susceptibility (categorical agreement) 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's percentage concordance. The total categorical concordance score was calculated by assigning a score of five for results the same as the consensus, four for a minor fault (susceptible or resistant miscategorised as intermediate or vice versa), three for a major fault (susceptible miscategorised as resistant), and one for a very major fault (resistant miscategorised as susceptible).

Intralaboratory concordance was examined using the triplicate (strain four) and two duplicate strains (strains two and six). All MIC results for these strains were assigned a score: five if the same as the other results, four if one MIC doubling dilution different (minor fault), three if two MIC doubling dilutions different (major fault) and zero if greater than two MIC doubling dilutions different (very major fault). These results were then averaged for the total number of results observed and given a percentage error score by comparison to the maximum score possible if there were no faults i.e. 5 = ((5+5+5)/3) + (5+5/2) + (5+5/2))/3). The higher the percentage, the more consistent the laboratory MIC test results were.

#### 3. Results

#### 3.1 QA20 panel strain characteristics

Table 2 shows the overall consensus category, the modal/range MIC for all tests, and the percentage concordance for each strain in the EQA panel. Consensus category of susceptibility for each strain tested are also shown. The strains tested demonstrated a range of phenotypes:

- Two strains were multidrug-resistant with high-level resistance to ciprofloxacin, one also had resistance to cefixime (Strain 1; 6342), and the other had resistance to ceftriaxone, cefixime, and MIC greater than the azithromycin ECOFF (>256 mg/L) (Strain 2; G7944 (WHO Q)).
- Two strains were high-level resistant to ciprofloxacin, one was also a beta-lactamase producer (Strain 6; EST\_11\_14), and the other was susceptible to the other antimicrobials tested (Strain 3; VMN11-4).
- One strain had an MIC above the azithromycin ECOFF (Strain 5; VMN11-65).
- One strain was susceptible to all antimicrobials tested (Strain 4; 500461).

#### 3.2 Susceptibility testing methods

In September 2020, 27 laboratories in 26 Euro-GASP participating countries received 10 gonococcal isolates (QA20) for susceptibility testing from UK NEQAS. Twenty-five Euro-GASP collaborating laboratories in 24 countries returned results to UK NEQAS (Figure 1). This is three countries fewer than in the 2019 EQA, as Luxembourg no longer participates in Euro-GASP and both Cyprus and Latvia were not able to retrieve any *N. gonorrhoeae* from the freeze-dried vials. All laboratories provided details on the methodology and breakpoints/guidelines (Table 3) used to test the isolates in the EQA. MIC gradient strip tests (96.0%) were the most common testing methodology, with BioMerieux the most common brand used (two laboratories reported using Liofilchem-branded gradient strips only, with a further two using a mixture of both brands). GC agar (44.0%) was the most common medium used, with Becton Dickinson the most common brand used, followed by chocolatised blood agar with both BioMerieux and Oxoid the main brands used.

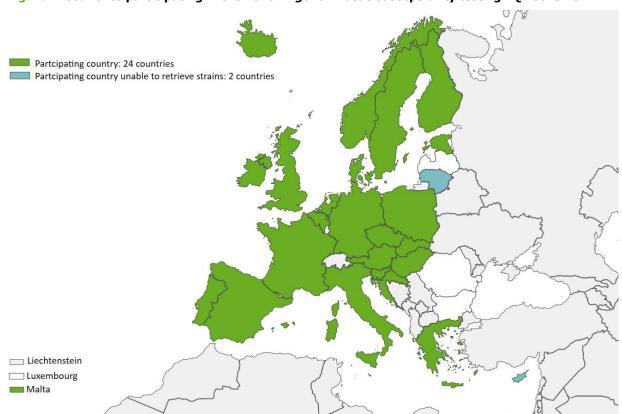


Figure 1. Countries participating in the 2020 N. gonorrhoeae susceptibility testing EQA scheme

Note: 25 laboratories participated in the 2020 EQA scheme; the United Kingdom had two participating laboratories.

Table 2. Consensus category, modal MIC (range) for gradient strip test and agar dilution (mg/L) and the percentage concordance of susceptibility category for the 2020 EQA panel

Strain		Azithromycin consensus	Cefixime consensus	Ceftriaxone consensus	Ciprofloxacin consensus	Gentamicin consensus	Spectinomycin consensus	Beta-lactamase consensus
	Consensus category	S	R	S	R	N/A	S	NEG
Charles 4 - C242 - (245)	Modal MIC (range)	0.25 (0.064-0.5)	0.5 (0.064-2)	0.125 (0.016-0.5)	>32 (16- >32)	4 (2-8)	8 (1-32)	N/A
Strain 1: 6342 (316/- 11) CfmR. CipR	Susceptibility category concordance (%)	100	87.5	70.8	100	N/A	100	95.5
	Reference MIC*	0.25	0.5	0.25	>32	4	8	N/A
	Consensus category	R	R	R	R	N/A	S	NEG
Strain 2: 6343/6344 (G7944 (WHO Q)) Az	Modal MIC (range)	>256 (>256)	2 (1-4)	0.5 (0.25-1)	>32 (8->32)	4 (2-8)	16 (4-32)	N/A
>256 mg/L, CfmR, CroR, CipR	Susceptibility category concordance (%)	100	100	100	100	N/A	100	95.7
	Reference MIC*	>256	1	1	>32	4	8	N/A
	Consensus category	S	S	S	R	N/A	S	NEG
Strain 3: 6345	Modal MIC (range)	0.5 (0.25-1)	0.064 (0.016-0.25)	0.125 (0.032-0.125)	>32 (≥32)	4 (1-8)	8 (4-32)	N/A
(VNM11-4) CipR	Susceptibility category concordance (%)	95.2	96	100	100	N/A	100	95.7
	Reference MIC*	0.25	0.125	0.125	>32	4	8	N/A
	Consensus category	S	S	S	S	N/A	S	NEG
Strain 4: 6346/6347/6348	Modal MIC (range)	0.25 (0.064-1)	≤0.016 (≤0.016-0.125)	≤0.016 (0.002-0.064)	0.008 (0.008-0.016)	4 (1-8)	8 (4-32)	N/A
(500461)	Susceptibility category concordance (%)	100	100	100	100	N/A	100	95.5
	Reference MIC*	0.25	≤0.016	0.032	0.008	4	8	N/A
	Consensus category	R	S	S	S	N/A	S	NEG

Strain		Azithromycin consensus	Cefixime consensus	Ceftriaxone consensus	Ciprofloxacin consensus	Gentamicin consensus	Spectinomycin consensus	Beta-lactamase consensus
	Modal MIC (range)	2 (0.5-4)	≤0.016 (≤0.016-0.032)	≤0.002 (≤0.002-0.016)	0.004 (≤0.002-0.064)	4 (1-8)	8 (4-32)	N/A
Strain 5: 6349 (VNM11-65) Az>1 mg/L	Susceptibility category concordance (%)	52.2	100	100	96	N/A	100	95.7
	Reference MIC*	2	≤0.016	≤0.016	0.004	4	16	N/A
	Consensus category	S	S	S	R	N/A	S	POS
Strain 6: 6350/6351	Modal MIC (range)	0.5 (0.032-1)	≤0.016 (≤0.016-0.064)	≤0.016 (0.004-0.064)	>32 (4->32)	4 (0.25-8)	8 (0.5-32)	N/A
(EST_11_14) CipR, BLP	Susceptibility category concordance (%)	95.1	100	100	100	N/A	100	100
	Reference MIC*	1	≤0.016	≤0.016	>32	2	16	N/A

<sup>\*</sup>MICs taken from UK NEQAS reference MIC results. Note: No consensus category of susceptibility was assigned to gentamicin as there are currently no published breakpoints for this antimicrobial. N/A: not available; MIC: minimum inhibitory concentration; WHO: World Health Organization; Az: azithromycin; CfmR: cefixime-resistant; CroR: ceftriaxone-resistant; CipR: ciprofloxacin-resistant; R: resistant; BLP: beta-lactamase producer; NEG: negative; POS: positive.

#### 3.3 Interpretation of MICs

All 25 laboratories reported adherence to the EUCAST breakpoints (Table 1) with only one laboratory reporting using the previous (v9.0) version and the others using the most recent one (v10.0) [5]. Most laboratories that tested gentamicin did not interpret categories of susceptibility as there are currently no internationally defined interpretive criteria for this antimicrobial. However, one laboratory did submit categories of susceptibility for gentamicin, using local interpretive criteria; these data were not analysed in this report.

Table 3. Susceptibility methods used by participating laboratories, September 2020 EQA

	Number of participa	ting laboratories (%)
Type of susceptibility test used	2019	2020
MIC gradient strip tests	27 (96.4%)	24 (96.0%)
Agar dilution	2 (7.1%)	2 (8%)
Testing guidelines used		
EUCAST	28 (100%)	25 (100%)
Agar base used		
GC agar base	11 (39.3%)	11 (44%)
Chocolatised blood agar	9 (32.1%)	8 (32%)
Diagnostic sensitivity agar	3 (10.7%)	2 (8%)
Thayer-Martin/Mueller-Hinton	2 (7.1%)	2 (8%)
Other	3 (10.7%)	2* (8%)

<sup>\*:</sup> includes one unknown (not recorded).

Please note that countries that reported using agar dilution also reported use of some gradient strips.

#### 3.4 Coded breakdown of concordance

Due to the confidential nature of the EQA scheme, only coded laboratory breakdowns for beta-lactamase assessment concordance, category of susceptibility concordance and MIC values for MIC gradient strip tests and agar dilution method are shown in the Annex (Tables A1.6 - A1.12). Analysis of the breakdown of results has highlighted that six laboratories reported isolates with MICs greater than two doubling dilutions different from the modal MIC. One laboratory reported more than 5% of results greater than two doubling dilutions from the modal MIC, this laboratory used diagnostic sensitivity agar. This laboratory participates in the Euro-GASP sentinel study via centralised testing, so its results will not have an impact on the Euro-GASP. However, the laboratory has been contacted and will be supported to improve the quality of its susceptibility testing.

In the 2019 EQA (QA19), also one laboratory reported more than 5% of results greater than two doubling dilutions from the modal MIC. This laboratory improved its results in the QA20 EQA with 97.8% of results within two doubling dilutions of the modal MICs showing the problems identified in QA19 have been rectified. This laboratory also currently still participates via centralised testing.

#### 3.5 Susceptibility category concordance

Susceptibility category data for cefixime, ceftriaxone and ciprofloxacin were submitted from all 25 laboratories, azithromycin and beta-lactamase production from 23 laboratories and spectinomycin from 19 laboratories. Four laboratories submitted incomplete susceptibility category results.

Incomplete data were submitted for:

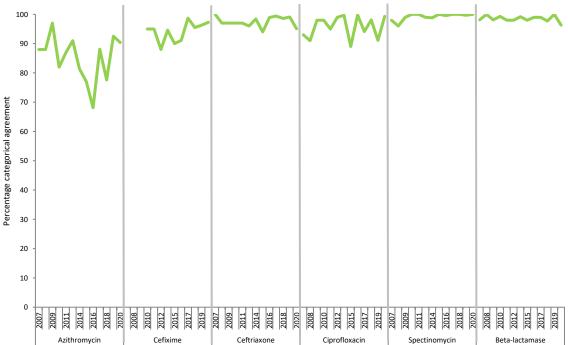
- Azithromycin (laboratory 92624 (isolates 6342,6345-8 and 6350-6351 all below ECOFF) and laboratory 92626 (isolates 6342,6345-8 and 6350-6351 all below ECOFF));
- Cefixime (laboratory 94938 (isolate 6351 only)) and laboratory 92626 (isolate 6347 only));
- Ceftriaxone (laboratory 92630 (isolate 6349 only), laboratory 94602 (isolate 6350 only) and laboratory 96244 (isolate 6344 only));
- Ciprofloxacin (laboratory 92627 (isolate 6347 only));
- Spectinomycin (laboratory 96244 (isolate 6346 only)); and
- Beta-lactamase production (laboratory 94602 (isolate 6348 only)).

Laboratories 92629 and 94938 did not interpret any azithromycin MICs (Table A1.1). Laboratory 92630 was not able to culture strain 6351, laboratory 94602 was not able to culture strain 6342 and laboratory 95588 was unable to culture both strains 6347 and 6348. Six laboratories did not test for spectinomycin susceptibility (92613, 92629, 92630, 94936, 94938 and 95589) (Table A1.10), two laboratories did not test for the production of beta-lactamases (Table A1.12), and eight laboratories did not test for gentamicin susceptibility (92613, 92623, 92628, 92629, 92945, 94936, 94938 and 95589) (Table A1.11).

The highest levels of categorical agreement were seen for spectinomycin (100%), ciprofloxacin (99.3%), and cefixime (97.3%). The lowest level was seen for azithromycin, with 90.4% concordance (Figure 2 and Tables A1.1, A1.3, A1.5 and A1.7). Consensus susceptibility categories were not assigned for gentamicin, as there are currently no published breakpoints for interpretation of results.

When categorical agreement data are compared with previous EQA distributions from both ESSTI (QA2007, QA2008 and QA2009) [6] and ECDC Euro-GASP (QA2010-19) [7-15], there was a slight decrease in concordance for azithromycin, ceftriaxone and beta-lactamase production whereas concordance for cefixime and ciprofloxacin increased (Figure 2). Spectinomycin concordance remained constant at 100% (Figure 2).

Figure 2. Longitudinal comparison of EQA inter-laboratory antimicrobial categorical agreement, EU/EEA, 2007-2020



Note: Cefixime was added to the EQA scheme in 2010.

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

The number of laboratories participating in the EQA changed over time: 19 laboratories (2007 and 2008), 16 laboratories (2009), 18 laboratories (2010), 20 laboratories (2011), 19 laboratories (2012), 21 laboratories (2014), 26 laboratories (2015), 27 laboratories (2016), 28 laboratories (2017), 27 laboratories (2018), 28 laboratories (2019), 25 laboratories (2020).

#### 3.6 MIC concordance

Overall, MIC essential agreement (MIC results within one doubling dilution of the modal MIC recorded) was at 93.8% for all antimicrobials tested (Table 4), which is comparable to the level of essential agreement achieved with the previous EQA panel distribution in 2019 (94.7%) [15]. In a change from the previous two EQA distributions (QA18 and QA19), the highest level of essential agreement was seen for gentamicin (97.0%) rather than cefixime, which was the lowest in this distribution (90.2%) (Table 4). For all MICs combined, 98.9% were within two doubling dilutions of the modal MIC. Cefixime had the highest proportion of isolates with a MIC greater than two doubling dilutions of the modal MIC (1.6%) and ceftriaxone had the lowest (0.4%).

When MIC concordance data are compared with previous ECDC Euro-GASP EQA distributions (QA2010-19) [7-15], the MIC concordance for azithromycin, ceftriaxone and gentamicin has increased, whereas ciprofloxacin, cefixime and spectinomycin have decreased (Figure 3).

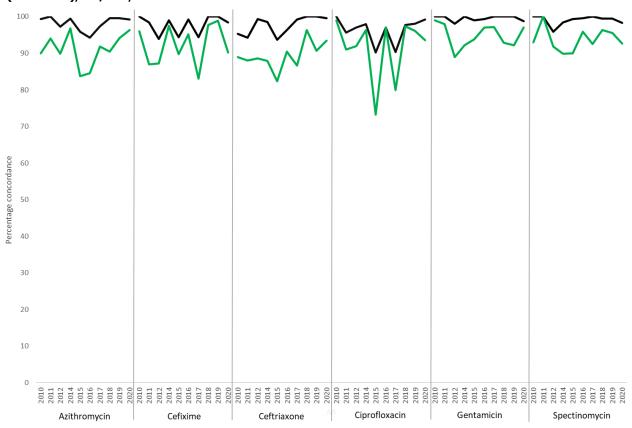
Table 4. Variation from modal MIC for EQA QA20

QA20	Azithr	omycin	Cefi	xime	Ceftr	iaxone	Ciprof	loxacin	Gent	amicin	Specti	nomycin	Tot	tal
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Within +/- 1 doubling dilution	237	96.3	221	90.2	230	93.5	221	93.6	161	97.0	164	92.7	1 234	93.8
Within +/- 2 doubling dilutions	7	2.8	20	8.2	15	6.1	13	5.5	3	1.8	10	5.6	68	5.2
More than +/- 2 doubling dilutions	2	0.8	4	1.6	1	0.4	2	0.8	2	1.2	3	1.7	14	1.1
Total no. of isolates with MIC data	2	46	2	45	2	46	2	36	1	66	1	77	1 3	16

No.: Number of isolates with MIC data.

Some percentages may not add up to 100% due to rounding.

Figure 3. Longitudinal comparison of EQA interlaboratory MIC concordance, percentage of essential agreement (green line) and percentage of results within two doubling dilutions of the modal MIC (black line), EU/EEA, 2010-2020

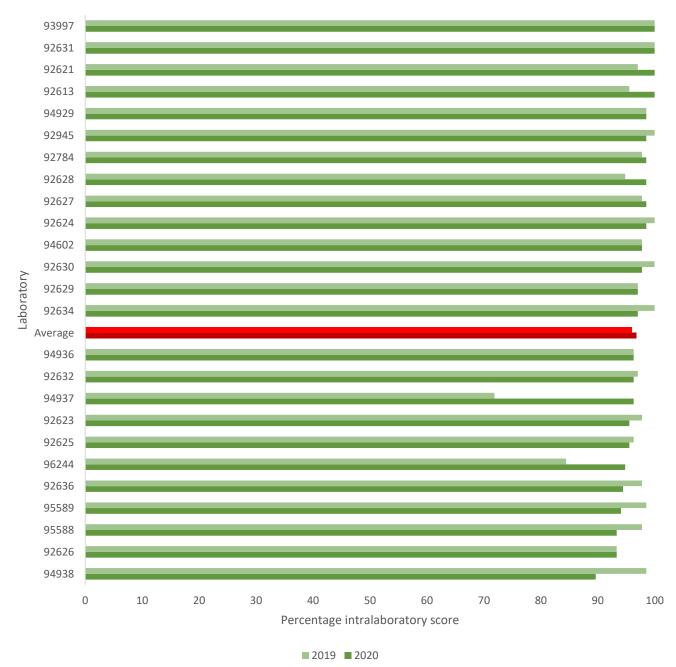


Note: The number of laboratories participating in the EQA changed over time: 18 laboratories (2010), 20 laboratories (2011), 19 laboratories (2012), 21 laboratories (2014), 26 laboratories (2015), 27 laboratories (2016), 28 laboratories (2017), 27 laboratories (2018), 28 laboratories (2019), 25 laboratories (2020).

#### 3.7 Intralaboratory concordance

Intralaboratory concordance was examined using the triplicate (strain four) and two duplicate strains (strains two and six). Figure 4 shows the results for the 2019 and 2020 concordance scores. Most laboratories performed well, with 76% of laboratories (19/25) scoring 95% or higher, including four laboratories obtaining a perfect score of 100%. Of the six laboratories scoring less than 95%, only one had a major fault and none had any very major faults; their lower scores were caused only by minor faults, with all MICs in essential agreement. Therefore, there are no issues with the data they provided for the TESSy database. The average score for the 2020 distribution improved to 96.8% from 96.0% in 2019. Two laboratories which had an intralaboratory score of less than 95% in the 2019 distribution also had a below 95% score in the 2020 distribution. Compared to the 2019 distribution, in 2020 five laboratories improved their intralaboratory concordance with at least 1% and for eleven laboratories there was a less than 1% change in the result.

Figure 4. Intra-laboratory MIC concordance percentage, 2019 vs 2020



#### 3.8 Overall EQA scores

Figure 5 shows the overall MIC scores for the 2020 EQA versus the 2019 EQA with the average score shown in red (2020: 90.3% (dark red); 2019: 89.6% (light red)). For the 2020 EQA, nine laboratories scored a below average result, none of which had greater than 5% of results greater than two doubling dilutions from the modal MIC for the core antimicrobials (azithromycin, ceftriaxone, and ciprofloxacin). The scores for overall categorical agreement are shown in Figure 6. The total score achieved by each laboratory out of a potential 150 is shown by the bars which are coloured to show the composition of the score by none, minor, major, and very major faults with the overall percentage score as a data label.

Figure 5. EQA overall MIC scores, 2019 vs 2020

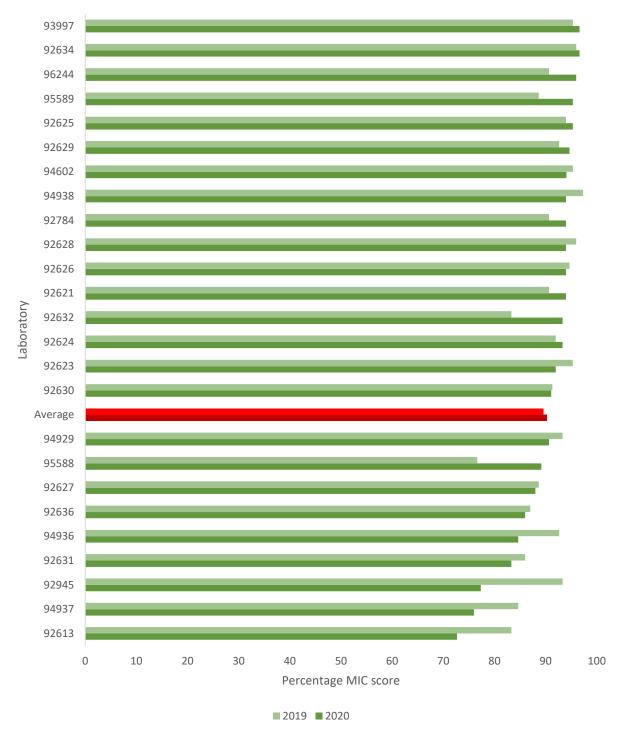


Figure 6. EQA overall categorical agreement scores, 2020



Overall percentage score is shown in the left of each bar. Maximum score for all laboratories was 150 unless otherwise specified.

<sup>\*:</sup> Maximum score was 130 as there were no results for strain 6351 as it was not culturable.

<sup>\*\*:</sup> Maximum score was 130 as there were no results for strain 6342 as it was not culturable.

*<sup>‡:</sup> Maximum score was 120 as there were no results for strains 6347 and 6348 as they were not culturable.* 

<sup>#:</sup> Maximum score was 115 as an SIR category was not assigned to azithromycin MICs below the ECOFF.

<sup>~:</sup> Maximum score was 145 as one ciprofloxacin MIC was not interpreted.

<sup>~~:</sup> Maximum score was 145 as one ceftriaxone MIC was not interpreted.

b: Maximum score was 100 as no SIR category was assigned to azithromycin MICs.

#### 4. Discussion

The 2020 Euro-GASP EQA distribution was sent out to 27 laboratories in 26 participating countries. Unfortunately, two laboratories were unable to retrieve any of the 10 strains received, but the remaining 25 laboratories reported results for all or most of the requested tests. Most laboratories (96.0%) used MIC gradient strip tests to perform antimicrobial susceptibility testing in *N. gonorrhoeae*, which was a comparable level to the previous year. EUCAST guidelines were used by all the participating laboratories to interpret MIC results. These results show the continuing implementation of the EUCAST guidelines and of MIC gradient strip tests across the Euro-GASP participating laboratories. The media used by participating laboratories remained constant from 2019 with GC agar base the most common followed by chocolatised blood agar.

Categorical agreement remained comparable to the previous distribution (QA19) with slight increases for both cefixime (96% to 97%) and ciprofloxacin (91% to 99%) and slight decreases for azithromycin (93% to 90%), ceftriaxone (99% to 95%) and beta-lactamase production (100% to 96%). For both cefixime and ciprofloxacin no strains were included in the OA20 distribution with MICs around their breakpoints. For azithromycin there was one strain included in the QA20 distribution one doubling dilution above the ECOFF (modal MIC= 2 mg/L) which may have caused the decrease in susceptibility category concordance this year. There was still some confusion with how to interpret the EUCAST ECOFF for azithromycin as a SIR category with two laboratories not reporting a category for strains with MICs below the ECOFF and a further two not interpreting the MICs at all. As this is only the second year with the azithromycin ECOFF, some issues as laboratories adjust and local interpretation of the quidelines may vary from those used in the EQA were to be expected. An e-mail was distributed to those laboratories that did not submit or only partially submitted azithromycin SIR results in QA20 to clarify the interpretation used in Euro-GASP. There was one strain in the OA20 distribution with an MIC around the ceftriaxone breakpoint (modal MIC=0.125 mg/L) which may have caused the decrease in susceptibility category concordance this year. The decrease in beta-lactamase production concordance was due to one laboratory's issues with testing this year with all ten strains incorrectly detected as beta-lactamase producers. This laboratory was contacted to investigate their issue with beta-lactamase detection in this distribution.

Overall categorical agreement scores were high, with no laboratory scoring less than 95% for the core antimicrobials which is an improvement on the previous year when one laboratory scored less than 95%. Eleven laboratories had a very major fault (calling a resistant isolate susceptible) all of which were due to a minor fault in the azithromycin MIC for strain 6349 (modal MIC one doubling dilution above the ECOFF). This was a challenging strain and, although the categorical interpretation was scored as a very major fault, all the laboratories correctly interpreted the MIC result they obtained, which was below the ECOFF. The average categorical concordance for the core antimicrobials remained comparable to the previous distribution at 98.2%. Essential MIC agreement was high at 94.0% maintaining the level observed in 2019 (94.7%).

Breakdown of EQA susceptibility testing results by laboratory allowed for detailed analysis of individual laboratory performance. In the 2020 EQA, in general, laboratories performed well, with a good level of interlaboratory and intralaboratory concordance of results. Consistent with the 2019 distribution, one laboratory reported more than 5% of results greater than two doubling dilutions from the modal MIC although the laboratory with below 95% concordance was different from the previous distribution. The laboratory that reported more than 5% variation from the modal MIC for the QA19 distribution improved and this year they achieved 95.7% essential agreement and 97.8% within two doubling dilutions of the modal MIC.

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 recent years. A full analysis of the different methods and breakpoints used in Euro-GASP EQAs over the years is publicly available [16].

## 5. Conclusion

The laboratories participating in the QA20 EQA scheme for susceptibility testing of *N. gonorrhoeae* showed good levels of competency and capability in testing strains of unknown phenotype. Two of 27 laboratories could not retrieve received strains hindering assessment of susceptibility testing competency of these laboratories. Both inter- and intralaboratory essential agreement for the different strains remained at comparable level to those from the QA19 EQA distribution, allowing confidence in Euro-GASP de-centralised susceptibility testing and comparison of surveillance data from the members of the Euro-GASP network. These results indicate that the Euro-GASP antimicrobial surveillance quality is of a good standard. The improvements observed in laboratories with results out of range in previous distributions demonstrate that appropriate troubleshooting and implementation have led to improvements in quality standards.

This Euro-GASP EQA is important to ensure that results from different submitting laboratories are comparable and that significant over- and under-reporting of resistance do not occur. It is also important that reference laboratories have access to appropriate internal quality control (IQC) strains such as the WHO control panel [17] to routinely ensure their own quality assurance in a variety of diagnostic and antimicrobial susceptibility testing. Antimicrobial susceptibility results from Euro-GASP contribute to the evidence base of gonorrhoea treatment guidelines and local susceptibility testing can be used for individual patient management, so confidence in reporting is essential.

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# **Annex. QA20 detailed results**

Table A1.1. Country coded category of susceptibility concordance – azithromycin

													Lab	oratory co	odes																	
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensus	Concordance (%)
1	6342	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	S	S	N	S	S	S	N	S	S	S	20	20	0	0	S	100.0
2	6343	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	R	R	R	R	R	N	R	R	R	46		_	AC		100.0
2	6344	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	R	R	R	R	R	N	R	R	R	46	"	U	46	ĸ	100.0
3	6345	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	- 1	S	S	S	S	S	N	S	S	S	21	20	1	0	S	95.2
	6346	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S						
4	6347	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	N	N	S	S	61	61	0	0	S	100.0
	6348	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	N	N	S	S						
5	6349	S	S	R	R	S	R	R	S	N	S	S	S	S	S	R	R	R	S	R	R	R	N	S	R	R	23	11	0	12	R	52.2
_	6350	S	S	S	N	S	N	S	S	N	S	S	S	S	S	S	I	S	S	S	S	S	N	S	S	S	41	39	2	0		95.1
О	6351	S	S	S	N	S	N	S	S	N	N	S	S	S	S	S	1	S	S	S	S	S	N	S	S	S	41	39	2	U	3	95.1
																															Total	90.4

N: no result; not retrieved or susceptibility category not supplied.

Table A1.2. Country coded MIC values (mg/L) – azithromycin

													Lab	oratory co	des																
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dilutions different
1	6342	0.5	0.25	0.125	0.25	0.125	0.125	0.5	0.125	0.125	0.25	0.125	0.25	0.25	0.25	0.25	0.25	0.25	N	0.25	0.5	0.064	0.125	0.125	0.125	0.125	0.25	0.064	0.5	1	0
2	6343 6344	>256 >256	>256 >256	>256 >256	256 256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 >256	>256 256	>256 >256	>256 >256	>256 >256	>256 >256	256 256	>256	256	>256	0	0
3	6345	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.25	0.5	1	0.25	0.5	0.5	0.5	0.5	0.5	0.25	1	0	0
4	6346 6347 6348	0.5 0.5 0.5	0.25 0.25 0.25	0.5 0.25 0.5	0.5 0.5 0.5	0.25 0.25 0.25	0.25 0.5 0.5	1 0.5 1	0.25 0.25 0.25	0.25 0.25 0.25	0.25 0.25 0.25	0.25 0.25 0.25	0.25 0.5 0.25	0.25 0.25 0.25	0.125 0.125 0.25	0.5 0.5 0.5	0.25 0.25 0.25	0.5 0.5 0.5	0.25 0.25 0.25	0.5 0.5 0.5	0.5 0.5 0.5	0.125 0.125 0.064	0.5 0.25 0.25	0.25 N N	0.5 0.25 0.5	0.25 0.5 0.25	0.25	0.064	1	3	0
5	6349	0.5	1	2	2	0.5	2	4	1	1	1	1	1	1	1	2	2	2	1	2	2	0.5	2	1	2	2	2	0.5	4	3	0
6	6350 6351	0.5 0.5	0.5 0.5	0.5 0.5	0.5 0.5	0.25 0.5	0.5 0.5	1 1	0.25 0.25	0.25 0.25	0.5 N	0.25 0.25	0.5 0.5	0.5 0.5	0.25 0.5	0.5 0.5	0.5 0.5	0.5 0.5	0.25 0.25	0.5 0.5	1 1	0.032 0.032	0.5 0.25	0.5 0.5	0.5 0.5	0.25 0.5	0.5	0.032	1	0	2

N: no result; not retrieved, not tested or MIC not supplied.

Note: Laboratory 92630 was not able to retrieve strain 6351, laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348.

Table A1.3. Country coded category of susceptibility concordance – cefixime

													Lab	oratory co	des																	
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensus	Concordanc e (%)
1	6342	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	N	R	R	R	S	S	R	R	24	3	0	21	R	87.5
2	6343 6344	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	R R	50	0	0	50	R	100.0
3	6345	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	25	24	0	1	S	96.0
4	6346 6347 6348	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 S	S S	S S	S S	S N	S S	S S	72	72	0	0	S	100.0						
5	6349	5	<u>s</u>	<u> </u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u> </u>	<u> </u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u>s</u>	<u> </u>	<u>s</u>	S	<u> </u>	5	25	25	0	0	S	100.0
6	6350 6351	S	S S	S N	S S	S S	S S	S S	S S	S S	S S	S S	S S	S S	S S	S N	S S	S S	S S	48	48	0	0	S	100.0							
																															Total	97.3

N: no result; not retrieved or susceptibility category not supplied.

Table A1.4. Country coded MIC values (mg/L) – cefixime

													Lab	oratory co	odes																
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dilutions different
1	6342	2	1	0.5	0.5	0.5	0.25	0.5	0.25	0.25	0.25	0.5	0.125	0.5	1	1	0.5	0.5	N	0.5	0.5	0.5	0.125	0.064	0.25	0.25	0.5	0.064	2	3	1
,	6343	2	2	2	2	2	2	1	2	2	2	2	1	2	2	4	2	2	2	2	1	2	1	1	1	2	1	1	4	0	0
	6344	2	2	2	2	2	2	1	2	1	2	2	1	2	2	2	2	2	2	2	2	2	1	1	1	1		1	4	U	U
3	6345	0.064	0.064	0.064	0.064	0.064	0.032	0.125	0.064	0.064	0.032	0.064	0.064	0.25	0.064	0.064	0.125	0.032	0.125	0.125	0.125	0.064	0.032	0.016	0.125	0.064	0.064	0.016	0.25	2	0
	6346	0.125	0.064	0.032	0.016	0.016	≤0.016	0.016	≤0.016	0.016	≤0.016	0.016	≤0.016	0.032	0.064	0.032	0.064	0.016	0.064	0.032	0.016	≤0.016	≤0.016	N	0.032	0.016					
4	6347	0.125	0.032	0.064	0.016	0.016	≤0.016	0.016	≤0.016	≤0.016	≤0.016	0.016	≤0.016	0.016	0.064	0.016	0.064	0.016	0.064	0.032	0.064	0.016	≤0.016	N	0.016	0.016	≤0.016	≤0.016	0.125	13	3
	6348	0.125	0.032	0.032	0.016	0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.016	≤0.016	0.016	0.064	≤0.016	0.064	0.016	0.064	0.064	0.032	0.032	≤0.016	N	0.016	0.016					
5	6349	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.032	≤0.016	0.016	0.016	≤0.016	≤0.016	≤0.016	0.016	≤0.016	≤0.016	0.032	0	0
-	6350	≤0.016	0.032	0.016	≤0.016	≤0.016	≤0.016	≤0.016	≤0.016	0.016	≤0.016	≤0.016	≤0.016	0.032	0.032	≤0.016	0.064	≤0.016	0.032	0.016	0.016	0.016	≤0.016	≤0.016	≤0.016	0.016	<0.016	≤0.016	0.064	2	0
	6351	≤0.016	0.032	0.016	0.016	0.016	≤0.016	≤0.016	≤0.016	0.016	N	≤0.016	≤0.016	0.032	0.032	≤0.016	0.064	≤0.016	0.032	0.016	0.016	0.016	≤0.016	≤0.016	0.016	0.016	20.016	20.010	0.064	2	U

N: no result; not retrieved, not tested or MIC not supplied.

Note: Laboratory 92630 was not able to retrieve strain 6351, laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348 and no MIC result was submitted for strain 6346.

Table A1.5. Country coded category of susceptibility concordance – ceftriaxone

													Lab	oratory co	des																	
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensus	Concordance (%)
1	6342	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	R	S	Ν	R	R	S	S	S	S	S	24	17	0	7	S	70.8
	6343	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	49			49		100.0
2	6344	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	N	49	0	U	49	K	100.0
3	6345	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	25	25	0	0	S	100.0
	6346	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
4	6347	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S	73	73	0	0	S	100.0
	6348	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S						
5	6349	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	24	24	0	0	S	100.0
_	6350	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	40	48	_	_		100.0
6	6351	S	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	48	48	١	"	3	100.0
																															Total	95.1

N: no result; not retrieved or susceptibility category not supplied.

Table A1.6. Country coded MIC values (mg/L) – ceftriaxone

													Labo	oratory co	des																
																											Modal			2	>2
																											MIC	Min MIC	Max MIC	dilutions	dilutions
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	IVIIC			different	different
1	6342	0.5	0.25	0.125	0.25	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.064	0.125	0.125	0.25	0.125	0.125	Ν	0.25	0.25	0.125	0.064	0.016	0.125	0.064	0.125	0.016	0.5	1	1
,	6343	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	1	1	0.5	0.5	0.5	1	0.25	0.5	0.5	0.5	0.25	0.5	0.5	0.25	1	0	0
	6344	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	1	1	0.5	0.5	0.5	1	0.5	0.5	0.5	0.25	0.25	0.5	0.5	0.25	1	U	U
3	6345	0.032	0.125	0.125	0.064	0.125	0.032	0.125	0.064	0.064	0.032	0.064	0.064	0.125	0.032	0.125	0.125	0.064	0.125	0.125	0.125	0.064	0.032	0.032	0.125	0.064	0.125	0.032	0.125	6	0
	6346	0.032	0.032	0.032	0.032	0.016	0.004	0.016	0.016	0.008	≤0.016	0.016	0.016	0.032	0.032	0.016	0.064	0.016	0.032	0.032	0.064	0.016	0.008	0.002	0.016	0.032					
4	6347	0.032	0.032	0.032	0.016	0.016	0.008	0.016	0.016	0.008	≤0.016	0.016	0.016	0.016	0.032	0.032	0.032	0.016	0.032	0.064	0.032	0.016	0.008	N	0.016	0.016	0.016	0.002	0.064	6	0
	6348	0.032	0.032	0.064	0.016	0.016	0.004	0.016	0.016	0.016	≤0.016	0.016	0.016	0.016	0.032	0.016	0.064	0.016	0.032	0.032	0.064	0.016	0.002	N	0.016	0.016					
5	6349	≤0.002	≤0.016	≤0.002	≤0.002	≤0.016	≤0.002	≤0.002	≤0.002	≤0.002	≤0.016	≤0.002	≤0.002	≤0.002	≤0.004	≤0.002	≤0.016	≤0.016	0.016	≤0.016	0.002	0.002	≤0.002	≤0.002	≤0.002	0.002	≤0.002	0.002	0.016	0	0
_	6350	0.016	0.032	0.016	0.016	≤0.016	0.004	0.016	0.008	0.016	≤0.016	0.008	0.008	0.032	0.016	0.008	0.064	≤0.016	0.016	0.016	0.032	0.004	0.008	0.008	0.016	0.016	0.016	0.004	0.064	2	0
6	6351	0.016	0.032	0.016	0.016	0.016	0.008	0.016	0.008	0.016	Ν	0.008	0.008	0.032	0.016	0.008	0.064	≤0.016	0.032	0.016	0.032	0.004	0.004	0.004	0.008	0.016	0.016	0.004	0.064	2	U

Note: no result; not retrieved, not tested or MIC not supplied

Note: Laboratory 92630 was not able to retrieve strain 6351, laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348. For ceftriaxone, MICs below 0.016 mg/L were not scored as not all laboratories tested dilutions below this value.

Table A1.7. Country coded category of susceptibility concordance – ciprofloxacin

													Labor	ratory cod	es												]					
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensus	Concordance (%)
1	6342	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	24	0	0	24	R	100.0
2	6343	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	50	0	0	50	R	100.0
	6344	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	30	Ů	ŭ	50		100.0
3	6345	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	25	0	0	25	R	100.0
	6346	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
4	6347	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S	72	72	0	0	S	100.0
	6348	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	N	S	S						
5	6349	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	25	24	0	1	S	96.0
6	6350	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	49	0	0	49	R	100.0
	6351	R	R	R	R	R	R	R	R	R	N	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	73	3	J	3	, n	100.0
																															Total	99.3

N: not retrieved or susceptibility category not supplied.

Table A1.8. Country coded MIC values (mg/L) – ciprofloxacin

													Laboi	ratory cod	es												Ī				
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Modal MIC	Min MIC		2 dilutions different	>2 dilutions different
1	6342	>32	>32	32	>32	>32	>32	>32	>32	>32	>32	>32	16	>32	N	>32	16	>32	N	>32	>32	16	>32	>32	32	32	>32	16	>32	0	0
,	6343	8	>32	>32	>32	>32	>32	>32	>32	>32	>32	8	32	>32	N	>32	8	>32	>32	>32	>32	>32	>32	32	32	32	>32	0	>32		0
	6344	8	>32	>32	>32	>32	>32	>32	>32	>32	>32	8	16	>32	N	>32	8	>32	>32	>32	>32	>32	>32	32	32	32	>32	٥	>32	О	U
3	6345	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	N	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	32	>32	32	>32	0	0
	6346	0.016	0.008	0.016	0.016	0.008	0.008	0.016	0.008	0.016	0.008	0.016	0.008	0.016	N	0.008	0.008	0.008	0.008	0.016	0.016	0.008	0.016	0.016	0.008	0.008					
4	6347	0.016	0.008	0.008	0.016	0.008	0.016	0.016	0.016	0.008	0.008	0.016	0.008	0.008	N	0.008	0.008	0.008	0.008	0.016	0.016	0.008	0.008	N	0.008	0.008	0.008	0.008	0.016	0	0
	6348	0.016	0.008	0.016	0.016	0.008	0.008	0.016	0.008	0.008	0.016	0.016	0.008	0.008	N	0.008	0.008	0.008	0.008	0.016	0.016	0.008	0.008	N	0.008	0.008					
5	6349	≤0.002	0.004	0.004	0.004	0.004	0.004	0.008	0.004	0.004	0.064	0.008	0.004	0.004	N	0.004	≤0.004	0.002	0.004	0.008	0.008	0.004	0.004	0.004	0.002	0.004	0.004	≤0.002	0.064	0	1
_	6350	8	>32	>32	>32	16	>32	>32	16	16	>32	8	16	>32	N	16	8	>32	>32	>32	>32	8	>32	>32	16	32	>32	4	>32	7	1
6	6351	8	>32	>32	>32	32	>32	>32	16	16	N	8	16	>32	N	16	8	>32	>32	>32	>32	4	>32	>32	32	32	>32	4	>32	,	1

N: no result; not retrieved or beta-lactamase result not supplied.

Note: Laboratory 92630 was not able to retrieve strain 6351, laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348. Laboratory 92636 tested ciprofloxacin susceptibility by breakpoint plates so their MIC results could not be scored.

Table A1.9. Country coded category of susceptibility concordance – spectinomycin

													Lab	oratory co	odes																	
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensus	Concordance (%)
1	6342	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	N	S	N	S	N	S	N	S	18	18	0	0	S	100.0
2	6343	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	38	38		0	_	100.0
2	6344	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	38	38	0	0	3	100.0
3	6345	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	19	19	0	0	S	100.0
	6346	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	N						
4	6347	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	N	N	S	54	54	0	0	S	100.0
	6348	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	N	N	S						
5	6349	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	19	19	0	0	S	100.0
_	6350	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	20	20		_	_	400.0
6	6351	N	S	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	S	N	S	N	S	N	S	38	38	0	0	5	100.0
_																															Total	100.0

N: not retrieved or susceptibility category not supplied.

Table A1.10. Country coded MIC values (mg/L) – spectinomycin

													Lab	oatory co	des																
	S	00540	00504	02522	22524	22525	22525	00507	22522	22522	22522	00504	00.500	22524	22525		222.45	22227	0.4500	0.4000	04005	0.400-7	0.4000	05500	05500		Modal MIC	Min MIC			>2 dilutions
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	_			different	different
1	6342	N	32	16	16	4	16	32	16	N	N	8	8	8	N	8	≤16	1	N	8	N	4	N	16	N	8	8	1	32	2	1
2	6343	N	32	8	16	8	16	16	16	N	N	8	8	16	N	16	≤16	8	8	16	N	16	N	16	N	8	16	4	32	1	0
	6344	N	32	16	16	8	16	16	16	N	N	8	8	16	N	16	≤16	8	4	8	N	16	N	16	N	8	10	4	32		٥
3	6345	N	32	16	8	8	16	16	8	N	N	8	8	8	N	8	≤16	8	4	8	N	8	N	16	N	8	8	4	32	1	0
	6346	N	32	16	16	8	8	16	8	N	N	8	8	8	N	16	≤16	4	8	16	N	4	N	16	N	4					
4	6347	N	32	16	16	8	16	16	8	N	N	8	8	8	N	16	≤16	4	8	16	N	8	N	N	N	8	8	4	32	3	0
	6348	N	32	16	16	8	8	16	8	N	N	8	8	8	N	16	≤16	8	8	16	N	4	N	N	N	8					
5	6349	N	32	16	8	8	8	16	8	N	N	8	8	8	N	8	≤16	8	4	16	N	4	N	16	N	8	8	4	32	1	0
_	6350	N	32	8	8	8	16	16	8	N	N	8	8	16	N	16	≤16	4	4	8	N	0.5	N	16	N	8		٥٢	32	2	
0	6351	N	32	8	8	8	16	16	8	N	N	8	8	16	N	16	≤16	4	4	8	N	0.5	N	8	N	8	8	0.5	32	2	2

Note: Laboratories 90984, 92613, 92624, 92629, 92630, 93997, 94936 and 95589 did not submit spectinomycin data.

N: no result; not retrieved, not tested or MIC not supplied.

Note: Laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strain 6347 and 6348. Laboratories 92613, 92629, 92630, 92636, 94936, 94938, and 95589 did not test for spectinomycin susceptibility. Laboratory 92945 tested spectinomycin susceptibility by breakpoint plates so their MIC results could not be scored.

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

													Lab	oratory co	des																
	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Modal MIC	Min MIC	Max MI		>2 dilutions different
1	6342	N	8	N	4	2	4	8	N	N	8	4	2	8	4	2	N	4	N	4	N	2	N	4	N	4	4	2	8	0	0
2	6343 6344	N N	8 8	N N	4 4	2 2	8 4	8 8	N N	N N	4	4	4 2	4 4	4	4	N N	4 4	4 4	4	N N	4 4	N N	8 8	N N	4	4	2	8	0	0
3	6345	N	8	N	8	4	8	8	N	N	8	4	4	4	4	4	N	4	4	4	N	1	N	4	N	4	4	1	8	1	0
4	6346 6347 6348	N N N	8 8 8	N N N	8 8 8	4 4 4	8 8 8	8 8 8	N N N	N N N	8 8 8	4 4 4	2 4 4	4 4 4	≤2 ≤2 ≤2	4 4 4	N N N	4 4 4	4 4 4	4 4 4	N N N	2 1 2	N N N	8 N N	N N N	4 4 4	4	1	8	1	0
5	6349	N	8	N	4	2	4	8	N	N	8	8	2	4	≤2	4	N	4	4	4	N	1	N	4	N	4	4	1	8	1	0
6	6350 6351	N N	8 8	N N	8 8	4 4	8 8	8 8	N N	N N	8 8	8 4	4 8	8 4	4 4	4 4	N N	4 4	2 4	4 0.5	N N	0.25 4	N N	8 N	N N	4 4	4	0.25	8	0	2

N: no result; not retrieved, not tested or MIC not supplied.

Note: Laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348. Laboratories 92613, 92623, 92628, 92629, 92945, 94936, 94938 and 95589 did not test for gentamicin susceptibility. Laboratory 95588 did not test strain 6351 for gentamicin susceptibility.

**Table A1.12.** Country coded concordance – beta-lactamase

	Strain	92613	92621	92623	92624	92625	92626	92627	92628	92629	92630	92631	92632	92634	92636	92784	92945	93997	94602	94929	94936	94937	94938	95588	95589	96244	Total	No. S	No. I	No. R	Consensu s	Concordance (%)
1	6342	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	N	S	R	S	S	S	N	S	22	21	0	1	S	95.5
2	6343 6344	S S	N N	S S	R R	S S	S S	S S	N N	S S	46	44	0	2	s	95.7																
3	6345	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	R	S	S	S	N	S	23	22	0	1	S	95.7
	6346	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	R	S	S	S	N	S						
4	6347	s	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	R	S	S	N	N	S	66	63	0	3	S	95.5
	6348	s	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	N	S	R	S	S	N	N	S						
5	6349	S	S	S	S	S	S	S	S	N	S	S	S	S	S	S	S	S	S	S	R	S	S	S	N	S	23	22	0	1	S	95.7
6	6350 6351	R R	N N	R N	R R	N N	R R	45	0	0	45	R	100.0																			
																															Total	96.4

N: no result; not retrieved or beta-lactamase result not supplied.

Note: Laboratory 92630 was not able to retrieve strain 6351, laboratory 94602 was not able to retrieve strain 6342, laboratory 95588 was not able to retrieve strains 6347 and 6348. Laboratories 92629 and 95589 did not test for beta-lactamase production. Laboratory 94602 did not submit a beta-lactamase result for strain 6348.

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