TECHNICAL REPORT

External quality assurance scheme for *Streptococcus pneumoniae*

2012

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ECDC TECHNICAL REPORT

External quality assurance scheme for *Streptococcus pneumoniae* – 2012

As part of the IBD-labnet surveillance network

The report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Adoración Navarro Torné and produced by Dr Mary Slack (Health Protection Agency, London, UK) on behalf of the IBD-labnet consortium participants (referring to specific contract ECDC 3335).

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Abbreviations

CAP	Community-acquired pneumonia
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
EQA	External quality assurance
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HPA	Health Protection Agency, UK
I	Intermediate
IPD	Invasive pneumococcal disease
MIC	Minimum inhibitory concentration
PCR	Polymerase chain reaction
PCV7	7-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PPV23	23-valent plain pneumococcal polysaccharide vaccine
QMS	Quality management systems
R	Resistant
S	Susceptible
SDRU	Streptococcus & Diphtheria Reference Unit

Executive summary

Streptococcus pneumoniae (pneumococcus) is the causative agent of a wide spectrum of diseases ranging from upper respiratory tract infections, including otitis media and sinusitis, to severe invasive disease. *S. pneumoniae* is the most frequently isolated respiratory pathogen in community-acquired pneumonia (CAP). Invasive pneumococcal disease (IPD), which is defined as the isolation of pneumococci or the detection of pneumococcal nucleic acid in normally sterile body fluids (blood, CSF, joint fluid, etc.), may present as meningitis, bacteraemic pneumonia, septic arthritis, or peritonitis [1].

Almost all strains of the pneumococcus have a polysaccharide capsule, which is a major virulence determinant contributing to evasion of the host immune system [3]. It also forms the basis for pneumococcal serotyping. Currently, 93 distinct serotypes have been identified. Overall, 20 serotypes of pneumococcus account for more than 80% of cases of IPD.

Prevention of invasive pneumococcal disease can be achieved by vaccination. There are two types of pneumococcal vaccine: a 23-valent plain polysaccharide vaccine and conjugate vaccines which contain an immunogenic non-pneumococcal protein conjugated to the pneumococcal polysaccharides. Currently, there are three conjugated pneumococcal vaccines, PCV7, PCV10 and PCV13, which target seven, ten and thirteen pneumococcal serotypes, respectively.

The implementation of infant immunisation with PCV7 resulted in a dramatic decline in IPD caused by vaccine serotypes, both among those targeted for the vaccine (direct effect) but also in older age groups (herd effect) by reducing nasopharynhgeal carriage of vaccine serotypes. The nasopharynx acts as a reservoir of pneumococci from which the organisms may be transmitted to other individuals [6]. However, the use of PCV7 was also accompanied by a significant increase in the circulation of non-vaccine serotypes (serotype replacement), notably serotypes 1, 3, 6A, 6C, 7F and 19A [9] and an increase in non-vaccine serotype IPD [8, 11, 5].

Surveillance of *S. pneumoniae* continues to be of importance, not only to establish the serotypes of *pneumococcus* causing invasive disease and monitor the impact of the newer pneumococcal conjugate vaccines (PCV10 and PCV13), but also to assess the long-term effectiveness of pneumococcal immunisation programmes. An integrated surveillance for this pathogen entails both epidemiological and laboratory surveillance. Epidemiological surveillance systems for IPD currently vary widely across Europe [2], making comparison of data difficult.

ECDC promotes the performance of external quality assurance (EQA) schemes, in which laboratories are sent simulated clinical specimens or bacterial isolates for testing by routine and/or reference laboratory methods. EQA schemes or laboratory proficiency testing provide information about the accuracy of different characterisation and typing methods as well as antimicrobial susceptibility testing; they also measure the sensitivity of the methods in place to detect certain pathogens or novel resistance patterns.

In May 2012, a collection of five strains of *Streptococcus pneumoniae* and two simulated samples of cerebrospinal fluid (CSF) (one containing *Streptococcus pneumoniae*, one containing *Neisseria meningitidis)* was sent to 29 participating reference laboratories in the IBD-labnet surveillance network for quality assurance testing. The laboratories were asked to perform standard laboratory protocols for the methods usually used by the laboratory: species identification, serotyping by serological methods and/or PCR. Antimicrobial susceptibility testing and β-lactamase testing was also requested for those laboratories that perform antimicrobial susceptibility testing of the isolates on a routine basis.

The results of this EQA exercise have shown that European pneumococcal reference laboratories differ in the level of characterisation of strains, ranging from speciation, serogrouping and serotyping to genotypic characterisation of isolates. All but one laboratory routinely serotype isolates. Eight laboratories perform genotypic characterisation of isolates; twenty-six laboratories routinely perform antimicrobial susceptibility testing.

Overall, out of 298 results submitted, there were only five errors in phenotypic identification of the strains (1.7%). One error was at the species level, where the strain of *S. pneumoniae* was identified as *S. mitis/oralis.* In one case, the serotype 8 pneumococcus was incorrectly identified as serotype 3, and in two cases the wrong serotype within a serogroup was identified. One laboratory reported auto-agglutination for one of the strains. These results show a marked improvement over the previous EQA distribution in 2010, where there were 13.1% errors.

Molecular typing by MLST but was not specifically requested for this EQA, but six laboratories did return sequence types (ST) for the strains and assigned the STs to clonal complexes (CC). All of the results were in agreement.

The antimicrobial susceptibility testing indicated that the majority of laboratories have little difficulty in identifying susceptible or resistant strains. Fifteen laboratories are using the EUCAST criteria, while nine are still using Clinical and Laboratory Standards Institute (CLSI) guidelines. Two laboratories reported antimicrobial susceptibilities based on their own national guidelines. This makes the comparison of results difficult. It is recommended that all European Reference laboratories move to using EUCAST guidelines as soon as possible.

A number of laboratories stated that the interpretation of MIC results for β-lactam antibiotics should be based on the source of the isolate (meningitis or non-meningitis) when using CLSI guidelines. All of the strains included in this EQA distribution were non-meningitis blood culture isolates. In future EQAs, the organisers of the EQA distribution will state the source of the isolates. In 2008, CLSI changed the interpretative standard for benzyl penicillin and *S. pneumoniae* [10] but the discrepancy with EUCAST breakpoints remains.

Two simulated CSF samples were included in the quality assurance panel to assess methods used for the non-culture detection of *S. pneumoniae.* Nineteen laboratories reported their results for these samples. The results submitted were very good. Seventeen (89%) laboratories correctly identified *S. pneumoniae* DNA in sample 1398. One laboratory stated that it contained either *S. pneumoniae or S. mitis* DNA.

Sample 1399 contained *N. meningitidis* DNA. Seven laboratories (37%) correctly identified this, and 10 laboratories (53%) stated that it was 'not *S. pneumoniae'.* Since the EQA samples had been sent to pneumococcal reference laboratories, this result should also be regarded as correct. Thus, a correct answer was obtained by 89% of the laboratories. One laboratory stated that sample 1399 was negative. One laboratory identified *S. tiguvinus/S. cristatus* in both sample 1389 and sample 1399.

In conclusion, the EQA results show a marked improvement over the previous distribution in 2010. Regular EQA distributions for the European pneumococcal reference laboratories are recommended to ensure that the improved quality of surveillance and epidemiological reporting is maintained. Electronic reporting is planned for the next EQA distribution, which should also facilitate the analysis of the results.

Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union agency with a mandate to operate dedicated surveillance networks 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 851/2004[1]).

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

- the assessment of the general standard of performance ('state of the art');
- the assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration);
- the evaluation of individual laboratory performance;
- the identification and justification of problem areas;
- the provision of continuing education; and
- the identification of needs for training activities.

Streptococcus pneumoniae (pneumococcus) is a common commensal of the upper respiratory tract and is a cause of local and invasive infections. Local infections of the respiratory tract include otitis media, sinusitis and pneumonia. Invasive pneumococcal disease (IPD) may present as a pneumonia, meningitis, septic arthritis or a bacteraemia without obvious focus. Young children, immunocompromised individuals and the elderly are at major risk of developing IPD. The World Health Organization estimates that more than 1.6 million people die of pneumococcal infection every year and half of these deaths are in children aged less than five years of age. Of the 93 different serotypes of pneumococcus that have to date been identified, only 20 to 30 are responsible for the majority of pneumococcal infections worldwide. There is some evidence of an association between serotype and severity of disease. Serotype prevalence varies between geographic regions and may change over time in response to selective vaccine pressure or clonal spread. Furthermore, capsular switching may occur, allowing the survival of specific clones and evasion of vaccine-induced immunity.

A number of pneumococcal vaccines are now available. The first to be introduced was the 23-valent plain pneumococcal polysaccharide vaccine (PPV23). This vaccine is indicated for use in children over the age of two years within risk groups and for the elderly. The first pneumococcal conjugate vaccine (PCV7) was licensed in the United States in 2000 and in Europe in 2001.This vaccine contains purified capsular polysaccharide of seven pneumococcal serotypes. The introduction of PCV7 led to a dramatic fall in the incidence of IPD in young children caused by these seven serotypes. In addition, the vaccination of infants with PCV7 reduced the nasopharyngeal carriage of these serotypes, resulting in a decline in the incidence of IPD due to these serotypes in older age groups through a 'herd effect'. However, the use of PCV7 was associated with an increase in other serotypes, not included in the vaccine (serotype replacement), notably 19A and 7F. More recently, a 10-valent (PCV10) and a 13-valent (PCV13) vaccine have been introduced.

Laboratory diagnostics and molecular epidemiology of *S. pneumoniae* are extremely important for the effective surveillance of this organism. Since the introduction of PCV10 and PCV13 in European countries, surveillance should monitor the impact of these vaccines in order to compare the different vaccine schedules adopted by Member States and to detect and study changes in serotype distribution and any possible serotype replacement due to vaccine pressure.

The implementation of laboratory surveillance activities – namely the external quality assurance (EQA) activities and training – have been outsourced by framework contract no. ECDC/08/008 to a consortium of European experts (the European Monitoring Group on Meningococci and other experts in *S. pneumoniae*, *H. influenzae* and *N. meningitidis*), coordinated by Prof Dr Matthias Frosch, University of Würzburg, Germany.

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

The specific objectives of this EQA exercise are:

- Further harmonisation of molecular typing of *S. pneumoniae*
- Further harmonisation of methods for antimicrobial susceptibility testing of *S. pneumoniae*
- Training and dissemination of methods for the laboratory surveillance of invasive bacterial infections
- Assisting the countries in capacity building, when required
- Supporting ECDC in linking laboratory surveillance data and epidemiological data.

1 Material and methods

The objectives of this exercise were:

- to design an EQA scheme utilising a small panel of material containing viable *Streptococcus pneumoniae* isolates and non-viable simulated clinical samples for phenotypic and genotypic characterisation (where possible) in all EU Member States and candidate countries with suitable reference facilities ; and
- to improve the quality of data, assisting in the standardisation of techniques and thereby facilitating consistent epidemiological data for submission to the ECDC TESSy database.

1.1 Study design

The design of the project allowed individual reference laboratories to test the material using their routinely available techniques in order to complete some or all of the requested criteria (Table 1) in the allocated time period.

An anonymised summary was produced, showing the submitted results, the consensus by interpretation, and the number of laboratories with each submitted result.

The EQA distribution used the availability of the large collection of *S. pneumoniae* isolates and expert knowledge of the Health Protection Agency's (HPA) Streptococcus and Diphtheria Reference Unit (SDRU, Microbiology Services Division: Colindale, HPA, London, UK) together with the expert knowledge of Dr Vivienne James (UK NEQAS for Microbiology) and facilities in the External Quality Assurance Department (eQAD) HPA: Colindale, London.

It should be noted that UK NEQAS for Microbiology undertake several international EQA schemes for other organisms that also require freeze-drying, distribution, results analysis and web-based reporting. The samples for the EQA scheme were selected by the coordinators (Prof Caugant, Dr Slack, Dr Lambertsen and Dr van der Ende) from the HPA collection by agreement of the University of Würzburg, as coordinator of the IBD-labnet project.

It was requested that all five strains be tested using standard laboratory protocols for the methods normally used by the laboratory to characterise submitted isolates of *S. pneumoniae*, namely species identification, serogrouping and serotyping.

It was also requested that antimicrobial susceptibility testing (penicillin, erythromycin, clindamycin, ceftriaxone and ciprofloxacin) be carried out using normal laboratory procedures. For the antimicrobial susceptibility testing participants were asked to perform MIC determinations and to provide an interpretation of their results – namely whether the strains were susceptible (S), resistant (R) or of intermediate susceptibility (I).

The characterisations (test results) requested of the participating laboratories are shown in Table 1.

Table 1. Tests requested from the participating laboratories

Procedure	Tests requested	
	Bacterial isolates	**Non-culture samples (simulated CSF)**
Phenotypic identification	Species	
	Serotype	
	Antimicrobial susceptibility testing	
Genotypic identification	Species	Detection of *Streptococcus pneumonia*
	Capsule type	

This was the first EQA exercise for *Streptococcus pneumonia* organised for IBD-labnet by eQAD, and web-based reporting was not available. It is anticipated that future pneumococcal EQAs will be reported using a web-based reporting system via the UK NEQAS website (www.uknegasmicro.org.uk).

1.2 Participants

The list of participating laboratories can be found in Annex 1.

All participants were contacted prior to the EQA distribution to confirm the address and contact details for despatch of the potentially hazardous material. It was envisaged that the reference laboratories would wish to store the viable cultures and retain any unused material for their own quality processes. It was hoped that the distribution of the well-characterised material would become a resource within and between the reference laboratories.

1.3 Timelines

The timelines for this EQA distribution are summarised in Table 2.

Table 2. Timelines for the EQA exercise

Event	Dates
Selection of EQA strains	March 2012
Assessment of material	April 2012
Transfer of material to eQAD NEQAS	April 2012
Pre-despatch checks (HRU and eQAD (NEQAS))	April 2012
Distribution of EQAC panel UK NEQAS EQA Distribution 2802	8th May 2012
Deadline for receipt of results	6th July 2012
Analysis and collation of results	July–August 2012
Interim report to participants	September 2012
Interim report at EUROVAC meeting, Barcelona, Spain	November 2012
Individual results released on UKNEQAS website at https:results.uknequas.org.uk	September 2012
Summary report and recommendations	December 2012

1.4 The EQA panel material

The EQA panel comprised five viable bacterial isolates (to test participating laboratories' abilities to identify and characterise live cultures) plus two non-viable simulated CSF samples (to test their ability to detect *S. pneumoniae* in clinical specimens using non-culture detection methods).

Bacterial isolates

Five viable isolates of *S. pneumoniae* were selected for the panel. These were selected to be representative of the major disease-causing serogroups and serotypes, and to include strains demonstrating a range of MICs to other commonly used antimicrobials.

The isolates were selected and pre-screened by staff at the HPA's Streptococcus and Diophtheria Reference Unit (SDRU) and Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL). They were then grown up, aliquoted, freeze-dried and distributed at ambient temperature by UK NEQAS for Microbiology. The samples were accompanied by instructions for their revival.

Non-culture simulated meningitis samples

The two simulated CSF (non-culture) samples for PCR were prepared from heat-killed suspensions of isolates obtained from the UK National Collection of Type Cultures (NCTC). One sample contained *S. pneumoniae* DNA. The other contained a *Neisseria meningitidis* DNA. This would allow laboratories capable of identifying this organism to report this information; the sample also acted as a negative control for the pneumococcal PCR.

Stock solutions of the bacterial cultures were prepared containing ≈ $2x10^8$ cfu/ml. The cultures were killed by heating to 100° C for 10 minutes and then diluted 1/100 in simulated CSF solution. The simulated CSF contained 6% sucrose and 1.1% bovine serum albumin. These simulated CSF samples were also distributed by UK NEQAS for Microbiology at ambient temperature, with instructions to handle them in the same way as clinical specimens.

2 Results

The strains were processed as requested, and the results were returned to NEQAS by 28 laboratories.

A summary of consensus results was released to participants via the UK NEQAS for Microbiology website in September 2012. An analysis of results from all participants was subsequently generated by UK NEQAS for Microbiology and SDRU. This was released to all participants via the UK NEQAS for Microbiology website in September 2012. Each participant received a customised report containing an analysis of their own results, plus a summary of the overall results from all participants. A copy of this report is included in Annex 3. The summary of overall results contained in Annex 3 is intended to complement the analysis of data in the following sections. The participation of each laboratory in the various parts of the EQA exercise is shown in Table 3.

It must be noted that each laboratory did not necessarily submit a result for all samples for a given test. Hence the total number of participants for a given test varies by sample (see Table 5).

A summary of the results of the EQA exercise was presented at the EUROVAC meeting, which was held in Barcelona in November 2012.

Table 3. Summary of tests for which each laboratory submitted results[a]

Laboratory identifier	Viable isolates							Non-culture detection
	Phenotypic identification			Genotypic identification			AST	Species ID
	Species ID	Serogroup	Serotype	Species ID	Serotype	MLST		
NM02	+	+	+					+
NM05	+	+	+		+		+	
NM06	+	+	+	+		+	+	+
NM07	+	+	+	+	+		+	+
NM08	+	+	+	+	+	+	+	+
NM09	+	+	+					
NM10	+	+	+				+	+
NM11	+	+	+			+	+	+
NM12	+	+	+			+	+	+
NM14	+	+	+				+	
NM17	+	+	+			+	+	+
NM20	+	+	+				+	+
NM23				+	+		+	+
NM26	+	+	+				+	+
NM33A	+	+	+		+		+	+
NM34	+	+	+			+	+	
NM36	+	+	+				+	+
NM37	+	+	+				+	
NM38	+	+						+
NM39	+	+	+	+			+	+
NM40	+	+	+				+	
NM41	+	+	+	+	+		+	+
NM43	+	+	+	+			+	+
NM45	+	+	+				+	
NM47	+			+			+	+
NM48	+	+	+				+	+
NM52	+	+					+	
NM54	+						+	
Total	**27**	**25**	**23**	**8**	**5**	**6**	**26**	**19**

[a] *Laboratories did not necessarily submit a result for all samples for a given test.*
Laboratory NM51 did not submit results.

2.1 Part 1: Characterisation of viable isolates

All participants confirmed that the five bacterial isolates were viable following the revival procedure. A summary of the number of laboratories reporting results for each sample by method is shown in Table 5.

The intended results for Part 1 of the analysis are shown in Table 4. Table 5 shows the ratio of laboratories which successfully reported the intended result for each test. It also lists the results that did not match the intended result.

The percentage of participants reporting the intended result for each test is also shown in Figures 1 to 4. In all tests for Part 1 of the study, the consensus of the submitted results matched the intended result. The percentage match varied between 75% and 100%. A detailed description of the results broken down by test is given below.

Table 4. Intended results for Part 1: Characterisation of viable isolates

EQA sample	Phenotypic species ID	Serogroup	Serotype	MLST	CC
1393	*S. pneumoniae*	8		*53*	CC62
1394	*S. pneumoniae*	14		*156*	CC156
1395	*S. pneumoniae*	6	B	*90*	CC156
1396	*S. pneumoniae*	9	N	*405*	CC218
1397	*S. pneumoniae*	1		*306*	CC306

Abbreviations: ID, identification; MLST, multi locus sequence typing; CC, clonal complex

Table 5. Results for Part 1: Phenotypic identification of viable isolates

Sample number	Intended result	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
Phenotypic species identification			
1393	*S. pneumoniae*	26/26 (100%)	
1394	*S. pneumoniae*	26/26 (100%)	
1395	*S. pneumoniae*	26/26 (100%)	
1396	*S. pneumoniae*	26/26 (100%)	
1397	*S. pneumoniae*	25/26 (96%)	*S.mitis/oralis (1)*
Phenotypic serogrouping			
1393	8	24/25 (96%)	3 (1)
1394	14	24/25 (96%)	Auto-agglutination (1)
1395	6	25/25 (100%)	
1396	9	25/25 (100%)	
1397	1	23/24 (96%)	
Phenotypic serotyping			
1393	NA		
1394	NA		
1395	B	21/22 (95%)	A (1)
1396	N	21/22 (95%)	Y (1)
1397	NA		
Genotypic species identification			
1393	*S. pneumoniae*	8/8 (100%)	
1394	*S. pneumoniae*	8/8 (100%)	
1395	*S. pneumoniae*	8/8 (100%)	
1396	*S. pneumoniae*	8/8 (100%)	
1397	*S. pneumoniae*	8/8 (100%)	
Genotypic capsular typing			
1393	8	3/4(75%)	Non-typed (1)
1394	14	5/5 (100%)	
1395	6[a]	5/5 (100%)	
1396	9N	3/ 4 (75%)[a]	Non-typed (1)
1397	1	5/5 (100%)	

Sample number	Intended result	Ratio of labs reporting the intended result (%)	Results not matching intended result (frequency)
MLST/CC			
1393	53/ CC62	6/6 (100%)	
1394	156/ CC156	6/6 (100%)	
1395	90/ CC156	6/6 (100%)	
1396	405/ CC218	6/6 (100%)	
1397	306/ CC306	6/6 (100%)	

Abbreviations: NA, not applicable.

[a] *One laboratory reported the serotype for sample 1396 as 9N/L.*

Figure 1: Phenotypic strain characterisation: consensus in per cent

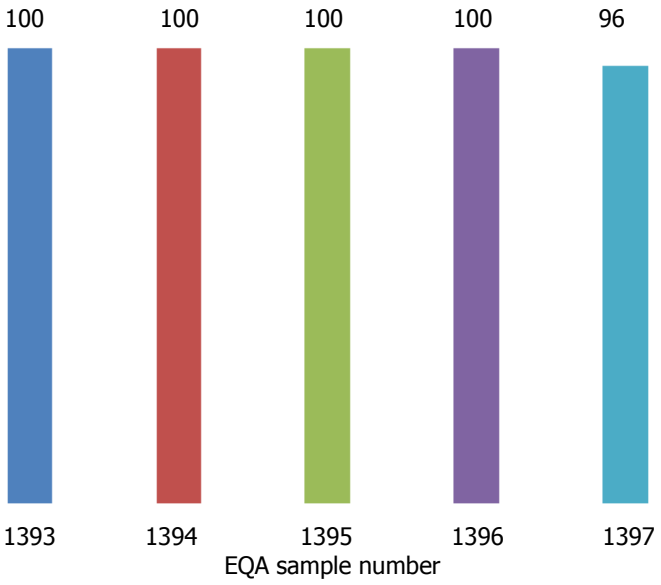
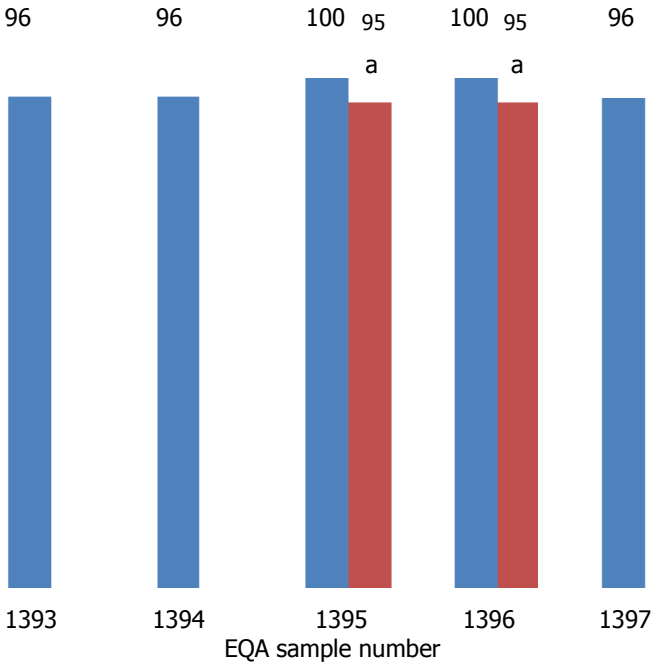


Figure 2: Phenotypic serogrouping and serotyping: consensus in per cent



[a] *Consensus % for serotyping of strains 1395 and 1396*

Figure 3: Genotypic species identification: consensus in per cent

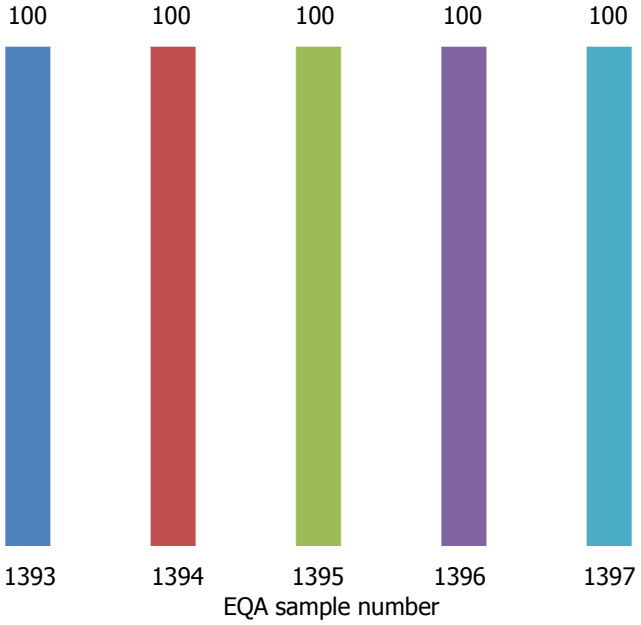
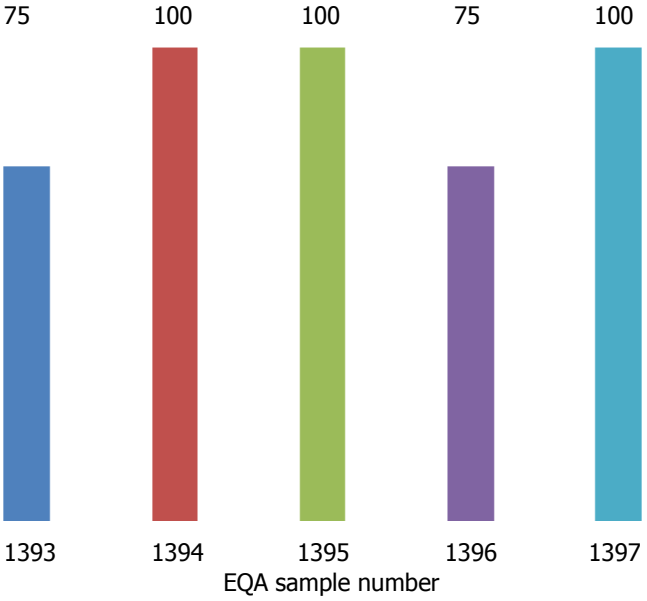


Figure 4: Genotypic capsular typing: consensus in per cent



Phenotypic species identification

Twenty-six laboratories performed phenotypic species identification. Four of the samples were correctly identified as *S. pneumoniae* by all participants. One participant incorrectly identified sample number 1397 as *S. mitis/oralis.* This laboratory reported using a combination of optochin susceptibility, bile solubility, pneumococcal agglutination and Vitek2 for speciation of the samples, so the reason for this discrepancy is unclear.

The most common methods used for phenotypic species identification are shown in Table 6.

Table 6. Phenotypic species identification methods reported by participating laboratories

Lab ID	ID method 1	ID method 2	ID method 3	Additional methods
NM02				
NM05	Optochin			
NM06	Optochin	Colonial morphology	Bile solubility	
NM07	Colonial morphology			

Lab ID	ID method 1	ID method 2	ID method 3	Additional methods
NM08	Optochin	Colonial morphology		
NM09	Optochin	Haemolysis		
NM10	Optochin	Bile solubility	Colonial morphology	
NM11	Optochin			
NM12	Optochin	Bile solubility		
NM14	Optochin	Bile solubility	Latex agglutination	
NM17				
NM20	Biomerieux slide agglutination			
NM23				
NM26	Optochin	Neufeld		
NM33A	Optochin			
NM34	Optochin	Bile solubility		
NM36	Conventional			
NM37	Optochin	Bile solubility		
NM38	Optochin	Bile solubility		
NM39	Classic biochemical tests			
NM40	Optochin	Bile solubility		
NM41	Optochin	Bile solubility	Vitek 2	
NM43	API	Optochin	Gram	
NM 45	API 20 Strep			
NM47	Optochin	MALDI-TOF		
NM47	Vitek2	Optochin		
NM52	Vitek 2	Optochin	Bile solubility	Pneumococcal agglutination
NM52	Vitek 2			

Phenotypic serotyping

Twenty-five laboratories undertook serotyping using a combination of agglutination (15 participants), Neufeld Quellung (13), gel diffusion (1), PCR sequencing (1), dot blot (1) and capsular reaction (1). Two laboratories reported erroneous results: one laboratory reported sample number 1393 as serotype 3 rather than 8, sample number 1395 as 6A rather than 6B, and sample number 1396 as 9Y rather than 9N. Another laboratory reported auto-agglutination for sample number 1394. Serotypes 6A and 6B can be difficult to differentiate, particularly if a molecular method is used for typing. However, both laboratories reporting these errors were using conventional agglutination and Quellung methods for phenotypic typing. The report of sample 1396 as 9Y rather than 9N may represent a data entry error rather than an actual error in serotyping since 9Y does not exist.

If serotyping is performed using conventional Quellung or agglutination methods it is important to use all of the necessary factor antisera, and to include a positive and a negative control. As an example, four factor antisera are used to serotype pneumococci in serogroup 9 (Table 7).

Table 7. Identification of serotypes within serogroup 9 pneumococci

Serotype	Reactions in factor antiserum			
	9b	9d	9e	9g
9A	-	+	-	-
9L	+	-	-	-
9N	+	-	+	-
9V	-	+	-	+

Genotypic species identification

Eight laboratories used a PCR-based method to identify the strains, and all reported the correct result (Table 4). Extraction methods included boil (4), salt precipitation (1), Qiagen DNA minikit (4) and other commercially available kits (4). The most common gene targets were *lytA (5),* and *ply (3).* Other gene targets were *cpsA(1), sodA(1)* and *psaA (1).* Capsular typing was undertaken by five laboratories, with four reporting results using the use of multiplex PCR (one of which was stated to be the CDC method). All six laboratories performing MLST reported the consensus result.

2.2 Part 2: Antimicrobial susceptibility testing

Antimicrobial susceptibility testing

The intended results for the antimicrobial susceptibility testing are shown in Table 8.

Participants were asked to provide information on the guidelines and MIC methods used to test susceptibilities for specimens 1393 to 1397. The results obtained by participants are shown in Table 9.

EUCAST guidelines were used by 15 laboratories: one used microdilution, two used automated methods (Sensititre and VITEK 2), 10 used gradient MIC (bioMerieux (6), Liofilchem (3), Oxoid (1)); one did not state the method and another used disc diffusion.

CLSI guidelines were used by nine participants with five of these stating they used EUCAST for ciprofloxacin. Of the nine following CLSI guidelines one used microdilution. The other eight laboratories used gradient MIC (bioMerieux (6), Liofilchem (2)).

Two participants used the agar dilution method according to national methods and guidelines.

Although participants were requested to report MIC results one laboratory reported interpretations based on disc diffusion methods for all agents. Two laboratories stated they routinely used disc diffusion for clindamycin to detect inducible resistance. Another two laboratories used disc diffusion for ciprofloxacin, clindamycin and erythromycin. These results have been included in the interpretations tabulated below. One participant reporting MIC results did not provide any interpretations.

Table 8. Intended results for antimicrobial susceptibility testing of bacterial isolates

Sample number	Antimicrobial susceptibility (S)/ intermediate (I), resistance (R)
1393	Ciprofloxacin I[a]
1394	Penicillin R, Erythromycin R, Clindamycin R, Ciprofloxacin I[a]
1395	Penicillin R, Erythromycin R, Clindamycin R, Ciprofloxacin I[a]
1396	Ciprofloxacin I[a]
1397	Ciprofloxacin I[a]

[a] CLSI does not give an interpretative criterion for ciprofloxacin susceptibility testing of pneumococci. EUCAST gives a breakpoint for ciprofloxacin of S≤ 0.12 R>2 µg/ml but states that wild type S. pneumoniae are not considered to be susceptible to ciprofloxacin and are therefore categorised as intermediate.

Three laboratories used cefotaxime rather than ceftriaxone and one of these also tested levofloxacin (these results have been excluded from this analysis).

Ciprofloxacin was specifically requested in the contract for this EQA exercise and was therefore included. However wild-type *S. pneumoniae* strains are not considered to be susceptible to ciprofloxacin and are therefore categorised as being of intermediate susceptibility. CLSI guidelines do not specify any interpretative criteria for ciprofloxacin and *S. pneumoniae.* For these reasons the results for ciprofloxacin should be ignored.

Table 9. Antimicrobial susceptibility testing results

Antimicrobial agent	Specimen 1393				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.004 to 0.016 (21)	0.016	S	22/22	
Ciprofloxacin	0.12 to 3 (14)	0.5	I	13/17	S (3), R (1)
Clindamycin	0.047 to 0.25 (16)	0.125	S	22/22	
Erythromycin	0.023 to 0.25 (23)	0.064	S	25/25	
Penicillin	0.008 to 0.47 (25)	0.016	S	26/26	

Antimicrobial agent	Specimen 1394				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.12 to 0.75	0.5	S	21/23	I (2)
Ciprofloxacin	0.25 to 2	0.5	I	14/17	S (2), R (1)
Clindamycin	>1 to >256	>256	R	22/22	
Erythromycin	>1 to >256	>256	R	25/25	

Antimicrobial agent	Specimen 1394				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Penicillin	0.075 to 2	1	I/R*		

Antimicrobial agent	Specimen 1395				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.12 to 1	0.5	S	20/23	I (3)
Ciprofloxacin	0.5 to 3	0.5	I	14/17	S (2), R (1)
Clindamycin	>1 to >256	>256	R	21/22	I (1)
Erythromycin	>1 to >256	>256	R	25/25	
Penicillin	0.25 to >2	1	I/R*		

Antimicrobial agent	Specimen 1396				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.003 to 0.12	0.016	S	22/22	
Ciprofloxacin	0.19 to 2	0.25	I	15/17	S (2)
Clindamycin	0.032 to >8	0.094	S	21/22	R (1)
Erythromycin	0.032 to >16	0.125	S	24/25	R (1)
Penicillin	0.006 to 1	0.016	S	25/26	I (1)

Antimicrobial agent	Specimen 1397				
	MIC range (n)	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.004 to 0.023	0.016	S	22/22	
Ciprofloxacin	0.25 to 2	0.25	I	16/17	R (1)
Clindamycin	0.032 to 0.125	0.094	S	22/22	
Erythromycin	0.023 to 4	0.125	S	23/25	I (1), R (1)
Penicillin	0.004 to <0.06	0.016	S	26/26	

The tables show the MIC range, mode consensus interpretation (includes disc susceptibility interpretations) and non-consensus results reported for each specimen and agent combination.

** Eleven participants qualified their responses for penicillin for specimens 1394 and 1395 and stated that they would interpret the susceptibility as resistant if the sample was from a case of meningitis, or reported they assumed the sample was not from a case of meningitis. One of these participants stated that the interpretation was also dose dependant.*

The use of different guidelines (EUCAST and CLSI) for interpreting antimicrobial susceptibility makes comparison of results problematic. There are major differences between the EUCAST and CLSI, both in terms of media, and defined breakpoints for a number of antimicrobials. This is especially true for the β-lactam antimicrobials, but there are also differences for other classes of antimicrobials, including the macrolides. Recently, the CLSI interpretative guidelines were modified for benzyl penicillin and pneumococci [7 ,10]. This has brought the breakpoint for determining penicillin resistance closer to that stated in the EUCAST guidelines (Table 10), but there is still a discrepancy between the level determining resistance for both meningitis (EUCAST >0.06 µg/ml ; CLSI ≥ 0.12 µg/ml) and non-meningitis (EUCAST >2µg/ml; CLSI 8≥ µg/ml).

Table 10. Comparison of interpretative standards for MIC determinations (µg/ml) with *S. pneumoniae* in EUCAST and CLSI guidelines

Antimicrobial agent	EUCAST MIC breakpoint (mg/L)		CLSI MIC Interpretative standard (mg/L)		
	S≤	R>	S≤	I	R≥
Penicillin parenteral (meningitis)	≤ 0.06	> 0.06	≤ 0.06		≥ 0.12
Penicillin parenteral (non-meningitis)	≤ 0.06	> 2	≤ 0.06	4	≥ 8
Ceftriaxone (meningitis)	≤ 0.5	> 2	≤ 0.5	1	≥ 2

Antimicrobial agent	EUCAST MIC breakpoint (mg/L)		CLSI MIC Interpretative standard (mg/L)		
	S≤	R>	S≤	I	R≥
Ceftriaxone (non-meningitis)	≤ 0.5	> 2	≤ 0.5	2	≥ 4
Erythromycin	≤ 0.25	.> 0.5	≤ 0.25	0.5	≥ 1
Ciprofloxacin	≤ 0.12	> 2			
Clindamycin	≤ 0.5	> 0.5	≤ 0.25	0.5	≥ 1

Overall, the antimicrobial susceptibility testing results were good. Most of the discrepancies arose with the use of different interpretative guidelines for the β-lactams (EUCAST and CLSI).

2.3 Part 3: Non-culture detection of *S. pneumoniae*

Two simulated CSF samples (1398 and 1399) were included in the EQA panel to test participants' ability to extract DNA from the clinical samples and assay for the presence of *S. pneumoniae* DNA. Participants were also encouraged to offer any further information that their assay was capable of elucidating about the samples. Sample 1398 contained 100cfu/µl of a heat-killed suspension of *S. pneumoniae* in simulated CSF. Sample number 1399 contained 100cfu/µl of a heat-killed suspension of a strain of *N. meningitidis*. The intended results and breakdown of submitted data are shown in Table 7.

With the exception of Qiagen, which was used by nine participants, the extraction methods quoted were different for all other laboratories and included manual and automated methods using commercial and non-commercial methods with magnetic and spin column technologies.

The amplification/detection methods used were RT PCR (7), RT PCR Taqman (4), PCR (with or without gel electrophoresis) (6) and Seegene PneumoBacter multiplex (2).

The gene targets included *ply* (10), *lytA* (10), *cpsA* (3), 16S rRNA (3), Seegene PneumoBacter multiplex (2), *ctrA* (1), 16S rDNA (1), *sodA* (1), *psaA* (1) and *crgA* (1) for *N. meningitidis*.

Nineteen participants reported non-culture detection results for these two samples.

Table 11. Intended and submitted results for non-culture detection of *S. pneumoniae*

Specimen number	Identification	Consensus %	Non-consensus results (n)
1398	*S. pneumoniae*	16/18	*S. pneumoniae/mitis/oralis** (1), *S. tiguvinus/S. cristatus* (1)
1399	*N. meningitidis* Not *S. pneumoniae*	17/19 Not *S. pneumoniae (10)* *N. meningitidis* (7)	Negative (1), *S. tiguvinus/S. cristatus* (1)

** No amplification of the specific genes (lytA, ply and cpsA).*
One laboratory reported specimen 1398 as S. pneumoniae *serotype 18C.*

Overall comments

The laboratory EQA has shown that the European pneumococcal reference laboratories vary in the level to which they characterise strains referred to them, ranging from full speciation and serogrouping to full serotyping and sequence typing.

Overall, the results show a significant improvement from the previous EQA, which was conducted in 2010. Errors in speciation and serotyping were down to 1.7%, compared with 13.2% in the previous distribution. One error was at the species level, where the strain of *S. pneumoniae* was identified as *S. mitis/oralis*. In one case, the serotype 8 pneumococcus was incorrectly identified as serotype 3, and in two cases the wrong serotype within a serogroup was identified. One laboratory reported auto-agglutination for one of the strains.

The EQA distribution has again indicated that some laboratories lack the necessary reagents to fully serotype isolates, and this renders surveillance of IPD difficult. Comprehensive data on serotype distribution are essential in order to establish the impact of the use of pneumococcal vaccines.

Only six laboratories reported molecular typing by MLST. This may be because this was not specifically requested for this EQA exercise.

The antimicrobial susceptibility testing results indicated that the majority of the laboratories have little difficulty in performing susceptibility testing. The major discrepancy was in the interpretation of the results because of the use of different interpretative guidelines. Some laboratories are using EUCAST guidelines while others are still using CLSI guidelines. There are major differences between the EUCAST and CLSI, both in terms of media and defined breakpoints for a number of antimicrobials. All EU reference laboratories should be moving towards using EUCAST guidelines.

Conclusions

A certain degree of heterogeneity exists in the level of characterisation of strains of *S. pneumoniae* among EU countries. This emphasises the need for consensus and agreement in methods for characterising and accurately defining this organism. Some countries still require some capacity building in this area.

It is recommended that all European laboratories adopt the EUCAST methods of antimicrobial susceptibility testing, which should facilitate better comparison of the results from different laboratories (http://www.EUCAST.org).

For the first time, two simulated clinical samples were included in the EQA panel to assess non-culture detection methods. The results were very encouraging, but a larger number of this type of sample will be required in future distributions to assess participants' proficiency more rigorously.

In future EQA distributions, an online form will be provided, which should facilitate both the return of results and subsequent analyses.

A validation process requiring regular submission of a sample of isolates to a supranational European reference laboratory for verification of serotype and MIC determination would be of great value in ensuring high-quality epidemiological data throughout Europe.

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Annex 1. Participating reference laboratories

Reference laboratories participating in the ECDC project entitled 'Laboratory surveillance and external quality assurance (EQA) of invasive bacterial diseases in EU'.

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Annex 2. Consensus results for the ECDC IBD-labnet survey for *Streptococcus pneumoniae* identification, typing and susceptibility testing

Distribution 3214

Table A2-1. Part 1: *Streptococcus pneumoniae* strain characterisation

Specimen number		1393	1394	1395	1396	1397
Phenotypic identification	**Species**	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
	Serotype	8	14	6	9	1
	Subtype			B	N	
Genotypic identification	**Species**	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
	Capsular type	8	14	6B	9N	1
	MLST	53	156	90	405	306
		CC62	CC156	CC156	CC218	CC306

Table A2-2. Part 2: Antimicrobial susceptibility testing

Table of MIC mode and consensus interpretation

Antimicrobial agent	1393		1394		1395		1396		1397	
	MIC (mg/L)	Result	MIC (mg/L)	Result	MIC (mg/L)	Result	MIC (mg/L)	Result	MIC (mg/L)	Result
Ceftriaxone	0.016	S	0.5	S	0.5	S	0.016	S	0.016	S
Ciprofloxacin	0.5	I	0.5	I	0.5	I	0.25	I	0.25	I
Clindamycin	0.125	S	>256	R	>256	R	0.094	S	0.094	S
Erythromycin	0.064	S	>256	R	>256	R	0.125	S	0.125	S
Penicillin	0.016	S	1	*	1	*	0.016	S	0.016	S

** Participants indicated different interpretations based on whether the isolate was from a meningitis or non-meningitis case.*

Table A2-3. Part 3: Results for non-culture detection of bacteria in a simulated CSF sample from a suspected meningitis case

Specimen number	ID
1398	*S. pneumoniae*
1399	Not *S. pneumoniae* (*N. meningitidis*)

Annex 3. Report generated by UK NEQAS

ECDC IBD-labnet survey for *Streptococcus pneumoniae* identification, typing and susceptibility testing – Distribution 3214

Specimens were sent to 29 laboratories, and results were returned by 28; 26 performed phenotypic species identification with 11 performing genotypic identifications. Six reported MLST results, with one laboratory reporting the clonal complexes. Antimicrobial susceptibility testing was performed by 27 of the laboratories. Identification of the non-culture specimens was undertaken by 19 laboratories.

Part 1: *Streptococcus pneumoniae* strain characterisation, phenotypic identification

Specimens 1393 to 1397 were sent as pure cultures, and participants were asked to identify and type each strain using their routine procedures. The most common methods used, as stated by the participants, for phenotypic species identification were Optochin susceptibility (20 participants), bile solubility (10), colony morphology (4), VITEK 2 (4), latex agglutination (2), API (2), with MALDI-TOF, haemolysis, Gram and biochemical identifications each mentioned once.

Serotyping was undertaken by 25 laboratories using combinations of agglutination (15), Neufeld Quellung (13), gel diffusion (1), PCR sequencing (1), dot blot (1), and capsular reaction (1).

Table A3-1. Results for phenotypic identification

Specimen number		1393	1394	1395	1396	1397
Phenotypic identification	**Species**	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
	Ratio reporting consensus	26/26	26/26	26/26	26/26	25/26
	Non-consensus results (n)					*S.mitis/ S. oralis* (1)
	Serogroup	**8**	**14**	**6**	**9**	**1**
	Ratio reporting consensus	24/25	24/25	25/25	25/25	23/23
	Non-consensus results (n)	3 (1)	auto-agglutination (1)			
	Serotype			**B**	**N**	
	Ratio reporting consensus			21/22	21/22	
	Non-consensus results (n)			A (1)	Y (1)	

Genotypic identification

Genotypic species identification on specimens 1393 to 1397 was undertaken by eight laboratories, and all reported correctly. Extraction methods included boil (4), salt precipitation (1), Qiagen DNA minikit (4) and other commercially available kits (4). The most common gene targets were *lytA* (5) and *ply* (3). Other gene targets were *cpsA* (1), *sodA* (1) and *psaA* (1). Capsular typing was undertaken by five laboratories, with four reporting the use of multiplex PCR, one of which stated the CDC method. All six laboratories performing MLST reported the consensus result.

Table A3-2. Results for genotypic identification

Specimen number		1393	1394	1395	1396	1397
Genotypic identification	**Species**	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
	Ratio reporting consensus	8/8	8/8	8/8	8/8	8/8
	Capsular type	**8**	**14**	**6**	**9N**	**1**
	Ratio reporting consensus	3/4	5/5	3/5	2/4	5/5
	Non-consensus results (n)	Non-typed (1)		6A/B (1) 6B (1)	Non-typed (1) 9N/L (1)	
	MLST	**53**	**156**	**90**	**405**	**306**
		CC62	**CC156**	**CC156**	**CC218**	**CC306**

Part 2: Antimicrobial susceptibility testing

Participants were asked to provide information on the guidelines and MIC methods used to test susceptibilities for specimens 1393 to 1397.

EUCAST guidelines were used by 15 laboratories: one used microdilution, two used automated methods (Sensititre and VITEK 2), 10 used gradient MIC (bioMerieux (6), Liofilchem (3), Oxoid (1)); one did not state the method, and another used disc diffusion.

CLSI guidelines were used by nine participants, with five of these stating they used EUCAST for ciprofloxacin. Of the nine, one laboratory used microdilution. The other eight laboratories used gradient MIC (bioMerieux (6), Liofilchem (2)).

Two participants used the agar dilution method, following national methods and guidelines.

Although participants were requested to report MIC results, one laboratory reported interpretations based on disc diffusion methods for all agents. Two laboratories stated that they routinely used disc diffusion for clindamycin to detect inducible resistance. Another two laboratories used disc diffusion for ciprofloxacin, clindamycin and erythromycin. These results have been included in the interpretations tabulated below. One participant reporting MIC results did not provide any interpretations.

Three laboratories used cefotaxime rather than ceftriaxone, and one of these also tested leuofloxacin (these results were excluded from this analysis).

The tables below display the MIC range, mode consensus interpretation (includes disc susceptibility interpretations) and non-consensus results reported for each specimen and agent combination.

Table A3-3. MIC range, mode consensus interpretation (includes disc susceptibility interpretations) and non-consensus results reported for each specimen and agent combination

Antimicrobial agent	Specimen 1393				
	MIC range (n)	**MIC (mg/L) mode**	**Consensus interpretation**	**Ratio reporting consensus**	**Non-consensus results (n)**
Ceftriaxone	0.004 to 0.016 (21)	0.016	S	22/22	
Ciprofloxacin	0.12 to 3 (14)	0.5	I	13/17	S (3), R (1)
Clindamycin	0.047 to 0.25 (16)	0.125	S	22/22	
Erythromycin	0.023 to 0.25 (23)	0.064	S	25/25	
Penicillin	0.008 to 0.47 (25)	0.016	S	26/26	

Antimicrobial agent	Specimen 1394				
	MIC range	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.12 to 0.75	0.5	S	21/23	I (2)
Ciprofloxacin	0.25 to 2	0.5	I	14/17	S (2), R (1)
Clindamycin	>1 to >256	>256	R	22/22	
Erythromycin	>1 to >256	>256	R	25/25	
Penicillin	0.075 to 2	1	I/R*		

Antimicrobial agent	Specimen 1395				
	MIC range	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.12 to 1	0.5	S	20/23	I (3)
Ciprofloxacin	0.5 to 3	0.5	I	14/17	S (2), R (1)
Clindamycin	>1 to >256	>256	R	21/22	I (1)
Erythromycin	>1 to >256	>256	R	25/25	
Penicillin	0.25 to >2	1	I/R*		

Antimicrobial agent	Specimen 1396				
	MIC range	MIC (mg/L) mode	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.003 to 0.12	0.016	S	22/22	
Ciprofloxacin	0.19 to 2	0.25	I	15/17	S (2)
Clindamycin	0.032 to >8	0.094	S	21/22	R (1)
Erythromycin	0.032 to >16	0.125	S	24/25	R (1)
Penicillin	0.006 to 1	0.016	S	25/26	I (1)

Antimicrobial agent	Specimen 1397				
	MIC range	MIC (mg/L mode)	Consensus interpretation	Ratio reporting consensus	Non-consensus results (n)
Ceftriaxone	0.004 to 0.023	0.016	S	22/22	
Ciprofloxacin	0.25 to 2	0.25	I	16/17	R (1)
Clindamycin	0.032 to 0.125	0.094	S	22/22	
Erythromycin	0.023 to 4	0.125	S	23/25	I (1), R (1)
Penicillin	0.004 to <0.06	0.016	S	26/26	

** Eleven participants qualified their responses for penicillin for specimens 1394 and 1395 and stated that they would interpret the susceptibility as resistant if the sample was from a case of meningitis or reported that they assumed the sample was not from a case of meningitis. One of these participants stated that the interpretation was also dose dependant.*

Part 3: Non-culture detection of bacteria in a simulated CSF samples from suspected meningitis cases

Specimens 1398 and 1399 were simulated CSF samples for detection of *S. pneumoniae* using molecular methods. Participants were asked to extract the DNA and analyse it using their routine methods. Identification of the non-culture specimens was undertaken by 19 laboratories.

With the exception of Qiagen, which was used by nine participants, the extraction methods quoted were different for all other laboratories and included manual and automated methods using commercial and non-commercial methods with magnetic and spin column technologies.

The amplification/detection methods used were RT PCR (7), RT PCR Taqman (4), PCR (with or without gel electrophoresis) (6) and Seegene PneumoBacter multiplex (2).

The gene targets included *ply* (10), *lytA* (10), *cpsA* (3), 16S rRNA (3), Seegene PneumoBacter multiplex (2), *ctrA* (1), 16S rDNA (1), *sodA* (1), *psaA* (1) and *crgA* (1) for *N. meningitidis*.

Specimen number	ID	Ratio reporting consensus	Non-consensus results (n)
1398	*S. pneumoniae*	16/18	*S. pneumoniae/ mitis/oralis** (1), *S. tiguvinus/S. cristatus* (1)
1399	Not *S. pneumoniae* (*N. meningitidis*)	17/19 Not *S. pneumoniae (10)* *N. meningitidis* (7)	Negative (1), *S. tiguvinus/S. cristatus* (1)

** No amplification of the specific genes (lytA, ply and cpsA).*
One laboratory reported specimen 1398 as S. pneumoniae *serotype 18C.*