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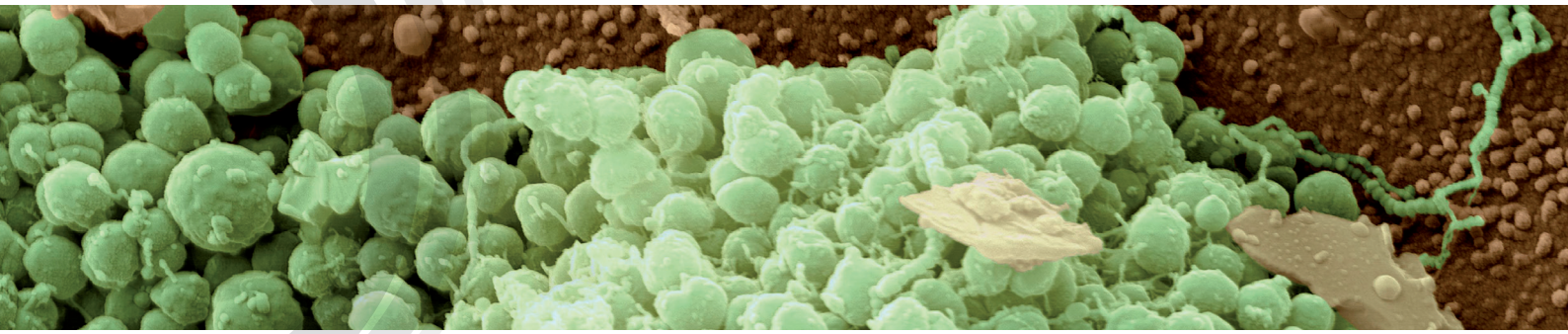

Gonococcal antimicrobial susceptibility surveillance in Europe

2009

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ECDC SURVEILLANCE REPORT

Gonococcal antimicrobial susceptibility surveillance in Europe 2009

This project was commissioned by the European Centre for Disease Prevention and Control and coordinated by Marita van de Laar (contract ECD.1699).

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Abbreviations

AMR	Antimicrobial resistance
EUCAST	European Committee on Antimicrobial Susceptibility Testing
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming Unit
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
DS	Decreased susceptibility
EEA	European Economic Area
EQA	External quality assurance
ESSTI	European Surveillance of Sexually Transmitted Infections Project
EU	European Union
Euro-GASP	European Gonococcal Antimicrobial Surveillance Programme
GC	Gonococcal
GUM	Genitourinary Medicine
GE	Genital Pathogens Scheme
GP	General practitioner
GRASP	Gonococcal Resistance to Antimicrobials Surveillance Programme
HPA	Health Protection Agency
ID	Identification
MIC	Minimum inhibitory concentration
MSM	Men who have sex with men
PBS	Phosphate buffered saline
PPNG	Penicillinase-Producing *Neisseria gonorrhoeae*
QA	Quality assurance
SRGA	Swedish Reference Group for Antibiotics
STD	Sexually transmitted disease
STI	Sexually transmitted infection
TSB	Trypticase Soy Blood
UK-NEQAS	United Kingdom National External Quality Assessment Service
WHO	World Health Organisation
WPR	Western Pacific Region

Executive summary

It is the overall aim of the European sexually transmitted infection (STI) surveillance network to strengthen the surveillance of gonococcal susceptibility in European Union/European Economic Area (EU/EEA) Member States (MS). Within this aim are the following two objectives, with an emphasis on gonococcal antimicrobial susceptibility surveillance and testing:

- To develop and implement sentinel surveillance of antimicrobial resistant gonorrhoea to a range of therapeutically relevant antimicrobials.
- To implement an external quality assurance (EQA) scheme for gonococcal antimicrobial susceptibility testing across Europe.

In 2009, 17 EU/EEA MS contributed with up to 110 consecutive gonococcal isolates to the European gonococcal antimicrobial surveillance programme (Euro-GASP). Susceptibility testing was performed by E-test or agar dilution for the following therapeutically relevant antimicrobials: cefixime, ceftriaxone, ciprofloxacin, azithromycin, spectinomycin and gentamicin. A total of 1366 isolates were collected and tested. The majority of gonococci (84%) were collected from men. The age range of the patients was less than 1 year to 88 years, with a median of 29 years; 32% of patients were younger than 25 years. Site of specimen was mainly genital (87%), followed by rectal (10%) and pharyngeal (3%). When information on previous diagnosis of gonorrhoea or concurrent STI was available, 19.5% had previously been diagnosed with gonorrhoea and 14% had a concurrent chlamydia. When sexual orientation was known, 63% stated that they were heterosexual and 37% were men who have sex with men.

Eighteen countries participated in the gonococcal antimicrobial resistance EQA scheme, which has shown high comparability between centres. This suggests that surveillance results, with respect to gonococcal antimicrobial susceptibility from the members of the European STI surveillance network, can be used with confidence and are comparable.

The European gonococcal antimicrobial surveillance programme has identified that 5% of tested isolates have decreased susceptibility to cefixime in 2009, using a cut-off of >0.125mg/L. Rates of ciprofloxacin and azithromycin resistance are high across Europe (63% and 13%, respectively) and these antimicrobials should not be used for treatment, unless isolates are known to be susceptible or local resistance rates are known to be less than 5%. Even though no breakpoint for resistance to gentamicin has been established, the minimum inhibitory concentration (MIC) distribution offers hope that gentamicin could be used for therapy in the future.

Decreased susceptibility to cefixime is extremely concerning as it is a recommended therapy for gonorrhoea across Europe, as is ceftriaxone. The continual upward drift in the MIC for ceftriaxone in the European gonococcal population therefore needs to be monitored carefully. Loss of cefixime as an oral treatment option across Europe may have major cost and compliance implications if parenterally administered ceftriaxone becomes the only viable option.

The European antibiotic resistance sentinel surveillance of *Neisseria gonorrhoeae* is essential to inform treatment guidelines, thereby preventing onward transmission and reducing patient morbidity. Currently, Euro-GASP is being implemented in additional EU/EEA MS to achieve greater representativeness and thus strengthen the network.

1 Introduction

Since 2009, the European Centre for Disease Prevention and Control (ECDC) has coordinated the enhanced surveillance of sexually transmitted infections (STI) in Europe. The Centre strives to ensure a high quality of standardised STI surveillance data from the countries in the European Union and the European Economic Area (EU/EEA). Until 2009, the STI surveillance in EU/EEA was coordinated by the European Surveillance of STI (ESSTI) project, funded by the European Commission (Directorate General for Health and Consumers) and the Health Protection Agency, United Kingdom. Evaluation and assessment of the ESSTI project concluded that all of its surveillance activities should be integrated into ECDC and that the laboratory and training component of its activities should be continued and outsourced. The evaluation also indicated that the STI microbiology component was extremely valuable but that the timeliness in reporting should improve and preferably be integrated with epidemiological data; strong links and collaboration between microbiologists and epidemiologists should be ensured.

The STI microbiology project—which is part of European STI surveillance—was launched in August 2009 and has been outsourced to an international team lead by the Health Protection Agency (UK) and includes the Statens Serum Institut (Denmark) and Örebro University Hospital (Sweden).

1.1 Background

The spread of drug-resistant gonorrhoea is of concern in the context of EU expansion and the growing relevance of imported gonococcal strains. The emergence of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* is a serious threat to the treatment and control of gonorrhoea. Numerous, formerly effective, therapeutic agents are no longer used due to the emergence of resistance and subsequent rapid global spread. High level resistance to penicillin, and later quinolone, first developed in the WHO West Pacific Region [1] and then disseminated globally [2, 3]. The therapeutic agents currently recommended in many countries worldwide, third generation cephalosporins, are amongst the last agents to remain effective. Unfortunately, evidence suggests a pattern similar to the one observed for penicillin and quinolone resistance is emerging for third generation cephalosporins. Reports of decreased susceptibility have been noted outside of this region in the USA [4] and Australia [5], and it is becoming increasingly common in European countries such as the United Kingdom [2], Greece [6], Spain [7] and Sweden [8].These observations highlight the need for an international perspective on gonococcal resistance to monitor and limit the spread of resistance and preserve therapeutic options.

Previously, the Euro-Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) was implemented by the ESSTI project as a sentinel surveillance programme in 16 EU countries from 2004 to 2008. Euro-GASP identified high levels of resistance to therapeutic agents [9] used across EU countries and has helped to inform European guidelines [10]. In particular, the following developments in gonococcal resistance were demonstrated:

- Ciprofloxacin resistance across Europe is at more than 5%, which demonstrates that this is no longer an appropriate agent for first-line empirical therapy.
- High-level azithromycin resistance in Scotland and Ireland.
- A drift towards higher ceftriaxone minimum inhibitory concentration (MIC) values in the gonococcal population in Europe; a worrying trend that highlights the need for continued and enhanced surveillance, including more countries and extension of the surveillance scheme to include additional third generation cephalosporins.

1.2 Objectives

It is essential to monitor AMR, particularly to detect the emergence of resistance to the currently recommended third generation cephalosporins, as there is a lack of new or alternative treatments once these are no longer effective.

It is the overall aim of the European STI surveillance network to strengthen the surveillance of gonococcal antimicrobial susceptibility in the EU/EEA MS. The following objectives are focused on achieving this aim:

- Developing and implementing sentinel surveillance of gonococcal antimicrobial susceptibility to a range of therapeutically relevant antimicrobials.
- Improving the timeliness of surveillance to allow more frequent reporting of developments in gonococcal antimicrobial susceptibility across Europe.
- Linking susceptibility data with epidemiological information.
- Implementing an external quality assurance (EQA) scheme for antimicrobial susceptibility testing across Europe.
- Providing training in gonococcal antimicrobial susceptibility testing.

This report presents the results from the 2009 gonococcal antimicrobial susceptibility surveillance and the 2010 EQA scheme.

A protocol for the implementation of more timely and decentralised gonococcal susceptibility surveillance has been developed and will be used for strain collection in 2010–2012.

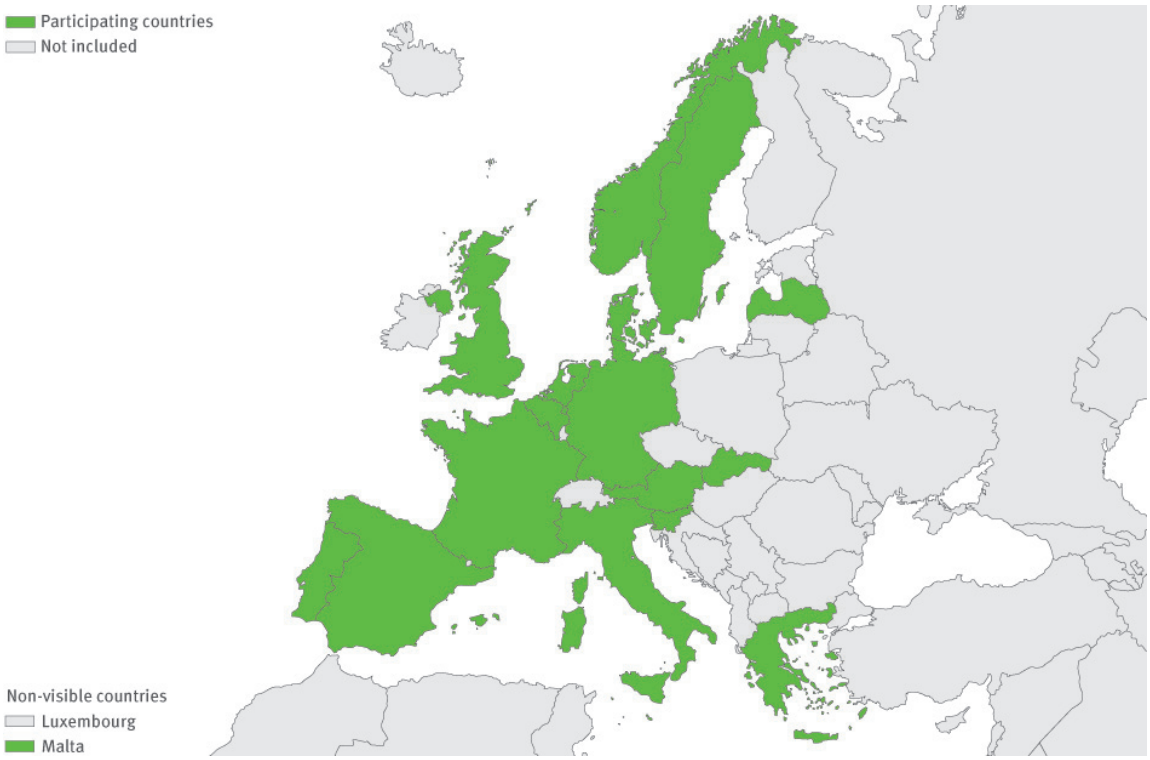
2 Methods

The gonococcal antimicrobial sentinel surveillance for 2009 followed a model similar to that of the former Euro-GASP [9, 11] as described below. Susceptibility testing was performed according to the protocol for centralised testing and is described in detail in Annex 1.

2.1 Participating laboratories

The contact points for STI surveillance in all EU/EEA MS were invited to participate in the strain collection as part of the Euro-GASP programme. The laboratory contact points in 17 countries agreed to participate in Euro-GASP as part of the European network for STI surveillance (Map 1).

Map 1: Participating countries in Euro-GASP, 2009



2.2 Strain collection

Each country was asked to contribute 110 isolates from October 1 2009, with the aim of retrieving and testing 100 isolates from each country. Some laboratories (Austria, Germany, Greece, Italy, Latvia, Malta, Norway, Portugal, Slovenia) collected isolates prior to this start date due to a low collection rate. Isolates from the UK were collected during the national gonococcal resistance to antimicrobials surveillance programme (GRASP) collection period in June and July 2009. Laboratories were requested to collect one isolate from each patient in the following order of preference when multiple sites were infected:

1. male rectal;
2. male urethral;
3. female cervical;
4. any other site.

If multiple isolates from the same patient were submitted for susceptibility testing, then isolates were selected using the same order of preference as stated above. Pure cultures, 18–24 hours old, were saved on Microbank beads and stored at -70°C. The isolates were then sent frozen on dry ice to one of the following three laboratories for susceptibility testing: Health Protection Agency (HPA), London, UK; Statens Serum Institut, Copenhagen, Denmark; or Örebro University Hospital, Örebro, Sweden.

2.3 Susceptibility testing

Susceptibility testing was performed using either a breakpoint technique that allows for isolates to be categorised as susceptible or resistant (including intermediate resistance where applicable), or E-tests to determine the minimal inhibitory concentration (MIC) to allow monitoring of drift in susceptibility.

The antimicrobials that were tested included those currently recommended for treatment (cefixime, ceftriaxone and spectinomycin), those considered potential alternatives (azithromycin and gentamicin) and those previously used for treatment (ciprofloxacin and penicillin, enzyme-mediated high-level resistance only).

The following methodologies were used for the individual antimicrobial agents:

- Ciprofloxacin (breakpoint);
- Azithromycin (breakpoint);
- Spectinomycin (breakpoint);
- Gentamicin (agar dilution/E-test);
- Cefixime (E-test);
- Ceftriaxone (E-test); and
- Penicillinase production (Nitrocefin).

Further details on the testing methodology and breakpoints can be found in Annex 1.

2.4 Background variables

For each isolate, the following data was collected where available: date specimen obtained; specimen site (rectum, cervix, urethra, pharynx, urethra-cervical, high vaginal swab, any other site in full); sex (male, female, unknown); age (in years); sexual orientation (heterosexual, homosexual, bisexual, unknown); previously diagnosed with gonorrhoea (yes, no, unknown); and concurrent STI diagnosed this episode (none, syphilis, chlamydia, herpes, warts, other, unknown).

Further information on the source of the data (STI clinics, dermatovenerology clinics, GPs, hospitals etc) and the level of coverage (comprehensive—all diagnosed gonorrhoea in the country; national—representations from all areas of the country but not every case of diagnosed gonorrhoea; and regional—coverage of a particular region) was also requested.

3 Results

3.1 Isolate and patient data

A total of 1471 isolates were collected over the 2009 collection period and after the removal of duplicate specimens 1366 isolates were retrieved and confirmed to be *Neisseria gonorrhoeae*. The overall retrieval rate was 93% (1366/1471). The number of isolates collected from each country varied from 11 (Latvia) to 146 (Norway). Information on the source of the data and the level of coverage is presented in Table 1. The level of coverage (number of isolates collected compared to the number of reported cases as part of the enhanced epidemiological surveillance of STI in 2009) ranged from 1% (UK) to 92% (Malta). Even though the range is large, only four countries had 5% or less collection coverage. Further isolate collection is being encouraged in Latvia and more isolates will be tested in 2010 and beyond for countries that have a higher number of cases (the Netherlands, Spain and the UK) to increase the level of representativeness.

Table 1: Participating laboratories Euro-GASP, coverage, specimen source, collected isolate number and number of cases, 2009

Country	Coverage	Specimen Source	Number of isolates collected	Number of cases reported* (% collected)
Austria	Regional	GPs and hospitals/Public Health department	110	143 (77)
Belgium	National	GPs, gynaecologists, hospitals and STI clinics	110	711 (15)
Denmark	Comprehensive	GPs and STI clinics	119	593 (20)
France	National	GPs, STI clinics, private and public hospital laboratories	110	342 (32)
Germany	Regional	Outpatients and medical emergency departments	48	N/A
Greece	National	Hospitals and STD outpatients clinic	110	164 (67)
Italy	Regional	Hospitals, GUM clinics, STI clinics, DV clinics, university/hospital microbiology units	74	154† (48)
Latvia	National	Latvian Infectiology Center	11	419 (3)
Malta	National	National GUM clinics, hospital gynaecology clinics and wards	57	62 (92)
Netherlands	Regional	GGD STI clinic and GPs	114	2426 (5)
Norway	National	University hospitals	146	269 (54)
Portugal	National	STI clinics and general, urology and gynaecology practices	85	114 (75)
Slovakia	Regional	DV, urology and gynaecology practices.	22	171 (13)
Slovenia	Regional	DV and microbiology department from university hospital.	24	30 (80)
Spain	National	STI clinics and general hospitals	103	1954 (5)
Sweden	Comprehensive	STI clinics	108	608 (18)
United Kingdom	Regional	GUM clinics and STI clinics	120	17001 (1)
		Total	**1471**	**25161 (5.9)**

Comprehensive coverage defined as all diagnosed gonorrhoea in the country. National defined as representations from all areas of the country but not every case of diagnosed gonorrhoea. Regional defined as good coverage of a particular region of the country but not national coverage.
** Number of gonorrhoea cases by year of diagnosis, 2009. Source: ECDC Surveillance Report: Sexually Transmitted Infections in Europe, 1990 – 2009. Stockholm, ECDC 2010.*
†2008 data used as 2009 data not available.

The majority of gonococci (84%) were collected from men. Gender was unknown for 24 cases (Table 2). The age range of the patients was less than 1 year to 88 years, with a mode and median age of 23 and 29 years respectively; a total of 32% of patients were younger than 25 years when age was known. Site of specimen was mainly genital (86.5%) followed by rectal, pharyngeal and other; site of infection was unknown for 21 cases. Information on previous diagnosis of gonorrhoea was available for 34% of the cases, of which 18% had a previous infection and 82% did not. Information on concurrent STI was available for 40% of cases. Fourteen percent of patients had concurrent chlamydia, 6% were infected with another STI and 79% were not co-infected with other STIs. Sexual orientation was available for 60% of the cases, of which 63% were heterosexual (17% females and 46% males), 37% were men who have sex with men (MSM). Further country specific data is presented in Annex 2 (Table A2.1).

Table 2: Overall patient characteristics, 2009

	Number	%
	1366	
Sex		
Male	1123	83.7
Female	219	16.3
Unknown	24	
Age (years)		
< 25	422	32.0
≥ 25	898	68.0
Unknown	46	
Sexual orientation		
Female heterosexual	117*	17.2
Male heterosexual	314	46.1
Men who have sex with men	251	36.8
Unknown	684	
Site of infection		
Genital	1164	86.5
Pharyngeal	34	2.5
Rectal	138	10.3
Other	9	0.7
Unknown	21	
Previously diagnosed		
Yes	84	18.1
No	379	81.9
Unknown	903	
Concurrent STI		
Concurrent Chlamydia	78	14.3
Concurrent other STI	35	6.4
No concurrent STI	433	79.3
Unknown	820	

** includes two bisexual females*

3.2 Antimicrobial resistance

The European guidelines for first-line empirical treatment of gonorrhoea [10] changed in 2009 to recommend usage of third generation cephalosporins (either the oral agent cefixime or the parenteral agent ceftriaxone) or spectinomycin. Surveillance of susceptibility of these agents is therefore essential to ensure efficient patient management. It must be kept in mind that a few countries were only able to contribute a small number of isolates in 2009 and it is hoped this will be increased when the Euro-GASP becomes more established.

The overall gonococcal resistance for each antimicrobial tested is described below (Table 3); country specific results with available demographic and epidemiological data are presented in Annex 2 (Table A2.2).

Table 3: Resistance to ciprofloxacin, azithromycin and penicillin antimicrobials and fully susceptible strains, 17 countries, 2009

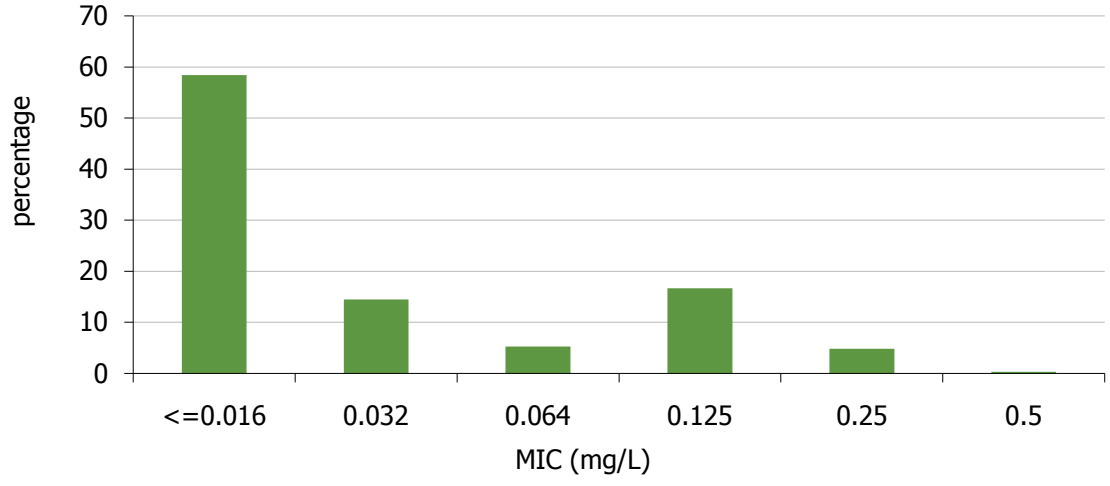
Country	Number of isolates tested	Resistance						Fully susceptible	
		Ciprofloxacin		Azithromycin		PPNG			
		No	%	No	%	No	%	No	%
Austria	104	83	80	30	29	9	9	21	20
Belgium	110	74	67	16	15	24	22	32	29
Denmark	119	83	70	55	46	7	6	30	25
France	104	45	43	19	18	6	6	53	51
Germany	45	33	73	0	0	3	7	12	27
Greece	110	74	67	9	8	4	4	32	29
Italy	70	53	76	20	29	5	7	16	23
Latvia	9	1	11	0	0	0	0	8	89
Malta	22	20	91	1	5	0	0	0	0
Netherlands	114	56	49	3	3	5	4	58	51
Norway	110	88	80	2	2	41	37	14	13
Portugal	79	27	34	0	0	13	16	46	58
Slovakia	15	15	100	1	7	1	7	0	0
Slovenia	24	19	79	2	8	3	13	3	13
Spain	103	67	65	6	6	7	7	33	32
Sweden	108	77	71	11	10	37	34	25	23
United Kingdom*	120	42	35	5	4	7	6	76	63
Total	**1366**	**857**	**63**	**180**	**13**	**172**	**13**	**459**	**34**
95% C.I.		(60.2 – 65.3)		(11.4 – 15)		(10.8 – 14.4)		(31.1 – 36.1)	

** 2009 isolates from the United Kingdom were only from England and Wales.*
C.I. confidence interval of the total % mean

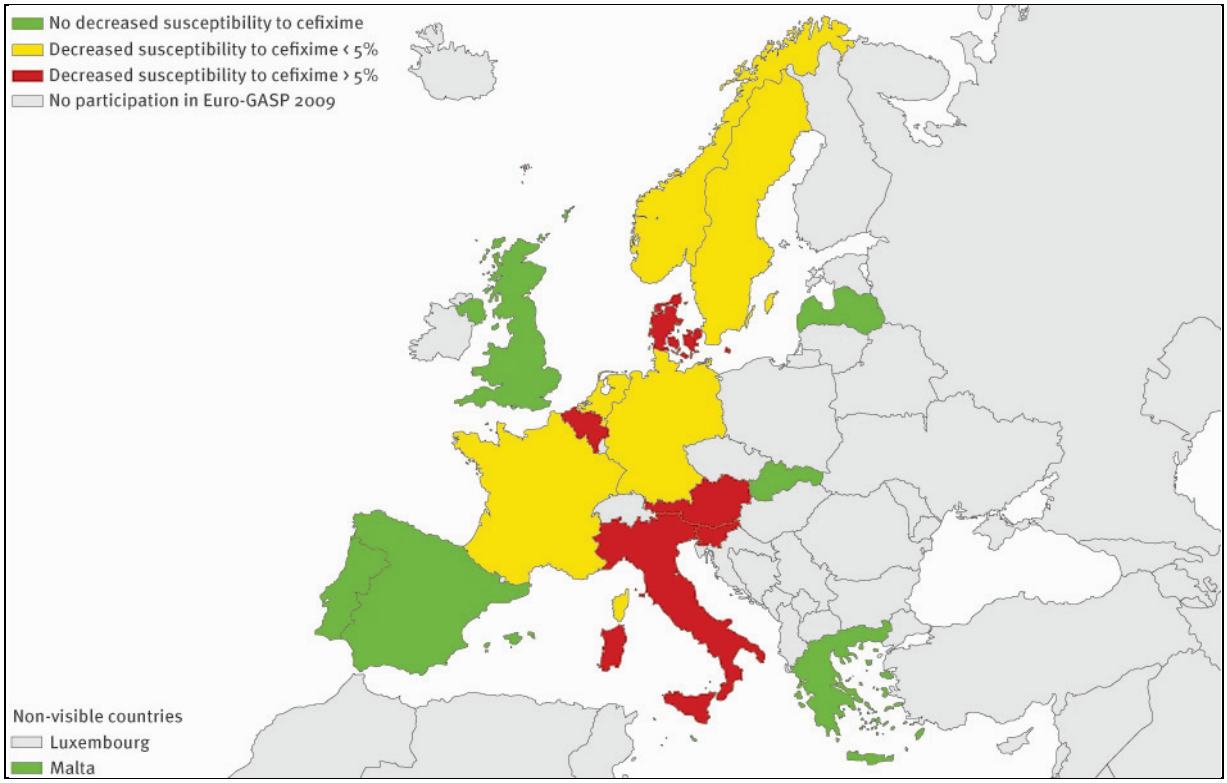
Cefixime

Five percent of the isolates displayed decreased susceptibility (>0.125 mg/L) to cefixime (Figure 1). As cefixime was included for the first time in the antimicrobial panel for Euro-GASP in 2009, it is not known if this has changed from previous years. Even though the majority of isolates showed low MICs of ≤0.016, it is of concern that isolates displaying an MIC of > 0.125 mg/L are apparent. All of these isolates with an MIC of > 0.125 mg/L (n=70) also displayed resistance to ciprofloxacin.

Figure 1: Distribution of MIC with respect to cefixime in Euro-GASP, 2009



Map 2: Geographical distribution of gonococcal isolates with respect to susceptibility to cefixime



Decreased susceptibility to cefixime was detected in ten countries, five of which had more than 5% decreased susceptibility (Table 4). Figure 3 displays the geographical distribution of these isolates and shows the geographical proximity of three (Italy, Austria and Slovenia) of the five countries with higher rates (5%) of decreased cefixime susceptibility.

Most of the isolates displaying decreased susceptibility to cefixime were from men (79%) and were predominantly heterosexually acquired (56%). However, there were differences across countries with respect to sexual orientation of the cases, as most were from MSM in Italy and heterosexuals in Austria and Denmark (Table 4) and based on rather low numbers.

Table 4: Countries with isolates displaying decreased susceptibility (DS) to cefixime and epidemiological information, 2009

Country	Isolates with DS-cefixime		Age				Gender						Sexual orientation					
			Age		<25 years		Males		Females		Unknown		MSM		Heterosexual		Unknown	
	No.	(%)	Mean	Mode	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Austria	22	(21.2)	29.6	25	7	(31.8)	18	(81.8)	4	(18.2)			1	(4.5)	21	(95.5)		
Italy	13	(18.6)	36.4	26	1	(7.7)	10	(76.9)			3	(23.1)	8	(61.5)	2	(15.4)	3	(23.1)
Denmark	18	(15.1)	25.8	21	11	(61.1)	13	(72.2)	5	(27.8)					16	(88.9)	2	(11.1)
Slovenia	2	(8.3)	23.5	N/A	1	(50)	2	(100)					1	(50)	1	(50)		
Belgium	7	(6.4)	35	40	1	(14.3)	6	(85.7)	1	(14.3)			2	(28.6)			5	(71.4)
Sweden	3	(2.8)	29.3	N/A			3	(100)									3	(100)
Germany	1	(2.2)	25	N/A			1	(100)									1	(100)
France	2	(1.9)	26	N/A			2	(100)									2	(100)
Norway	1	(0.9)	26	N/A			1	(100)									1	(100)
The Netherlands	1	(0.9)	40	N/A			1	(100)					1	(100)				
Total	70		29.9	26	21	(30)	57	(81.4)	10	(14.3)	3	(4.3)	13	(18.6)	40	(57.1)	17	(24.3)

N/A – not enough data to establish a modal age

Ciprofloxacin

Resistance (≥ 1mg/L) in 2009 ranged from 11% (Latvia) to 100% (Slovak Republic) with a mean of 63% (Table 4), all above the WHO recommendation of 5% as the level at which to consider changing first-line therapy.

Azithromycin

Resistance (≥ 1mg/L) ranged from 0% (Latvia and Portugal) to 46% (Denmark) with a mean of 13% (Table 3). However, there was considerable variation, with eight countries below 5%. No isolates displayed high-level resistance to azithromycin (>256 mg/L).

Penicillin

High-level plasmid-mediated-resistance to penicillin (penicillinase-producing *N. gonorrhoeae*, (PPNG)) ranged from 0% (Latvia and Malta) to 37% (Norway), with a mean of 13% (Table 3).

Ceftriaxone and spectinomycin

No decreased susceptibility to ceftriaxone (>0.12 mg/L) or resistance to spectinomycin (>0.64 mg/L) was detected in 2009.

Overall, 34% of isolates remained fully susceptible to all antimicrobial agents but again this ranged from 0% (Malta) to 89% (Latvia).

Gentamicin

Gentamicin has been used to successfully treat gonorrhoea in other parts of the world, notably in some African countries [12–15]. As the susceptibility of the European gonococcal population was unknown, and this agent could have future applications for treatment of persistent infections, gentamicin was included in the panel of antimicrobials tested as part of European AMR surveillance for the first time in 2009. No breakpoints for gentamicin have been established, but the overall MIC distribution was low in all European countries as the MIC_{50} and MIC_{90} of gentamicin in the isolates was 8 mg/L (MIC range observed was 1–16 mg/L).

3.3 Trends in antimicrobial resistant gonorrhoea

Ceftriaxone

Although no decreased susceptibility to ceftriaxone was detected in 2009, there was an upward drift in MIC compared to that observed in the annual report from Euro-GASP in 2008 [11] (Figure 4). The modal MIC of isolates to ceftriaxone was 0.008 mg/L in 2009 compared with ≤0.002 mg/L in 2004. Furthermore, the proportion of isolates that display MICs of ≥0.094mg/L is increasing annually and reached a level in 2009 (2.9%), which was considerably higher than previously observed.

Spectinomycin

No resistance to spectinomycin was demonstrated in either 2008 or 2009, the years when this agent was tested.

Ciprofloxacin

In spite of changes to treatment guidelines that recommend ciprofloxacin not be used as a first line therapy, resistance rates to this agent remain very high and increased by 12% between 2008 and 2009 (Figure 5).

Azithromycin

Azithromycin resistance increased from 2% in 2008 to 13% in 2009 (Figure 3), with no apparent trend between 2004 and 2009. While the resistance rate in 2009 was higher than rates observed in earlier years, the modal MIC of resistant isolates to azithromycin was 1 mg/L, which is the breakpoint used for categorising resistance. Isolates with an MIC on the breakpoint are just one doubling dilution from giving a susceptible category, which may explain the fluctuating resistance rates observed from 2004 to 2009.

Penicillin

High level resistance to penicillin (penicillinase-producing *N. gonorrhoeae,* PPNG) has remained fairly constant at 12–13% (Figure 3).

Figure 2: Distribution of MIC for ceftriaxone, 2004–09

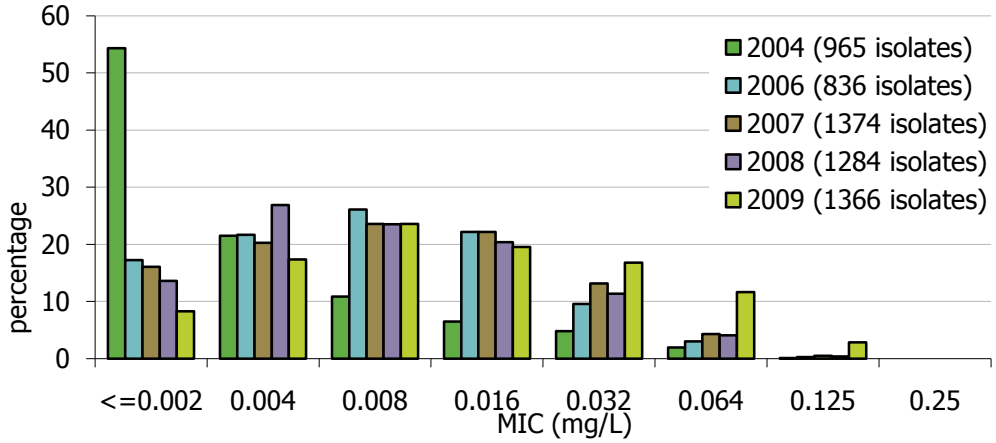
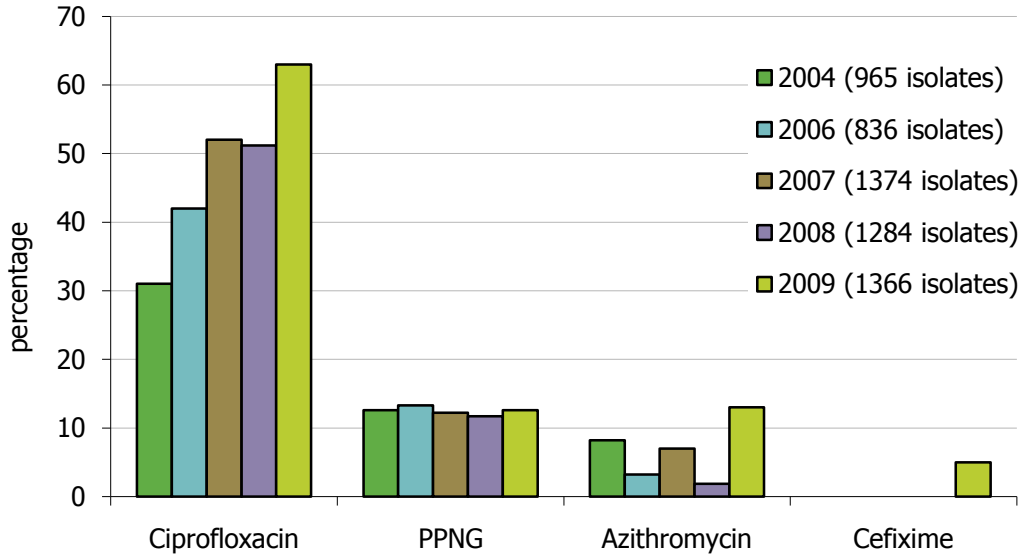


Figure 3: Overall mean incidence of resistant *Neisseria gonorrhoeae*, 2004–09



4 External quality assurance

4.1 Background

It is the overall aim of the European STI surveillance network to strengthen the surveillance of *N. gonorrhoeae* antimicrobial susceptibility in EU/EEA MS and the implementation of an EQA scheme for *N. gonorrhoeae* antimicrobial susceptibility is an important step towards achieving that aim. The EQA scheme is an essential component of any surveillance programme; ensuring comparability of data and successful performance in EQA will be essential for laboratories participating in future de-centralised testing as part of AMR surveillance across Europe. An EQA scheme is provided for gonococcal susceptibility testing for participating laboratories in the STI surveillance network.

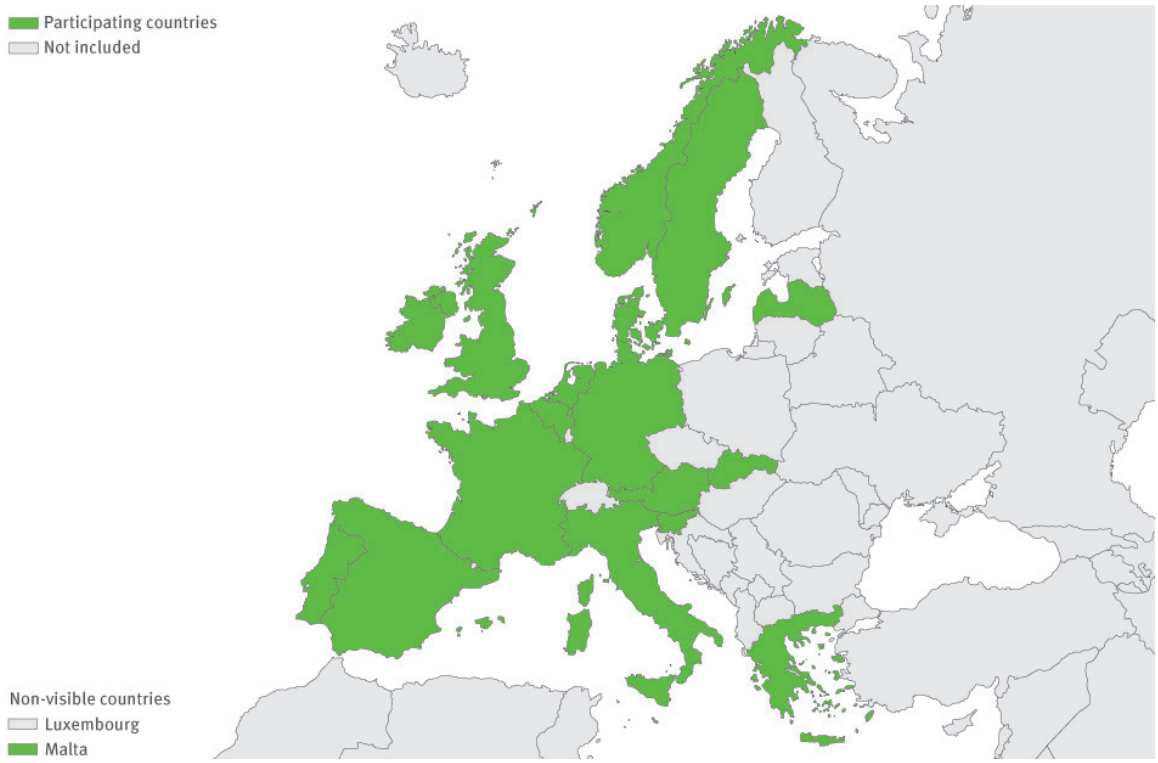
The United Kingdom National External Quality Assessment Service (UK-NEQAS) provides a genital pathogens scheme for pathogen identification and antimicrobial susceptibility testing. The scheme contains two pathogens per distribution, distributed three times each year in October, March and July. An additional panel of strains are incorporated into this scheme so more extensive susceptibility testing EQA can be implemented.

In March 2010, participating laboratories received two genital pathogens scheme pathogens for identification and susceptibility testing and these results were reported back to UK-NEQAS. In addition, ten gonococcal isolates for susceptibility testing were included in the distribution.

4.2 Antimicrobial susceptibility testing external quality assurance scheme

The panel consisted of ten cultures of *N. gonorrhoeae*, two of which were in duplicate (QA10–03/07 and QA10–04/10), resulting in eight different strains. The strains included in the panel were chosen to cover a range of susceptibilities to therapeutic antimicrobial agents. The strains were selected from a global panel of well characterised strains and from recently isolated clinical strains. The panel (QA2010) was received by 19 participating laboratories (Map 3).

Map 3: Participating countries of the 2010 *N. gonorrhoeae* susceptibility testing EQA scheme



Note: Eighteen countries participated; two EQA submissions from the United Kingdom. Ireland participated in the EQA and not the AMR surveillance programme.

The participating laboratories tested the panel of ten cultures using their own routine methodology, and the results were returned centrally for analysis. It was requested that participants test a minimum set of the following therapeutic antimicrobials against the panel:

- Ciprofloxacin
- Ceftriaxone
- Cefixime

Participants were also requested to test the following if possible:

- Azithromycin
- Gentamicin
- Spectinomycin
- Beta-lactamase testing

This range of antimicrobials is used in the AMR sentinel surveillance protocol. Collection of data on the laboratory performance using these antimicrobials will be critical in establishing decentralised testing in the future.

Susceptibility testing methods

Each laboratory reported details of the testing methodology used (Table 5) and described the breakpoints for determining the category of resistance (resistant, intermediate or susceptible) for each antimicrobial. For each strain, the laboratories reported the results as the category of resistance and the MIC or zone of inhibition for disc diffusion. After receipt, results were decoded and sent back to the laboratories so the centres could study their intra-laboratory reproducibility and start working on any identified problems immediately. To allow for differences in methods and breakpoints used, the analysis was performed using the category of resistance result. When all EQA results from participating laboratories were submitted, the consensus was ascertained by establishing the category of resistance that occurred most often.

Table 5: Details of susceptibility methods used by participating laboratories

Type of susceptibility test used	**Number (18*)**
E-test	10
Agar dilution	3
Disc diffusion	3
E-tests and agar dilution	2
Detection of beta-lactamase production	**Number (18**)**
Chromogenic cephalosporin nitrocefin	15
Penicillinase production well in identification kits	2
Hodge plate technique	1
*One country did not perform beta-lactamase testing	
Type of culture media used	**Number (18*)**
GC agar base	11
Chocolatised blood agar	5
Thayer-Martin agar	1
Diagnostic sensitivity agar & 5% horse blood	1
Inoculum size used	**Number (18*)**
0.5 MacFarland standard†	14
1.0 MacFarland standard	1
1:10 dilution of 0.5 MacFarland standard	1
1:10 dilution of 5 MacFarland standard	1
5 colony suspension	1
Diluent for suspension	**Number (18*)**
Saline	9
Broth (1x Mueller Hinton; 1x TSB, 1X Nutrient broth, 1X Bouillon Thioglycollate)	4
Water	2
PBS	3

**One country performed beta-lactamase testing only*
***One country did not perform beta-lactamase testing*
†Normally equivalent to 10^4 cfu/µl

The temperature and CO_2 range for incubation was 33°C to 37°C in 5% to 10% CO_2, for 16 to 24 hours, with one laboratory incubating agar dilution plates for 48 hours.

Eighteen laboratories returned category of resistance results and 18 laboratories performed beta-lactamase testing. In addition to the requested panel, the following antimicrobials were tested (number of centres):

- Tetracycline, n=9
- Penicillin, n=8
- Cefoxitine, n=1
- Cefotaxime n=1

Minimum inhibitory concentration breakpoints

Thirteen laboratories stated that they performed the susceptibility testing in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines [16], which include recommended breakpoints (Table 6). Two laboratories did not state the use of any particular guidelines but one was using the CLSI breakpoints. Three laboratories adhered to other susceptibility testing criteria (Gonococcal Resistance to Antimicrobials Surveillance Program (GRASP), England and Wales; European Committee on Antimicrobial Susceptibility Testing (EUCAST), France; Swedish Reference Group on Antibiotics (SRGA), Sweden).

Variations in breakpoints are shown in Table 7. The most variation was observed with azithromycin (Table 8) and gentamicin (Table 9) as no interpretive criteria have yet been set by CLSI [16] or EUCAST (gentamicin). However, four centres are now using the recently published EUCAST breakpoints for azithromycin [17]. Fourteen laboratories used an MIC method for determining azithromycin resistance.

Table 6: Clinical and Laboratory Standards Institute breakpoints

	MIC breakpoint (mg/L)			Zone diameters (mm)		
	R ≥	I	S ≤	R ≤	I	S ≥
Cefixime			0.25			31
Ceftriaxone	-	-	0.25			35
Ciprofloxacin	1	0.12 – 0.5	0.06	27	28–40	41
Spectinomycin	128	64	32	14	15–17	18

Note: Currently there are no interpretive criteria for azithromycin, but EUCAST [17] GRASP (UK) and CDC (USA, CDC brochure B88) use ≥ 1 mg/L for resistance. Strains with azithromycin zone diameters of ≤ 30mm are of interest to CDC

Table 7: Breakpoint variations

	MIC breakpoint (mg/L)		
	R ≥	I	S ≤
Ceftriaxone*		≥ 0.125 ≥ 0.250	0.064 0.125
Cefixime*	0.5	≥ 0.125 ≥ 0.250 ≥ 0.250	0.064 0.125 0.125
Ciprofloxacin	1 0.064	0.064 – 0.5	0.032 0.032
Spectinomycin	128 64		64 32

**Intermediate range = decreased susceptibility*

Table 8: Azithromycin breakpoints

	MIC breakpoint (mg/L)		
No. of centres	R ≥	I	S ≤
5	1		0.5
2	2		1
1	2	1	0.5
4	1	0.5	0.25
1	2	0.5 – 1	0.25
1	None given		

Table 9: Gentamicin breakpoints

	MIC breakpoint (mg/L)		
No. of centres	**R ≥**	**I**	**S ≤**
1	32	8–16	4
1	16	8	4
1	8		4
1	8	4	2
6	None given		

Disc diffusion zone diameters

All centres adhered to the CLSI zone diameters for interpreting category of resistance, except for one centre that declared a zone diameter of ≤30mm for azithromycin resistance. All centres used the CLSI recommended disc concentrations [16]: penicillin 10 units; tetracycline 30µg; ciprofloxacin 5µg; ceftriaxone 30µg; cefixime 5µg; and spectinomycin 100µg. There are no CLSI disc concentration recommendations for gentamicin and azithromycin; however, 10µg and 15µg disc concentrations were used respectively.

4.3 Results

Resistance categories concordance

The overall concordance of resistance categories (Table 10) was highest for spectinomycin (100%) and ciprofloxacin (98%). The lower concordance for azithromycin (82%) is most probably due to the variation in breakpoints. The disc diffusion method gave the highest concordance between the centres, although it should be noted that just three centres used this method. Overall concordance was not established for gentamicin as only four centres reported breakpoints, all of which were different (Table 9).

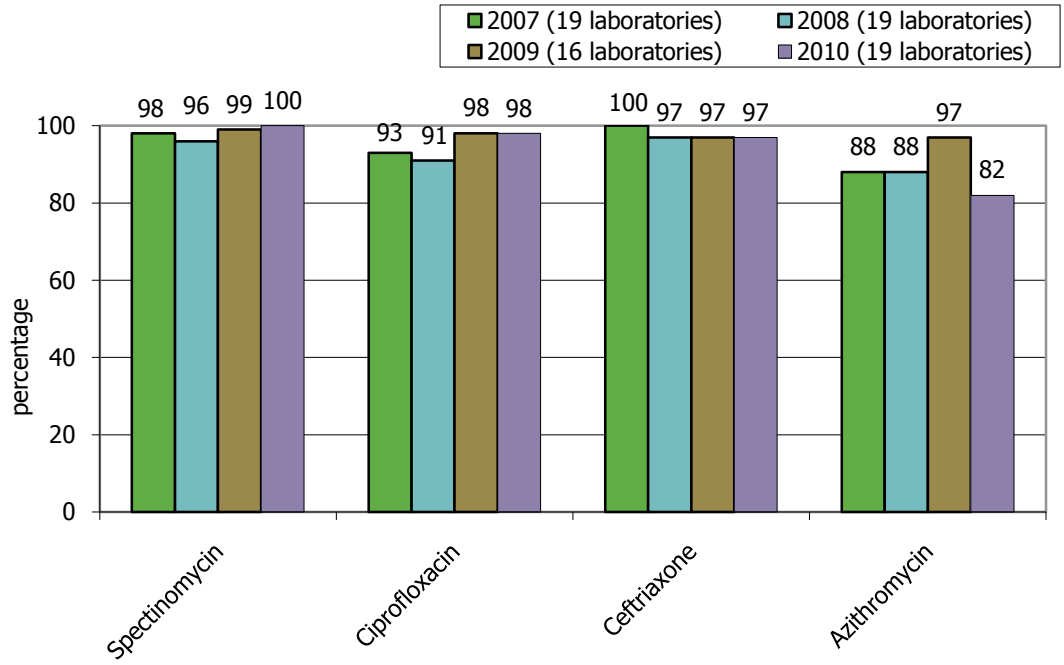
Table 10: Overall concordance (%) of resistance categories

	All	E-test	Agar dilution	Disc
Spectinomycin	100	100	100	100
Ciprofloxacin	98	97	100	100
Ceftriaxone	97	97	96	100
Cefixime	95	93	96	100
Azithromycin	82	79	100	NR

*Note: *NR = No result, as only one laboratory tested the panel against azithromycin*
Values in red = highest concordance

The comparison of the overall concordance from previous ESSTI QA panel distributions (QA2007, QA2008 QA2009 (Figure 4)) [18] shows that the concordance of resistance categories is very good over the four distributions. It should be noted that the ESSTI panels contained 30 isolates (10 in triplicate) and this current EQA only contained 10 isolates (two in duplicate).

Figure 4: Inter-laboratory concordance



Tables A3.1 to A3.5 (Annex 3) show the consensus for each strain when tested against each antimicrobial and each test method. For each strain, the category of resistance consensus was the same for all methods, other than on one occasion (azithromycin QA10–01) when the agar dilution consensus was sensitive and the consensus for E-test and Disc methods was resistant.

Beta-lactamase

Of the 18 centres that tested for beta-lactamase production, only one centre did not achieve fully concordant results (one specimen incorrectly identified as PPNG negative). The overall concordance for the detection of beta-lactamase production was 99.3%.

Minimum inhibitory concentration concordance

A high proportion of isolates MIC (agar dilution and E-tests) are within one dilution of the modal MIC (94%), and just 5% within two doubling dilutions (Table 11). On just eight occasions (1%), isolate MICs differed from the modal MICs by more than two dilutions, seven of which were ceftriaxone in the low dilution range. This is not unexpected as the differences in this low range are very small. Overall, the MIC concordance demonstrates the high level of comparability between the participating laboratories and these two methods (agar dilution and E-test).

QA panel characteristics

Table 12 shows the overall consensus category, percentage concordance, the modal/range MIC and mean/range disc zones for each strain in the QA10 panel. The final consensus phenotype and the identification of strains that have MICs on CLSI breakpoints are shown in Annex 3 (Table A3.6). Generally a lower concordance was demonstrated when the susceptibility of the strains is close to a breakpoint, in particular in the intermediate range.

Due to the confidential nature of an EQA scheme, a coded country breakdown for beta-lactamase, MIC values, disc zone diameters and category concordance is shown in Annex 3 (Tables A3.7–A3.24). The breakdown of these results shows that no particular country is performing unsatisfactorily.

Table 11: Variation from modal MIC

	Ciprofloxacin		Azithromycin		Spectinomycin		Ceftriaxone		Cefixime		Gentamicin		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Within 1 doubling dilution*	143	96	132	89	116	90	126	85	123	95	89	99	729	92
Within 2 doubling dilutions*	6	4	16	11	13	10	16	11	7	5	1	1	59	7
>2 doubling dilutions*	0	0	1	1	0	0	7	5	0	0	0	0	8	1
Total no. of isolates with MIC data	149		149		129		149		130		90		796	

**When compared to the modal MIC (see Table 12)*

Table 12: Consensus results from March 2010 EQA

Consensus category
Modal (Range) MIC for Etest* and agar dilution (mg/L)
Mean (Range) diameter for disc diffusion (mm)
% concordance of resistance category

Strain	Ciprofloxacin Consensus	Ceftriaxone Consensus	Cefixime Consensus	Azithromycin Consensus	Spectinomycin Consensus	Gentamicin Consensus	Beta-lactamase Consensus
	S	S	S	R (S*)	S		
QA10-01	0.004 (0.002-0.008)	<=0.016†(<0.002-0.064)	<0.016 (0.008 - 0.064)	2 (0.5-4)	16 (8-16)	8 (4-16)	NEG
(G07-1220)	51 (46-54)	46 (45-46)	39 (39-40)	28 (27-28)	29 (26-32)	18	100%
	100	100	100	67	100		
	S	S	S	S	S		
QA10-02	0.004 (0.002-0.008)	<0.002 (<0.002-<0.016)	<0.016 (<0.002-0.032)	0.125 (0.064-0.25)	16 (4-32)	4 (1-4)	NEG
(WHO F)	47 (46-48)	51 (48-53)	44 (40-47)	35 (34-36)	24 (21-27)	20	100%
	100	100	100	100	100		
	R	S	S	S	S		
QA10-03	>32 (>1 - 64)	0.064/0.125 (<0.002-0.25)	0.25 (0.064-0.5)	0.25 (0.064-1)	16 (4-32)	4 (4-8)	NEG
QA10-07	4 (0-6)	40 (39-42)	32 (30-34)	34 (31-36)	28 (25-30)	18	100%
(WHO K)	100	97	70	77	100		
	S	S	S	S	R		
QA10-04	0.016 (0.008-0.016)	0.032 (<0.002-0.064)	0.032 (0.008-0.064)	0.5 (0.125-1)	>1024 (>64->1024)	4 (2-8)	POS
QA10-10	49 (46-53)	47 (45-49)	40 (36-43)	36 (34-37)	3 (0-6)‡	18	100%
(WHO O)	100	100	100	72	100		
	R	S	S	S	S		
QA10-05	1 (0.5-4)	0.008 (<0.002-0.032)	<0.016 (<0.016-0.064)	0.064 (0.032-0.125)	4 (4-16)	4 (4-8)	POS
(G08-2946)	25 (23-26)	50 (46-53)	43 (41-45)	42 (39-45)	33 (29-37)	23	94%
	83	100	100	100	100		
	R	S	S	S	S		
QA10-06	>32 (>1->32)	0.125 (0.016-0.25)	0.125 (0.032-0.25)	0.5 (0.25-2)	16 (4-32)	4 (2-8)	NEG
(G08-1299)	11 (9-13)	44 (42-45)	33 (31-34)	33 (32-34)	30 (25-34)	18	100%
	100	100	94	67	100		
	R	S	S	S	S		
QA10-08	>32 (>1->32)	0.25 (0.016-0.5)	0.125 (0.032-0.25)	0.5 (0.064-2)	8 (4-32)	4/8 (2-8)	NEG
(WHO L)	4 (0-6)‡	44 (41-46)	40 (39-42)	39 (37-40)	34 (32-36)	21	100%
	100	82	94	73	100		
	S	S	S	R	S		
QA10-09	0.008 (0.008-0.016)	0.004/0.008 (<0.002-0.016)	<0.016 (0.008-0.064)	>256 (>2->256)	8 (4-32)	4 (2-8)	NEG
(G07-1454)	48 (46-51)	43 (43)	40 (36-44)	6 (6)	29 (26-32)	20	100%
	100	100	100	100	100		

*Etest values that are inbetw een doubling dilutions are rounded up.
† no consensus MIC available, but all MICs below value show n
**Agar dilution consensus
‡ Variation in interpretation of zones w ith no inhibition; centres either include disc diameter or do not.
Number of centres used to calculate disc diffusion mean diameter; ciprofloxacin & cefixime = 3; Ceftriaxone, azithromycin and spectinomycin = 2; gentamicin = 1.

4.4 United Kingdom national external quality assessment service genital pathogens scheme external quality assurance

Seventeen centres received the UK-NEQAS genital pathogens scheme EQA.

- Three centres did not return results.
- Two centres stated they did not examine the specimens.
- Twelve centres correctly reported for the positive specimen (*N. gonorrhoeae* in specimen 9679), however two centres reported the presence of a pathogen in the negative specimen.

Susceptibility testing was performed on specimen 9679 and the results are shown in Table 13. Concordance of resistance categories is 100% for all antimicrobials, other than for azithromycin (88%) and beta-lactamase testing (80%).

Table 13: Analysis of the antimicrobial susceptibility results for specimen 9679

Antimicrobial	S	I	R
Azithromycin	7	1	0
Cefixime	6	0	0
Cefpodoxime	1	0	0
Ceftriaxone	10	0	0
Ciprofloxacin	0	0	10
Gentamicin	2	0	0
Penicillin	0	0	3
Spectinomycin	7	0	0
Tetracycline	0	0	2
Beta-Lactamase	Pos (4); Neg (1)		

5 Conclusions

5.1 Gonococcal antimicrobial resistance

Cefixime is a recommended therapy for gonorrhoea across Europe [10] and so the 5% level of decreased susceptibility identified in five countries (Austria, Italy, Denmark, Slovenia and Belgium) is of great concern. Most of the isolates displaying decreased susceptibility to cefixime were from men (79%) and overall were predominantly heterosexually acquired (56%). However, there were differences across countries in the sexual orientation of the cases, as most were from MSM in Italy and heterosexuals in Austria and Denmark. While the relationship between cefixime MIC and treatment failure remains poorly understood, there is a possibility that treatment failure could become an increasing problem within Europe if MICs continue to increase. Countries with circulating strains that demonstrate decreased susceptibility to cefixime and use cefixime for treatment should consider sending an alert to all appropriate health professionals to monitor treatment failure. The loss of cefixime as an oral treatment option for gonorrhoea across Europe may have severe implications as use of the parenterally administered ceftriaxone will be more expensive, overall patient compliance to treatment may reduce, and new alternative treatments are lacking.

Ceftriaxone is an appropriate treatment for gonorrhoea in Europe as all isolates tested were susceptible. However, an upward ceftriaxone MIC drift is present, demonstrating that this situation needs to be monitored carefully.

Rates of ciprofloxacin and azithromycin resistance are high across Europe and these antimicrobials should not be used for empirical treatment, unless isolates are known to be susceptible or local resistance rates are known to be less than 5%.

Even though no breakpoint for resistance to gentamicin has been established, the MIC distribution offers hope that gentamicin could be used for therapy in the future.

5.2 Implementing Euro-GASP

European gonococcal antimicrobial surveillance is essential to inform treatment guidelines thereby preventing onward transmission and reducing patient morbidity. Currently, Euro-GASP is being implemented in EU/EEA MS. In the 2009 AMR surveillance, 17 of the 30 EU/EAA countries participated, which does not provide the full representativeness of the EU/EEA. In 2009, several laboratory experts in MS were trained as part of the ECDC STI surveillance activities in STI laboratory diagnostics including the methods for gonococcal antimicrobial testing. All MS will be encouraged to participate in Euro-GASP. It is essential to monitor for emerging, increasing and high-level resistance and to inform treatment guidelines so optimum treatment therapies are administered not only at national level but also at the EU level. Immediate public health action should involve the close monitoring for treatment failure in individuals who have been administered cefixime for gonorrhoea treatment.

Euro-GASP will also contribute to the global surveillance of gonococcal AMR and the development of a WHO action plan for emerging third generation cephalosporin resistance. The action plan was discussed at a WHO/CDC joint consultation meeting in April 2010 and will include the following elements:

- enhanced surveillance;
- strengthening laboratory capacity;
- epidemiological support;
- clinical support;
- advocacy, programme coordination and collaboration; and
- enhanced gonorrhoea control.

Further work is underway to strengthen the Euro-GASP and includes:

- increased number of participating countries;
- improved timeliness; and
- encouraging decentralised testing;

5.3 Quality assurance

The susceptibility testing EQA in March 2010 has shown that there are common features in the methodology used across Europe, such as the use of GC agar base, suspensions equivalent to a 0.5 MacFarland standard and adherence to CLSI breakpoints. The overall concordance is high (>90%) for all antimicrobials other than azithromycin. The concordance of each strain was very good for determining resistance category, with the concordance lowering due to strains that straddle or are close to breakpoints. This high level of comparability

allows comparison of surveillance data from the members of STI surveillance network with confidence and gives more evidence to support de-centralised testing as a viable option for the future Euro-GASP.

Further participation with the UK-NEQAS genital pathogens scheme EQA should be encouraged to help build capability in the laboratory isolation and identification of *Neisseria gonorrhoeae*.

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Annex 1: Protocol for gonococcal susceptibility testing

- Isolates are shipped frozen to one of three testing centres:
  - Health Protection Agency (HPA), London, UK
  - Statens Serum Institut, Copenhagen, Denmark
  - Örebro University Hospital, Örebro, Sweden
- The isolates are stored at -70°C or in liquid nitrogen.
- Isolates are retrieved onto non-selective agar (such as GCVIT with 1% Vitox), and incubated for 18–24 hours at 36°C in 5% CO_2.
- The purity and the identity of the isolates are confirmed using Gram stain, oxidase and the *N. gonorrhoeae* MicroTrak (Trinity Biotech) test. A further sub-culture is performed.
- Repeat retrieval onto selective agar is performed if a high level of contamination is demonstrated.
- Susceptibility testing is performed using the agar dilution breakpoint technique for ciprofloxacin, spectinomycin and azithromycin, and the full agar dilution technique for gentamicin. Suspensions of cultures aged 18–24 hours are prepared equivalent to McFarland standard 0.5 (approximately 10^4 cfu/µl) in saline. Using a multipoint inoculator, suspensions are inoculated onto GC agar plates with 1% Vitox, containing a panel of antimicrobials at the following breakpoint concentrations:

Table A1.1: Concentrations (mg/L) of antimicrobials used for the agar dilution breakpoint technique and the full agar dilution technique

Antimicrobial	Intermediate	Resistant
Ciprofloxacin	0.06	0.5
Azithromycin		0.5
Spectinomycin		64
Gentamicin (no breakpoint determined yet)	1, 2, 4, 8, 16	

- The ceftriaxone and cefixime MICs are determined using E-tests according to the manufacturer's instructions.
- All isolates are tested for penicillinase production using the chromogenic reagent nitrocefin.
- E-tests are performed on isolates that are resistant to azithromycin using the agar dilution breakpoint technique.
- E-tests are performed on isolates are > 8 mg/L to gentamicin using the agar dilution technique.
- The following control strains [19] are tested on the poured agar dilution plates and each batch of E-tests:
  - WHO G (QA07–10)
  - WHO K (QA09–03)
  - WHO M (QA09–09)
  - WHO O (QA09–10)
  - WHO P (QA09–05)
- Bacterial growth is recorded for the agar dilution plates and the MIC is recorded from the E-tests plated. The category of resistance is determined using the following breakpoints:

Table A1.2: MIC breakpoints for specific antimicrobials

Antimicrobial	MIC breakpoint (mg/L)		
	R ≥	I	S ≤
Azithromycin	1	-	0.5
Ceftriaxone*	0.25		
Cefixime*	0.25		
Gentamicin	To be determined		
Ciprofloxacin	1	0.12 – 0.5	0.06
Spectinomycin	128		64

*Note: * Decreased susceptibility*

European Committee on Antimicrobial Susceptibility Testing breakpoints [17] have been used, other than for ciprofloxacin and azithromycin intermediate resistance. The ciprofloxacin resistance breakpoint in this study is more clinically relevant to treatment failure. Azithromycin intermediate resistance has not been recorded as the clinical significance of this is currently unknown.

- Isolates that are contaminated in the original vial or are slow to grow are re-saved.

Annex 2: Patient characteristics and antimicrobial resistance testing results

Table A2.1: Patient characteristics; all countries and by country, 2009

	All countries		Austria		Belgium		Denmark		France		Germany		Greece		Italy		Latvia	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	1366		104		110		119		104		45		110		70		9	
Sex																		
Male	1123	82.2	78	75.0	90	81.8	90	75.6	79	76.0	43	95.6	107	97.3	56	80.0	6	66.7
Female	219	16.0	26	25.0	20	18.2	29	24.4	22	21.2	2	4.4	3	2.7	1	1.4	3	33.3
Unknown	24	1.8						0.0	3	2.9					13	18.6		
Age (years)																		
<25	422	30.9	31	29.8	15	13.6	52	43.7	33	31.7	12	26.7	23	20.9	10	14.3	4	44.4
>=25	898	65.7	73	70.2	93	84.5	67	56.3	55	52.9	33	73.3	84	76.4	46	65.7	5	55.6
Unknown	46	3.4			2	1.8			16	15.4			3	2.7	14	20.0		
Sexual orientation																		
Heterosexual (male and female)	431**	31.6	94	90.4	1	0.9	82	68.9			2	4.4	75	68.2	20	28.6	6	66.7
Male heterosexual	314	23.0	68	65.4	1	0.9	53	44.5			1	2.2	74	67.3	19	27.1	3	33.3
MSM	251	18.4	6	5.8	4	3.6	14	11.8			1	2.2	29	26.4	37	52.9		
Unknown	684	50.1	4	3.8	105	95.5	23	19.3	104	100.0	42	93.3	6	5.5	13	18.6	3	33.3
Site of infection																		
Genital	1164	85.2	98	94.2	104	94.5	108	90.8	92	88.5	44	97.8	110	100.0	55	78.6	9	100.0
Pharyngeal	34	2.5					3	2.5							4	5.7		
Rectal	138	10.1	6	5.8	1	0.9	5	4.2	7	6.7					8	11.4		
Other	9	0.7			1	0.9	1	0.8			1	2.2						
Unknown	21	1.5			4	3.6	2	1.7	5	4.8					3	4.3		
Previously diagnosed																		
Yes	84	6.2	8	7.7	1	0.9	5	4.2					24	21.8				
No	379	27.7	3	2.9	14	12.7	114	95.8					77	70.0				
Unknown	903	66.1	93	89.4	95	86.4			104	100.0	45	100.0	9	8.2	70	100.0	9	100.0
Concurrent STI																		
Concurrent CT	78	5.7	8†	7.7							5	11.1					2	22.2
Concurrent other	35	2.6	1	1.0	1	0.9					1	2.2	3	2.7	6	8.6		
No concurrent STI	433	31.7	95	91.3							37	82.2	11	10.0	50	71.4	7	77.8
Unknown	820	60.0			109	99.1	119	100.0	104	100.0	2	4.4	96	87.3	14	20.0		

***Includes two bisexual females*

† *Includes one patient with concurrent infection with* Mycoplasma genitalium *and* Ureaplasma urealyticum

Table A2.1: Patient characteristics; all countries and by country, 2009 (continued)

	Malta		Netherlands		Norway		Portugal		Slovakia		Slovenia		Spain		Sweden		UK	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	22		114		110		79		15		24		103		108		120	
Sex																		
Male	15	68.2	101	88.6	92	83.6	70	88.6	10	66.7	22	91.7	87	84.5	90	83.3	87	72.5
Female	5	22.7	12	10.5	18	16.4	9	11.4	5	33.3			14	13.6	17	15.7	33	27.5
Unknown	2	9.1	1	0.9							2	8.3	2	1.9	1	0.9		
Age (years)																		
<25	9	40.9	29	25.4	28	25.5	37	46.8	4	26.7	8	33.3	23	22.3	42	38.9	62	51.7
>=25	10	45.5	84	73.7	82	74.5	40	50.6	11	73.3	14	58.3	77	74.8	66	61.1	58	48.3
Unknown	3	13.6	1	0.9			2	2.5			2	8.3	3	2.9				
Sexual orientation																		
Heterosexual (male and female)	15	68.2	30*	26.3			25	31.6	6	40.0	8	33.3					67*	55.8
Male heterosexual	11	50.0	18	15.8			18	22.8	2	13.3	8	33.3					38	31.7
MSM	1	4.5	83	72.8	1	0.9	23	29.1			12	50.0					40	33.3
Unknown	6	27.3	1	0.9	109	99.1	31	39.2	9	60.0	4	16.7	103	100.0	108	100.0	13	10.8
Site of infection																		
Genital	20	90.9	58	50.9	94	85.5	71	89.9	15	100.0	13	54.2	85	82.5	90	83.3	98	81.7
Pharyngeal			8	7.0	4	3.6	1	1.3			1	4.2	3	2.9	7	6.5	3	2.5
Rectal	2	9.1	48	42.1	6	5.5	4	5.1			8	33.3	14	13.6	10	9.3	19	15.8
Other					1	0.9	3	3.8					1	0.9	1	0.9		
Unknown					5	4.5					2	8.3						
Previously diagnosed																		
Yes	1	4.5					14	17.7			1	4.2					30	25.0
No	17	77.3			10	9.1	34	43.0	14	93.3	21	87.5					75	62.5
Unknown	4	18.2	114	100.0	100	90.9	31	39.2	1	6.7	2	8.3	103	100.0	108	100.0	15	12.5
Concurrent STI																		
Concurrent CT	1	4.5	25	21.9			8	10.1	1	6.7							28	23.3
Concurrent other			17	14.9			1	1.3			2	8.3					3	2.5
No concurrent STI	16	72.7	72	63.2	6	5.5	39	49.4	11	73.3	20	83.3					76	63.3
Unknown	5	22.7			104	94.5	31	39.2	3	20.0	2	8.3	103	100.0	108	100.0	13	10.8

**Includes one bisexual female*

Table A2.2: Resistance to ciprofloxacin and azithromycin, by country, 2009

	All countries						Austria						Belgium						Denmark					
	All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR	
	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%
	1366		857	62.7	180	13.2	104		83	79.8	30	28.8	110		74	67.3	16	14.5	119		83	69.7	55	46.2
Sex																								
Male	1123	82.2	716	83.5	147	81.7	78	75.0	61	73.5	26	86.7	90	81.8	64	86.5	15	93.8	90	75.6	60	72.3	39	70.9
Female	219	16.0	125	14.6	28	15.6	26	25.0	22	26.5	4	13.3	20	18.2	10	13.5	1	6.3	29	24.4	23	27.7	16	29.1
Unknown	24	1.8	16	1.9	5	2.8																		
Age (years)																								
<25	422	30.9	241	28.1	58	32.2	31	29.8	22	26.5	6	20.0	15	13.6	6	8.1			52	43.7	41	49.4	28	50.9
>=25	898	65.7	588	68.6	114	63.3	73	70.2	61	73.5	24	80.0	93	84.5	67	90.5	16	100.0	67	56.3	42	50.6	27	49.1
Unknown	46	3.4	28	3.3	8	4.4							2	1.8	1	1.4								
Sexual orientation																								
Heterosexual (male and female)	431**	31.6	272*	31.7	89	49.4	94	90.4	76	91.6	25	83.3	1	0.9	1	1.4			82	68.9	66	79.5	48	87.3
Male heterosexual	314	23.0	209	24.4	68	37.8	68	65.4	54	65.1	21	70.0	1	0.9	1	1.4			53	44.5	43	51.8	32	58.2
MSM	251	18.4	145	16.9	27	15	6	5.8	4	4.8	4	13.3	4	3.6	3	4.1	1	6.3	14	11.8	7	8.4	2	3.6
Unknown	684	50.1	440	51.3	64	35.6	4	3.8	3	3.6	1	3.3	105	95.5	70	94.6	15	93.8	23	19.3	10	12.0	5	9.1

Table A2.2: Resistance to ciprofloxacin and azithromycin, by country, 2009 (continued)

	France						Germany						Greece						Italy					
	All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR	
	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%
	104		45	43.3	19	18.3	45		33	73.3	0	0.0	110		74	67.3	9	8.2	70		53	75.7	20	28.6
Sex																								
Male	79	76.0	37	82.2	17	89.5	43	95.6	31	93.9			107	97.3	73	98.6	8	88.9	56	80.0	42	79.2	16	80.0
Female	22	21.2	7	15.6	1	5.3	2	4.4	2	6.1			3	2.7	1	1.4	1	11.1	1	1.4	1	1.9		0.0
Unknown	3	2.9	1	2.2	1	5.3													13	18.6	10	18.9	4	20.0
Age (years)																								
<25	33	31.7	11	24.4	6	31.6	12	26.7	8	24.2			23	20.9	19	25.7	2	22.2	10	14.3	8	15.1	4	20.0
>=25	55	52.9	28	62.2	10	52.6	33	73.3	25	75.8			84	76.4	53	71.6	6	66.7	46	65.7	34	64.2	12	60.0
Unknown	16	15.4	6	13.3	3	15.8							3	2.7	2	2.7	1	11.1	14	20.0	11	20.8	4	20.0
Sexual orientation																								
Heterosexual (male and female)							2	4.4	1	3.0			75	68.2	55	74.3	8	88.9	20	28.6	18	34.0	7	35.0
Male heterosexual							1	2.2					74	67.3	54	73.0	7	77.8	19	27.1	17	32.1	7	35.0
MSM							1	2.2					29	26.4	16	21.6	1	11.1	37	52.9	26	49.1	10	50.0
Unknown	104	100.0	45	100.0	19	100.0	42	93.3	32	97.0			6	5.5	3	4.1			13	18.6	9	17.0	3	15.0

Table A2.2: Resistance to ciprofloxacin and azithromycin, by country, 2009 (continued)

	Latvia						Malta						Netherlands						Norway					
	All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR	
	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%
	9		1	11.1	0	0.0	22		20	90.9	1	4.5	114		56	49.1	3	2.6	110		88	80.0	2	1.8
Sex																								
Male	6	66.7	1	100.0			15	68.2	15	75.0	1	100.0	101	88.6	51	91.1	3	100.0	92	83.6	73	83.0	2	100.0
Female	3	33.3					5	22.7	4	20.0			12	10.5	5	8.9			18	16.4	15	17.0		
Unknown							2	9.1	1	5.0			1	0.9										
Age (years)																								
<25	4	44.4	1	100.0			9	40.9	7	35.0	1	100.0	29	25.4	16	28.6			28	25.5	24	27.3	1	50.0
>=25	5	55.6					10	45.5	10	50.0			84	73.7	40	71.4	3	100.0	82	74.5	64	72.7	1	50.0
Unknown							3	13.6	3	15.0			1	0.9										
Sexual orientation																								
Heterosexual (male and female)	6	66.7					15	68.2	14	70.0			30*	26.3	11*	19.6	1	33.3						
Male heterosexual	3	33.3					11	50.0	11	55.0			18	15.8	6	10.7	1	33.3						
MSM							1	4.5	1	5.0			83	72.8	45	80.4	2	66.7	1	0.9	1	1.1		
Unknown	3	33.3	1	100.0			6	27.3	5	25.0	1	100.0	1	0.9					109	99.1	87	98.9		

Table A2.2: Resistance to ciprofloxacin and azithromycin, by country, 2009 (continued)

	Portugal						Slovakia						Slovenia						Spain					
	All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR		All patients		CipR		AzR	
	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%	No.	%	No	%	No	%
	79		27	34.2	0	0.0	15		15	100.0	1	6.7	24		19	79.2	2	8.3	103		67	65.0	6	5.8
Sex																								
Male	70	88.6	26	96.3			10	66.7	10	66.7	1	100.0	22	91.7	17	89.5	2	100.0	87	84.5	54	80.6	4	66.7
Female	9	11.4	1	3.7			5	33.3	5	33.3									14	13.6	12	17.9	2	33.3
Unknown													2	8.3	2	10.5			2	1.9	1	1.5		
Age (years)																								
<25	37	46.8	10	37.0			4	26.7	4	26.7			8	33.3	5	26.3	2	100.0	23	22.3	18	26.9	2	33.3
>=25	40	50.6	17	63.0			11	73.3	11	73.3	1	100.0	14	58.3	12	63.2			77	74.8	46	68.7	4	66.7
Unknown	2	2.5											2	8.3	2	10.5			3	2.9	3	4.5		
Sexual orientation																								
Heterosexual (male and female)	25	31.6	5	18.5			6	40.0	6	40.0			8	33.3	7	36.8								
Male heterosexual	18	22.8	5	18.5			2	13.3	2	13.3			8	33.3	7	36.8								
MSM	23	29.1	11	40.7									12	50.0	8	42.1	2	100.0						
Unknown	31	39.2	11	40.7			9	60.0	9	60.0	1	100.0	4	16.7	4	21.1			103	100.0	67	100.0	6	100.0

Table A2.2: Resistance to ciprofloxacin and azithromycin, by country, 2009 (continued)

	Sweden						UK					
	All patients		CipR		AzR		All patients		CipR		AzR	
	No.	%	No	%	No	%	No.	%	No	%	No	%
	108		77	71.3	11	10.2	120		42	35.0	5	4.2
Sex												
Male	90	83.3	63	81.8	8	72.7	87	72.5	38	90.5	5	100.0
Female	17	15.7	13	16.9	3	27.3	33	27.5	4	9.5		
Unknown	1	0.9	1	1.3		0.0						
Age (years)												
<25	42	38.9	29	37.7	3	27.3	62	51.7	12	28.6	3	60.0
>=25	66	61.1	48	62.3	8	72.7	58	48.3	30	71.4	2	40.0
Unknown												
Sexual orientation												
Heterosexual (male and female)							67*	55.8	12	28.6		
Male heterosexual							38	31.7	9	21.4	5	100.0
MSM							40	33.3	23	54.8		
Unknown	108	100.0	77	100.0	11	100.0	13	10.8	7	16.7		

Annex 3: External quality assurance detailed results

Table A3.1: **Ceftriaxone – overall concordance for each EQA strain from 17 participants**

Strain no.	All methods (n=169)		E-test (n=120)		Agar dilution (n=29)		Disc (n=20)	
	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance
QA10–01	S	100	S	100	S	100	S	100
QA10–02	S	100	S	100	S	100	S	100
QA10–03/QA10–07	S	97	S	96	S	100	S	100
QA10–04/QA10–10	S	100	S	100	S	100	S	100
QA10–05	S	100	S	100	S	100	S	100
QA10–06	S	100	S	100	S	100	S	100
QA10–08	S	82	S	83	S	67	S	100
QA10–09	S	100	S	100	S	100	S	100
Overall		97		97		96		100

Table A3.2: **Cefixime – overall concordance for each EQA strain from 16 participants**

Strain no.	All methods (n=158)		E-test (n=98)		Agar dilution (n=30)		Disc (n=30)	
	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance
QA10–01	S	100	S	100	S	100	S	100
QA10–02	S	100	S	100	S	100	S	100
QA10–03/QA10–07	S	70	S	61	S	67	S	100
QA10–04/QA10–10	S	100	S	100	S	100	S	100
QA10–05	S	100	S	100	S	100	S	100
QA10–06	S	94	S	90	S	100	S	100
QA10–08	S	94	S	90	S	100	S	100
QA10–09	S	100	S	100	S	100	S	100
Overall		95		93		96		100

Table A3.3: **Ciprofloxacin – overall concordance for each EQA strain from 18 participants**

Strain no.	All methods (n=179)		E-test (n=120)		Agar dilution (n=29)		Disc (n=30)	
	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance
QA10–01	S	100	S	100	S	100	S	100
QA10–02	S	100	S	100	S	100	S	100
QA10–03/QA10–07	R	100	R	100	R	100	R	100
QA10–04/QA10–10	S	100	S	100	S	100	S	100
QA10–05	R	83	R	75	R	100	R	100
QA10–06	R	100	R	100	R	100	R	100
QA10–08	R	100	R	100	R	100	R	100
QA10–09	S	100	S	100	S	100	S	100
Overall		98		97		100		100

Table A3.4: **Spectinomycin – overall concordance for each EQA strain from 15 participants**

Strain no.	All methods (n=149)		E-test (n=100)		Agar dilution (n=29)		Disc (n=20)	
	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance
QA10–01	S	100	S	100	S	100	S	100
QA10–02	S	100	S	100	S	100	S	100
QA10–03/QA10–07	S	100	S	100	S	100	S	100
QA10–04/QA10–10	R	100	R	100	R	100	R	100
QA10–05	S	100	S	100	S	100	S	100
QA10–06	S	100	S	100	S	100	S	100
QA10–08	S	100	S	100	S	100	S	100
QA10–09	S	100	S	100	S	100	S	100
Overall		100		100		100		100

Table A3.5: Azithromycin – overall concordance for each EQA strain from 17 participants

Strain no.	All methods (n=149)		E-test (n=110)		Agar dilution (n=29)		Disc (n=10)	
	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance	Consensus	% concordance
QA10–01	R	67	R	82	S	100	R	N/A
QA10–02	S	100	S	100	S	100	S	N/A
QA10–03/QA10–07	S	77	S	68	S	100	S	N/A
QA10–04/QA10–10	S	72	S	64	S	100	S	N/A
QA10–05	S	100	S	100	S	100	S	N/A
QA10–06	S	67	S	55	S	100	S	N/A
QA10–08	S	73	S	64	S	100	S	N/A
QA10–09	R	100	R	100	R	100	R	N/A
Overall		82		79		100		

Table A3.6: Phenotype and breakpoints of QA10 panel

Strain	Phenotype*	Breakpoints*†
QA10–01 (G07–1220)	AzR	
QA10–02 (WHO F)	Fully susceptible	
QA10–03 QA10–07 (WHO K)	CipR	Cefixime
QA10–04 QA10–10 (WHO O)	SpecR, PPNG	Az
QA10–05 (G08–2946)	CipR, PPNG	Cip
QA10–06 (G08–1299)	CipR	Az
QA10–08 (WHO L)	CipR	Ceftriaxone, Az
QA10–09 (G07–1454)	AzR	

**MIC breakpoints – as described in Annex I*
† Where the modal MIC is on a category of resistance

Table A3.7: Country coded beta-lactamase results

		Country codes																		Consensus	% concordance
Group	Strain	4	7	10	15	12	2	5	8	11	16	3	6	9	14	17	18	13	19		
1	QA10–01	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100
2	QA10–02	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100
3	QA10–03	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100
3	QA10–07	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
4	QA10–04	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	100
4	QA10–10	POS	POS	POS	POS	POS		POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
5	QA10–05	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	94.4
6	QA10–06	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100
7	QA10–08	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100
8	QA10–09	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	100

Table A3.8: Country coded category of resistance concordance – CIPROFLOXACIN

		Country codes																		Total number	No. sensitive	No. intermediate	No. resistant	Consensus by category	% concordance
Group	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18						
1	QA10–01	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	18	18	0	0	S	100
2	QA10–02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	18	18	0	0	S	100
3	QA10–03	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	36	0	0	36	R	100
3	QA10–07	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R						
4	QA10–10	S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	35	35	0	0	S	100
4	QA10–04	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
5	QA10–05	R	R	R	R	R	R	R	R	R	I	I	R	R	R	R	I	R	R	18	0	3	15	R	83.3
6	QA10–06	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	18	0	0	18	R	100
7	QA10–08	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	18	0	0	18	R	100
8	QA10–09	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	18	18	0	0	S	100

Table A3.9: Country coded MIC values (mg/L) – CIPROFLOXACIN

		Country codes																		
Group	Strain	2	3	4	5	6	7	8	10	11	12	13	14	16	17	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dd
1	QA10–01	0.004	0.004	0.008	0.004	0.002	0.004	0.008	0.002	0.004	0.004	0.008	<=0.004	0.004	0.008	0.008	0.004	0.002	0.008		
2	QA10–02	0.004	0.004	0.008	0.004	0.004	<0.002	0.008	0.002	0.002	0.004	0.004	<=0.004	0.004	0.004	0.002	0.004	0.002	0.008		
3	QA10–03	16	>32	64	>32	>32.0	>32	32	>32	>32	>32	>32	>1	32	>32	>32	>32	>1	64	1	
3	QA10–07	32	>32	64	>32	>32.0	>32	32	>32	>32	>32	>32	>1	32	>32	>32					
4	QA10–10		0.008	0.016	0.016	0.016	0.016	0.016	0.008	0.008	0.008	0.016	0.008	0.008	0.008	0.016	0.016	0.008	0.016		
4	QA10–04	0.008	0.008	0.016	0.016	0.016	0.016	0.016	0.008	0.008	0.008	0.016	0.008	0.008	0.016	0.016					
5	QA10–05	1	1	4	1	2	4	1	0.5	0.5	1	2	1	0.5	1	2	1	0.5	4	2	
6	QA10–06	16	32	32	>32	>32.0	>32	32	>32	>32	>32	>32	>1	12	>32	>32	>32	>1	>32	2	
7	QA10–08	16	>32	32	>32	>32.0	>32	32	>32	>32	>32	>32	>1	32	>32	>32	>32	>1	>32	1	
8	QA10–09	0.008	0.016	0.016	0.008	0.016	0.016	0.016	0.008	0.008	0.008	0.016	0.008	0.008	0.008	0.016	0.008	0.008	0.016		

Highlighted cells indicate E-test values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

Table A3.10: Country coded disc diameters (mm) – CIPROFLOXACIN

		Country codes				
Group	Strain	1	9	15	Mean	Range
1	QA10–01	46	54	52	51	46 – 54
2	QA10–02	48	47	46	47	46 – 48
3	QA10–03	6	0	6	4	0 – 6
3	QA10–07	6	0	6		
4	QA10–10	47	46	53	49	46 – 53
4	QA10–04	50	48	52		
5	QA10–05	23	26	26	25	23 – 26
6	QA10–06	9	13	11	11	9 – 13
7	QA10–08	6	0	6	4	0 – 6
8	QA10–09	47	46	51	48	46 – 51

Table A3.11: Country coded category of resistance concordance – CEFIXIME

		Country codes																			
Group	Strain	1	3	4	5	6	7	8	9	10	12	13	14	15	16	17	18	Total number	No. sensitive	No. intermediate	No. resistant	Consensus by category	% concordance
1	QA10–01	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	16	0	0	S	100
2	QA10–02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	16	0	0	S	100
3	QA10–03	S	I	S	S	S		I	S	S	S	S	I	S	S	R	R	30	21	6	3	S	70
3	QA10–07	S	I	S	S	S		I	S	S	S	S	I	S	S	S	R						
4	QA10–10	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	32	32	0	0	S	100
4	QA10–04	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
5	QA10–05	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	16	0	0	S	100
6	QA10–06	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	15	1	0	S	93.75
7	QA10–08	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	15	1	0	S	93.75
8	QA10–09	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	16	16	0	0	S	100

Table A3.12: Country coded MIC values (mg/L) – CEFIXIME

		Country codes																
Group	Strain	3	4	5	6	7	8	10	12	13	14	16	17	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dd
1	QA10–01	<0.016	0.008	<0.016	<0.016	0.032	0.064	<0.064	<0.016	0.016	<=0.016	0.032	0.032	0.016	<0.016	0.008	0.064	1	
2	QA10–02	<0.016	<0.002	<0.016	<0.016	<0.016	0.032	<0.064	<0.016	0.002	<=0.016	<0.016	<-0.016	<0.016	<0.016	<0.002	0.032		
3	QA10–03	0.25	0.25	0.125	0.064	0.5	0.5	0.25	0.125	0.25	>0.125	0.25	0.5	0.5	0.25	0.064	0.5	1	
3	QA10–07	0.25	0.25	0.125	0.125	0.5	0.5	0.25	0.125	0.25	>0.125	0.25	0.25	0.5					
4	QA10–10	0.016	0.008	<0.016	<0.016	0.032	0.064	<0.064	<0.016	0.016	0.032	0.032	0.032	0.032	0.032	0.008	0.064	1	
4	QA10–04	0.016	0.016	<0.016	0.016	0.032	0.064	<0.064	0.016	0.016	0.032	0.032	0.032	0.032					
5	QA10–05	<0.016	0.016	<0.016	0.032	<0.016	0.064	<0.064	<0.016	0.016	<=0.016	0.016	0.032	0.032	<0.016	<0.016	0.064	1	
6	QA10–06	0.125	0.125	0.125	0.032	0.25	0.25	0.125	0.064	0.064	0.125	0.16	0.125	0.125	0.125	0.032	0.25	1	
7	QA10–08	0.25	0.125	0.125	0.064	0.125	0.125	0.25	0.032	0.064	0.125	0.125	0.125	0.125	0.125	0.032	0.25	1	
8	QA10–09	<0.016	0.008	<0.016	<0.016	0.032	0.064	<0.064	<0.016	0.016	<=0.016	0.016	0.032	<0.016	<0.016	0.008	0.064	1	

Highlighted cells indicate E-test values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

Table A3.13: Country coded disc diameters (mm) – CEFIXIME

		Country codes				
Group	Strain	1	9	15	Mean	Range
1	QA10–01	40	39	39	39	39 – 40
2	QA10–02	47	44	40	44	40 – 47
3	QA10–03	31	31	32	32	30 – 34
3	QA10–07	30	34	31		
4	QA10–10	41	36	43	40	36 – 43
4	QA10–04	43	38	41		
5	QA10–05	41	42	45	43	41 – 45
6	QA10–06	34	31	34	33	31 – 34
7	QA10–08	40	42	39	40	39 – 42
8	QA10–09	41	44	36	40	36 – 44

Table A3.14: Country coded category of resistance concordance – CEFTRIAXONE

		Country codes																			
Group	Strain	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	Total number	No. sensitive	No. intermediate	No. resistant	Consensus by category	% concordance
1	QA10–01	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	17	17	0	0	S	100
2	QA10–02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	17	17	0	0	S	100
3	QA10–03	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	34	33	1	0	S	97.1
3	QA10–07	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
4	QA10–10	S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	33	33	0	0	S	100
4	QA10–04	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
5	QA10–05	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	17	17	0	0	S	100
6	QA10–06	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	17	17	0	0	S	100
7	QA10–08	S	S	I	S	S	S	S	S	S	S	S	S	I	S	S	S	R	17	14	2	1	S	82.4
8	QA10–09	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	17	17	0	0	S	100

Table A3.15: Country coded MIC values (mg/L) – CEFTRIAXONE

		Country codes																		
Group	Strain	2	3	4	5	6	7	8	10	11	12	13	14	16	17	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dilutions different
1	QA10-01	0.016	0.004	0.008	0.002	0.002	0.016	0.016	0.004	<0.016	<0.016	0.064	<=0.016	0.008	<0.002	0.008	<=0.016*	0.002	0.064	1	
2	QA10-02	=<0.001	<0.002	<0.002	<0.002	<0.002	<0.002	0.002	0.002	<0.016	<0.016	0.002	<=0.016	<0.002	<0.002	<0.002	<0.002	<0.002	0.002		
3	QA10-03	0.064	0.125	0.064	0.064	0.032	0.125	0.032	0.125	0.064	0.064	0.25	0.125	0.064	<0.002	0.125	0.064 /	<0.002	0.25		1
3	QA10-07	0.125	0.064	0.064	0.064	0.032	0.125	0.125	0.125	0.125	0.064	0.25	0.125	0.064	0.064	0.125	0.125				
4	QA10-10		0.016	0.008	0.008	0.008	0.032	0.008	0.032	0.016	<0.016	0.064	0.032	0.016	<0.002	0.032	0.032	<0.002	0.064	7	2
4	QA10-04	0.032	0.016	0.008	0.008	0.016	0.032	0.008	0.032	0.032	0.016	0.064	0.032	0.016	<0.002	0.032					
5	QA10-05	0.016	0.008	0.008	0.008	0.008	0.016	0.016	0.008	<0.016	<0.016	0.032	<=0.016	0.008	<0.002	0.016	0.008	<0.002	0.032	1	
6	QA10-06	0.125	0.032	0.032	0.032	0.016	0.25	0.125	0.125	0.064	0.032	0.125	0.125	0.064	0.032	0.125	0.125	0.016	0.25	6	1
7	QA10-08	0.125	0.25	0.125	0.125	0.032	0.25	0.032	0.125	0.25	0.064	0.5	>0.125	0.25	0.016	0.25	0.25	0.016	0.5	1	3
8	QA10-09	0.004	0.002	<0.002	<0.002	0.004	0.008	0.004	0.002	<0.016	<0.016	0.016	<=0.016	0.008	<0.002	0.008	<=0.016*	<0.002	0.016		

Highlighted cells indicate Etest values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

*no consensus MIC available, but all MICs below value shown

Table A3.16: Country coded disc diameters (mm) – CEFTRIAXONE

		Country codes			
Group	Strain	1	15	Mean	Range
1	QA10–01	46	45	46	45 – 46
2	QA10–02	53	48	51	48 – 53
3	QA10–03	39	39	40	39 – 42
3	QA10–07	39	42		
4	QA10–10	45	49	47	45 – 49
4	QA10–04	46	46		
5	QA10–05	46	53	50	46 – 53
6	QA10–06	42	45	44	42 – 45
7	QA10–08	41	46	44	41 – 46
8	QA10–09	43	43	43	43

Table A3.17: Country coded category of resistance concordance – SPECTINOMYCIN

		Country codes																			
Group	Strain	2	4	5	6	7	8	9	10	11	13	14	15	16	17	18	Total number	No. sensitive	No. intermediate	No. resistant	Consensus by category	% concordance
1	QA10–01	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
2	QA10–02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
3	QA10–03	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	30	30	0	0	S	100
3	QA10–07	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S						
4	QA10–10		R	R	R	R	R	R	R	R	R	R	R	R	R	R	29	0	0	29	R	100
4	QA10–04	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R						
5	QA10–05	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
6	QA10–06	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
7	QA10–08	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
8	QA10–09	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100

Table A3.18: Country coded MIC values (mg/L) – SPECTINOMYCIN

		Country codes																
Group	Strain	2	4	5	6	7	8	10	11	13	14	16	17	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dilutions different
1	QA10–01	16	16	8	16	16	8	16	8	8	<=16	8	8	16	16	8	16		
2	QA10–02	16	32	8	16	16	8	16	8	8	<=16	16	4	16	16	4	32	1	
3	QA10–03	16	32	8	32	16	4	16	4	8	<=16	16	4	8	16	4	32	6	
3	QA10–07	16	32	8	8	16	4	32	4	8	<=16	8	4	16					
4	QA10–10		>64	>1024	>24	>1024	1024	>1024	>1024	>1024	>128	1024	>1024	>1024	>1024	>24	>1024		
4	QA10–04	>=128	>64	1024	>124	>1024	1024	>1024	>1024	>1024	>128	1024	>1024	>1024					
5	QA10–05	16	16	4	8	16	4	8	4	4	<=16	8	4	8	4	4	16	3	
6	QA10–06	16	32	8	8	16	8	16	4	8	<=16	16	8	16	16	4	32	1	
7	QA10–08	16	16	8	8	16	8	32	4	16	<=16	8	8	8	8	4	32	1	
8	QA10–09	16	32	8	8	16	8	16	4	8	<=16	16	4	8	8	4	32	1	

Highlighted cells indicate E-test values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

Table A3.19: Country coded disc diameters (mm) – SPECTINOMYCIN

		Country codes			
Group	Strain	9	15	Mean	Range
1	QA10–01	26	32	29	26 – 32
2	QA10–02	21	27	24	21 – 27
3	QA10–03	26	30	28	25 – 30
3	QA10–07	25	29		
4	QA10–10	0	6	3	0 – 6
4	QA10–04	0	6		
5	QA10–05	29	37	33	29 – 37
6	QA10–06	25	34	30	25 – 34
7	QA10–08	32	36	34	32 – 36
8	QA10–09	26	32	29	26 – 32

Table A3.20: Country coded category of resistance concordance – AZITHROMYCIN

		Country codes																		
Group	Strain	1	2	3	4	5	6	7	8	10	11	12	14	16	17	18	Total number	No. sensitive	No. intermediate	No. resistant	Consensus by category	% concordance
1	QA10–01	R	S	R	S	I	R	R	I	R	R	R	S	R	R	R	15	3	2	10	R	66.7
2	QA10–02	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
3	QA10–03	S	S	I	S	S	I	I	S	S	S	S	S	S	S	I	30	23	7	0	S	76.7
3	QA10–07	S	S	I	S	S	I	I	S	S	S	S	S	S	S	S						
4	QA10–10	S		I	S	S	I	R	S	S	S	S	S	S	S	I	29	21	6	2	S	72.4
4	QA10–04	S	S	I	S	S	R	I	S	S	S	S	S	S	S	I						
5	QA10–05	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	15	15	0	0	S	100
6	QA10–06	S	S	I	S	S	R	R	I	S	S	S	S	S	S	I	15	10	3	2	S	66.7
7	QA10–08	S	S	I	S	S	R	I	S	S	S	S	S	S	S	I	15	11	3	1	S	73.3
8	QA10–09	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15	0	0	15	R	100

Table A3.21: Country coded MIC values (mg/L) – AZITHROMYCIN

		Country codes																		
Group	Strain	2	3	4	5	6	7	8	10	11	12	13	14	16	17	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dd
1	QA10–01	0.5	2	0.5	0.5	4	2	0.5	1	1	4	2	1	1	2	2	2	0.5	4	4	
2	QA10–02	0.125	0.125	0.125	0.064	0.25	0.25	0.064	0.064	0.064	0.25	0.125	0.064	0.125	0.25	0.125	0.125	0.064	0.25		
3	QA10–03	0.125	0.5	0.25	0.25	1	0.5	0.064	0.5	0.125	1	0.25	0.25	0.25	0.25	0.5	0.25	0.064	1	5	
3	QA10–07	0.25	0.5	0.5	0.125	1	0.5	0.064	0.25	0.25	0.5	0.5	0.25	0.25	0.5	0.25					
4	QA10–10		0.5	0.25	0.125	1	1	0.25	0.5	0.25	0.5	0.5	0.25	0.25	0.5	0.5	0.5	0.125	1	3	
4	QA10–04	0.25	0.5	0.25	0.125	1	0.5	0.125	0.5	0.5	1	1	0.25	0.25	0.5	0.5					
5	QA10–05	0.064	0.064	0.125	0.032	0.125	0.125	0.032	0.032	0.032	0.064	0.032	0.064	0.064	0.064	0.032	0.032/ 0.064	0.032	0.125		
6	QA10–06	0.5	0.5	0.5	0.25	2	1	0.5	0.5	0.5	1	0.5	0.5	0.5	1	0.5	0.5	0.25	2	1	
7	QA10–08	0.125	0.5	0.5	0.064	2	0.5	0.125	0.25	0.25	0.5	0.25	0.25	0.25	0.5	0.5	0.5	0.064	2	3	1
8	QA10–09	>=2	>256	>2.0	>32	>256	>256	256	>256	>256	>256	>256	>2	256	>256	>256	>256	>=2	>256		

Highlighted cells indicate E-test values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

Table A3.22: Country coded disc diameters (mm) – AZITHROMYCIN

		Country codes			
Group	Strain	1	15	Mean	Range
1	QA10–01	27	28	28	27 – 28
2	QA10–02	36	34	35	34 – 36
3	QA10–03	36	31	34	31 – 36
3	QA10–07	34	34		
4	QA10–10	34	35	36	34 – 37
4	QA10–04	36	37		
5	QA10–05	39	45	42	39 – 45
6	QA10–06	32	34	33	32 – 34
7	QA10–08	37	40	39	37 – 40
8	QA10–09	6	6	6	6

Table A3.23: Country coded MIC values (mg/L) – GENTAMICIN

		Country codes													
Group	Strain	3	5	6	8	10	12	13	16	18	Modal MIC	Min MIC	Max MIC	2 dilutions different	>2 dilutions different
1	QA10–01	8	8	4	4	8	16	8	8	8	8	4	16		
2	QA10–02	4	2	4	1	4	4	4	4	4	4	1	4	1	
3	QA10–03	4	4	4	4	8	8	8	8	4	4	4	8		
3	QA10–07	4	4	4	4	8	8	4	8	4					
4	QA10–10	4	4	4	4	4	8	4	4	4	4	2	8		
4	QA10–04	4	2	4	4	4	8	4	4	4					
5	QA10–05	4	4	4	4	4	8	4	4	4	4	4	8		
6	QA10–06	4	4	4	2	8	8	4	4	4	4	2	8		
7	QA10–08	8	4	4	2	8	8	4	4	8	4/8	2	8		
8	QA10–09	4	4	4	2	8	8	8	4	4	4	2	8		

Highlighted cells indicate E-test values that have been rounded up to the next full MIC value, *i.e.* 0.003 = 0.004

Table A3.24: Country coded disc diameters (mm) – GENTAMICIN

		Country code
Group	Strain	15
1	QA10–01	18
2	QA10–02	20
3	QA10–03	18
3	QA10–07	17
4	QA10–10	18
4	QA10–04	18
5	QA10–05	23
6	QA10–06	18
7	QA10–08	21
8	QA10–09	20