JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2011, p. 1475–1482 Vol. 49, No. 4
0095-1137/11/$12.00 doi:10.1128/JCM.02365-10

International External Quality Assurance for Laboratory Identification and Typing of *Streptococcus agalactiae* (Group B Streptococci)▽

Baharak Afshar,[1]* Karen Broughton,[1] Roberta Creti,[2] Antoaneta Decheva,[3] Markus Hufnagel,[4] Paula Kriz,[5] Lotte Lambertsen,[6] Marguerite Lovgren,[7] Pierrette Melin,[8] Graziella Orefici,[2] Claire Poyart,[9] Andreas Radtke,[10] Javier Rodriguez-Granger,[11] Uffe B. Skov Sørensen,[12] John Telford,[13] Lea Valinsky,[14] Levantia Zachariadou,[15] members of the DEVANI Study Group,† and Androulla Efstratiou[1]

Respiratory and Systemic Infection Laboratory, Health Protection Agency, Microbiology Services Division, Colindale, London, United Kingdom[1]; Istituto Superiore di Sanità, Rome, Italy[2]; National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria[3]; Center for Pediatrics and Adolescent Medicine, University Medical Center Freiburg, Germany[4]; National Institute of Public Health, Prague, Czech Republic[5]; Neisseria and Streptococcus Reference Center, Statens Serum Institut, Copenhagen, Denmark[6]; National Centre for Streptococcus, Provincial Laboratory for Public Health, University of Alberta Hospital, Edmonton, Alberta, Canada[7]; Centre Hospitalier Universitaire de Liège, Liège, Belgium[8]; Centre National de Référence des Streptocoques, APHP Hôpital COCHIN, Université Paris Descartes, Paris, France[9]; St. Olavs University Hospital, Department of Medical Microbiology, Trondheim, Norway[10]; Servicio Andaluz de Salud, Hospital Universitario Virgen de las Nieves, Granada, Spain[11]; Department of Medical Microbiology and Immunology, Aarhus University, Aarhus, Denmark[12]; Novartis Vaccines and Diagnostics, Siena, Italy[13]; National Streptococcus Reference Centre, Jerusalem, Israel[14]; and Aghia Sophia Children's Hospital, Athens, Greece[15]

Received 23 November 2010/Returned for modification 22 December 2010/Accepted 4 February 2011

We report the results from the first international multicenter external quality assessment (EQA) studies for molecular and serological typing of group B streptococcus (GBS) strains as part of DEVANI (*De*sign of a *V*accine *a*gainst *N*eonatal *I*nfections), a pan-European program. A questionnaire-based surveillance was undertaken among eight laboratories participating in DEVANI and six laboratories not participating in DEVANI from 13 countries in order to assess their current microbiological procedures for GBS screening, diagnosis, and typing. GBS strains from three EQA distributions were characterized using molecular and serological methods based on GBS capsular polysaccharide typing. Participants were asked to test the first distribution using their current serotyping and genotyping methods. The Strep-B-Latex agglutination method was the most widely used method, with a typeability value of >90%. A multiplex PCR assay for GBS capsular gene typing was also used by 2 of 14 centers, which achieved a typeability value of 93%; this assay detected only 9 of 10 GBS capsular polysaccharide genes. From the second and third EQA studies, standardized protocols were prepared for serological and molecular typing of GBS strains based on the Strep-B-Latex agglutination method and a novel multiplex PCR assay that detected all 10 GBS capsular types (Ia to IX). These standardized protocols are being used by many European laboratories, and as the use of these methods increases, it is imperative to continuously improve and assess laboratory performance and offer training to any laboratories that have technical difficulties.

Streptococcus agalactiae (group B streptococcus [GBS]) is a leading cause of neonatal sepsis, meningitis, and pneumonia, affecting 0.5 to 2 newborns/1,000 live births in Europe (34), although the true burden of GBS disease in newborns could be significantly higher than that reported in some European studies. Case-fatality rates from neonatal GBS disease are estimated at between 4 and 10% (12, 29). Neonatal GBS disease can occur within the first 6 days of life (early-onset disease [EOD]) or between 1 week and 3 months of age (late-onset disease [LOD]). Since 1996, in the United States, the rate of EOD has decreased significantly, from 1.7/1,000 to 0.34/1,000 live births (70% reduction) after the implementation of screening guidelines recommended by the Centers for Disease Control and Prevention (CDC) and the use of antibiotic prophylaxis (29). In contrast, the best strategy in European countries is still a matter of debate, and reliable data are urgently required to make reliable comparisons between countries.

The serological classification of GBS is based upon the identification of capsular polysaccharides (CPS) and protein antigens (8, 16, 22). CPS is essential to the virulence of GBS, and

* Corresponding author. Mailing address: Streptococcus and Diphtheria Reference Unit, Respiratory and Systemic Infection Laboratory, Health Protection Agency, Microbiology Services Division, 61 Colindale Avenue, London NW9 5EQ, United Kingdom. Phone: 44 (0)208 327 7160. Fax: 44 (0)208 205 6528. E-mail: Baharak.Afshar@hpa.org.uk.

† The contributing members of the DEVANI (*De*sign of a *V*accine *a*gainst *N*eonatal *I*nfections) Study Group were J. Kozakova and J. Motlová (Czech Republic); M. Kilian and K. Poulsen (Denmark); R. Berner, F. Lander, and L. Karstens (Germany); L. Baldassarri, M. Imperi, and M. Pataracchia (Italy); and M. De La Rosa Fraile (Spain).

▽ Published ahead of print on 16 February 2011.

as antibodies against CPS provide type-specific protection, it is potentially an ideal target for most trial GBS vaccines. Currently, GBS can be differentiated into 10 distinct CPS serotypes (Ia, Ib, and II to IX) (13, 22, 31), and their distribution varies worldwide (9, 20, 21, 30, 36). Knowledge of serotype distribution is necessary for the selection and development of serotype-based vaccines in a given geographic area; therefore, a standardized method that is rapid, robust, and reproducible for identifying all 10 GBS capsular polysaccharide types would be useful for epidemiological studies across Europe and beyond.

Many GBS capsular polysaccharide typing methods have been described (1, 5, 10, 14, 18, 35, 36), with the most common methods based on serological tests, i.e., immunodiffusion and commercial latex agglutination methods. Other PCR-based molecular methods for typing GBS strains have also been described (19, 23, 24). In addition, whole-genome molecular typing methods, such as restriction fragment length polymorphism (RFLP) (11) and pulsed-field gel electrophoresis (PFGE) (26), have also been useful for epidemiological studies of GBS strains because of their discriminatory power and reproducibility; however, these methods are more time-consuming and laborious. More recently, a multilocus sequence-typing (MLST) method, based on ~500-bp fragments of seven housekeeping genes, has been used to investigate the clonal population structures and genetic lineages of GBS strains (17). A major part of the present study involved investigation of the current microbiological procedures for GBS characterization in order to harmonize and standardize serological and molecular methods and improve laboratory performance across selected European countries.

A European Commission Seventh Framework program known as DEVANI (*De*sign of a *V*accine *a*gainst *N*eonatal *I*nfections) was launched on 1 January 2008 (http://www.devaniproject.org). This pan-European program includes beneficiaries (i.e., DEVANI participants) from eight European countries: Belgium, Bulgaria, Czech Republic, Denmark, Germany, Italy, Spain, and the United Kingdom. The overall aim of this 3.5-year project is to assess European GBS epidemiology in order to facilitate the design of a new vaccine capable of conferring broad coverage to immunize neonates against GBS infections through a durable maternal immune response. The program is divided into eight specific work packages, one of which is specifically for method standardization and external quality assessment (EQA) schemes (work package 6). The main objectives of this work package were to (i) undertake questionnaire-based surveillance among all countries participating in DEVANI to assess the current microbiological procedures for GBS screening, diagnosis, and typing; (ii) to establish EQA studies for GBS characterization so as to test the abilities of participating centers to correctly "type" a panel of GBS strains using molecular and serological typing methods; and (iii) to harmonize and standardize GBS-typing methods for enhanced surveillance of neonatal infections in Europe. Other international streptococcal reference centers were also invited to take part in the first and second EQA studies.

In this study, we also reviewed the current laboratory methods for antenatal GBS screening, as well as existing policies and recommendations for the prevention of early-onset neonatal GBS disease among various European countries.

MATERIALS AND METHODS

Participants. Fourteen institutions representing 13 countries, i.e., Belgium, Bulgaria, Canada, Czech Republic, Denmark, France, Germany, Greece, Israel, Italy, Norway, Spain, and the United Kingdom, participated in the first two EQA distributions. Denmark was represented by two centers. The third EQA distribution was open only to the eight DEVANI collaborating laboratories. Most of these laboratories are National Streptococcus Reference Centers. These studies were coordinated by the Streptococcus and Diphtheria Reference Unit (SDRU), Respiratory and Systemic Infection Laboratory, Health Protection Agency Microbiology Services Division (HPAMSD) Colindale, London, United Kingdom.

Questionnaire assessment. A questionnaire for the assessment of microbiological procedures for group B streptococcal diagnosis, screening, and typing was sent to the DEVANI and non-DEVANI centers between April and July 2008. This questionnaire requested detailed information on the level of reference services provided, national GBS screening policies for women during pregnancy or at delivery, and current laboratory methods used for GBS screening and typing.

Bacterial isolates and dispatch. GBS isolates were selected from archived clinical isolates submitted by hospital laboratories in England and Wales to the Streptococcus and Diphtheria Reference Unit, HPAMSD Colindale, United Kingdom, from 2007 to 2009 and GBS serotype reference strains. The clinical isolates were obtained from male and female patients across different age groups.

Each distribution panel contained a duplicate set of strains and three fully sequenced reference strains, including strain A909 (Ia), strain NEM316 (III), and strain 2603V/R (V) (Table 1). The first distribution comprised 14 GBS isolates, the second distribution included 15 GBS isolates, and the third distribution consisted of 12 GBS isolates. The isolates were coded prior to dispatch to blind the participants to their identities. Replicates of each isolate were prepared on Columbia blood agar (CBA) slopes and then sent to each participating center by courier. The first EQA distribution was dispatched in September 2008, the second in February 2009, and the third in September 2009. With each distribution, the coordinating center provided detailed instructions for completion and submission of results.

EQA distributions. Participants were asked to test a panel of 14 coded GBS isolates for the first EQA distribution, using their current serotyping and genotyping methods (Table 2). For the second EQA distribution, each center was asked to serotype 15 GBS isolates using the standardized latex agglutination method based on the Strep-B-Latex kit (Diagnostica, Statens Serum Institut [SSI], Denmark), as agreed by the DEVANI participants (the modified method is described below). Participating centers were also asked to perform a multiplex PCR assay for GBS capsular gene typing (24). Only the eight beneficiaries within the DEVANI consortium took part in the third distribution, which involved testing a panel of 12 coded GBS isolates by the standardized Strep-B-Latex method, using the same batch of reagents (lot La-1-4) in all participating centers. In addition, participants were asked to genotype the strains using a novel multiplex PCR method developed by the DEVANI beneficiary from Italy (15). This PCR method was not published at the time of this distribution, and hence, the protocol could not be disseminated to other, non-DEVANI centers.

Reporting arrangements. Results were submitted to the coordinating center by completing and returning a questionnaire indicating the result for each coded GBS isolate. This form also detailed other information, such as the methodology used, detailed protocols, time taken to achieve a final result, and any problems participants encountered.

Evaluation of participants' results. The analysis of submitted data was undertaken by the coordinating center, and each participant was provided with a report summarizing the results of each EQA study. The participants were each given a confidential center number. Each intended GBS capsular type was scored as "1," and incorrect types were scored as "0" (the maximum scores were 14, 15, and 12 per center per EQA study). The typeability of each method was determined by using the following formula: $T = N_t/N$, where N_t is the number of isolates assigned a type and N is the number of isolates tested (33).

Latex serogrouping and serotyping methods. The modified method was developed and validated against all GBS serotype reference strains and numerous wild strains by SDRU, HPAMSD Colindale, United Kingdom (3). Each coded GBS strain was subcultured onto a CBA plate (or an equivalent growth medium) for single colonies and incubated overnight at 35 to 37°C in 5 to 10% CO_2. The Lancefield group B antigen was determined using a latex grouping kit, such as Prolex (Prolab Diagnostics) or Remel Streptex Latex Group B (Thermo Fisher Scientific), according to the manufacturer's recommendations. A heavy suspension of the test organism from CBA was prepared in saline buffer (250 μl), and a 20-μl aliquot was pipetted onto a glass microscope slide. A 1-μl loopful of each latex reagent (Ia to IX) from the Strep-B-Latex kit was added to each drop of

TABLE 1. Intended results for the GBS strains included in the three EQA distributions[a]

Study code[b]	Reference no.	Serotype[c]	Genotype[d]
First EQA distribution			
EQA1-1	H08 278 0114	Ib	Ib
EQA1-2	H08 310 0180	III	III
EQA1-3	H08 302 0227	II	II
EQA1-4	H08 282 0049	IV	IV
EQA1-5	**H08 302 0226**	**V**	**V**
EQA1-6	H07 392 0032	VI	VI
EQA1-7	**H08 302 0226**	**V**	**V**
EQA1-8	H08 282 0046	IX	IX
EQA1-9	H08 300 0098	Ia	Ia
EQA1-10	H07 496 0592	NT	Ia
EQA1-11	H07 498 0062	NT	V
EQA1-12	RR 0800 0895 (reference strain A909)	Ia	Ia
EQA1-13	RR 0800 1025 (reference strain NEM316)	III	III
EQA1-14	RR 0800 1203 (reference strain 2603V/R)	V	V
Second EQA distribution			
EQA2-1	H09 032 0548	II	II
EQA2-2	H09 024 0494	Ib	Ib
EQA2-3	H09 032 0799	III	III
EQA2-4	H09 026 0642	VI	VI
EQA2-5	H08 506 0226	NT	II
EQA2-6	**H08 510 0757**	**IV**	**IV**
EQA2-7	H08 440 0083	IX	IX
EQA2-8	H08 096 0562	VII	VII
EQA2-9	RR 0800 0895 (reference strain A909)	Ia	Ia
EQA2-10	RR 0800 1025 (reference strain NEM316)	III	III
EQA2-11	RR 0800 1203 (reference strain 2603V/R)	V	V
EQA2-12	**H08 510 0757**	**IV**	**IV**
EQA2-13	H07 318 0704	II	II
EQA2-14	H09 032 0798	Ia	Ia
EQA2-15	H09 030 0388	V	V
Third EQA distribution			
EQA3-1	RR 0800 0895 (reference strain A909)	Ia	Ia
EQA3-2	RR 0800 1025 (reference strain NEM316)	III	III
EQA3-3	RR 0800 1203 (reference strain 2603V/R)	V	V
EQA3-4	H09 278 0683	Ib	Ib
EQA3-5	H09 254 0277	IV	IV
EQA3-6	H09 326 0340	II	II
EQA3-7	H09 082 0835	VI	VI
EQA3-8	**SK1177**	**VIII**	**VIII**
EQA3-9	H08 440 0083	IX	IX
EQA3-10	NCTC 7271	VII	VII
EQA3-11	H09 272 0420	NT	III
EQA3-12	**SK1177**	**VIII**	**VIII**

[a] All GBS strains were from hospital laboratories in England and Wales, except strain SK1177, which was provided by the University of Aarhus, Denmark.
[b] Duplicate strains are indicated in boldface.
[c] Serotyping results are based on the standardized Strep-B-Latex method.
[d] Genotyping results are based on Multiplex PCR methods (15, 24).

suspension and mixed briefly. The slide was then rotated for 15 to 30 s, and a positive reaction was indicated by agglutination appearing within 30 s. If the reaction time exceeds 30 s, false-positive reactions may occur.

RESULTS

Results from the questionnaire. All eight DEVANI centers and six non-DEVANI centers completed their questionnaires and returned them to the coordinating center by August 2008.

(i) Details of reference services provided. Nine of 14 laboratories from Belgium, Bulgaria, Canada, Czech Republic, Denmark (Copenhagen), Israel, Norway, France, and the United Kingdom were designated National Streptococcus Reference Centers within their countries. Two centers, from the Czech Republic and the United Kingdom, were also designated WHO Collaborating Centers for Reference and Research on Streptococci. Apart from those of Germany, Greece, and Spain, 11 of 14 centers provided a streptococcal reference service for their entire country, three of which (from the Czech Republic, Denmark, and the United Kingdom) also provided reference services for streptococcal cultures referred from other countries. The majority of the countries, except Canada, Greece, and Italy, undertook routine national surveillance for invasive GBS disease.

The spectrum of group B streptococcal reference and diagnostic services that were offered by laboratories comprised GBS culture identification, antimicrobial susceptibility testing

TABLE 2. GBS-typing methods used by participating centers

Typing method	No of centers	% Typeability (no. of accurate results/total no. of tests performed)
First EQA distribution		
Serology		
Strep-B-Latex agglutination[a]	7	90 (88/98)
Standardized Strep-B-Latex agglutination[b]	4	95 (53/56)
Ouchterlony immunodiffusion with in-house-prepared antisera	2	88 (23/26)
Molecular typing		
Multiplex PCR assay[c]	2	93 (26/28)
Genotype identification by PCR[d]	1[e]	93 (13/14)
DNA polymorphism and subtyping of the GBS capsular gene cluster[f]	1	93 (13/14)
Second EQA distribution		
Serology		
Standardized Strep-B-Latex agglutination[b]	11[h]	88 (146/165)
Ouchterlony immunodiffusion with in-house prepared antisera	2	90 (27/30)
Molecular typing		
Multiplex PCR assay[c]	8	88 (88/100)
Novel multiplex PCR assay[g]	1	100 (15/15)
Genotype identification by PCR[d]	1	93 (14/15)
Third EQA distribution		
Serology		
Standardized Strep-B-Latex agglutination[b]	8[i]	100 (96/96)
Molecular typing		
Multiplex PCR assay[c]	4	90 (35/39)
Novel Multiplex PCR assay[g]	5	98 (59/60)

[a] Commercial kit manufactured by Statens Serum Institut, Denmark. Participants followed the method described by the manufacturer.
[b] This standardized method has been developed and validated against all group B type strains and numerous wild strains by the coordinating center (HPAMSD Colindale).
[c] The multiplex PCR assay detected 9/10 GBS capsular gene types (24).
[d] This method identified 6/10 GBS types directly by PCR and 8/10 types by sequencing (19).
[e] This center also used a dot blot hybridization method (2) and a PCR assay that detected GBS surface protein genes (4).
[f] PCR based on the capsular polysaccharide synthesis gene (*cps*) and restriction enzyme digestion (23).
[g] This novel multiplex PCR method detected all 10 GBS capsular gene types (Ia to IX) (15).
[h] Four centers used the same batch of Strep-B-Latex reagents (lot La-1).
[i] All centers used the same batch of Strep-B-Latex reagents (lot La-1-4).

(disk diffusion and Etest), CAMP tests, real-time PCR detection assays, genotyping for macrolide resistance genes, capsular polysaccharide typing and surface protein typing by PCR and in-house antisera, and other genotyping methods (i.e., PFGE and MLST).

(ii) GBS screening policies for women during pregnancy or at delivery. The questionnaire revealed that currently in Europe the majority of countries, except Bulgaria, Denmark, Greece, Norway, and the United Kingdom, offer universal antenatal screening for GBS between 35 and 37 weeks gestation. According to their national recommendations for the prevention of GBS EOD, intrapartum antibiotic prophylaxis is recommended and should be administered to all pregnant women who (i) are found to be GBS colonized between 35 and 37 weeks gestation or (ii) have one or more of the following recognized risk factors: a previous baby affected by GBS, GBS bacteriuria detected during the current pregnancy, preterm labor, prolonged rupture of the membranes, and fever in labor (7).

The types of maternal specimens received for GBS screening were predominantly rectal/vaginal swabs and urine. Neonatal samples, such as blood; cerebrospinal fluid (CSF); and lower respiratory tract, umbilical, gastric, ear, nose, and throat swabs, as well as surface samples for GBS diagnosis and further characterization, were also examined by at least 7 of 14 participating centers.

(iii) Laboratory methodologies for GBS screening. The majority of laboratories that received clinical samples for GBS screening and characterization used blood agar as the primary isolation medium. The types of selective media used were Granada (bioMérieux), Strep B Select (Bio-Rad), Columbia blood agar and Columbia broth with colistin and nalidixic acid supplement, blood agar supplemented with colistin and oxolinic acid, Todd-Hewitt broth with nalidixic acid and gentamicin sulfate, and Lim broth (Todd-Hewitt broth with nalidixic acid and colistin).

Other identification methods employed by centers included Lancefield serogrouping, the CAMP test, biotyping, latex agglutination, and Gram stain. One center also performed an "in-house" real-time PCR assay based on the *cylB* gene for the detection of GBS directly from clinical specimens (6).

(iv) Laboratory methodologies for GBS typing. The majority of centers (11/14 centers) used a commercial latex agglutination kit (Strep-B-Latex kit; SSI, Denmark) for serotyping of GBS isolates. One center routinely used the modified Strep-B-Latex method described in this study. Two centers also used in-house antisera raised against the three protein antigens c, R, and X. In addition, 9 of 14 centers were able to apply various molecular typing methods, such as a multiplex PCR assay for GBS antibiotic resistance genes, CPS, and surface protein typing, as well as PFGE and MLST.

First EQA distribution. GBS capsular typing results were obtained from 14 centers in 13 countries. All centers tested the entire GBS panel using one or more typing methods (Table 2) and were also able to identify the correct intended results for the duplicate strains (EQA1-5 and EQA1-7) and the three reference strains (EQA1-12 to EQA1-14). The majority of centers (11/14 centers) performed the Strep-B-Latex agglutination method, 4 of which employed the standardized Strep-B-Latex method with a typeability value of >90% (Table 2). Two centers used immunodiffusion with in-house-produced antisera (Ia to VIII). The overall typeability value for this method was 88%. A multiplex PCR assay (24) performed by two centers gave a typeability value of 93%. This PCR assay does not contain specific primers for the amplification of capsular gene type IX. One non-DEVANI center tested the entire panel using various molecular methods (2, 4, 19), and a DEVANI center also used a method based on DNA polymorphism and molecular subtyping of the capsular gene cluster (23). These molecular typing methods produced an overall typeability value of 93%.

Second EQA distribution. Results were received from 14 centers in 13 countries. The majority of centers (11/14 centers) used the standardized Strep-B-Latex agglutination method to test the entire panel. However, the typeability value of this method decreased from 95 to 88% in the second EQA study (Table 2). Of 15 strains tested using this method, only 7 strains, including the duplicate set (EQA2-6 and EQA2-12), were identified correctly by all centers. Most centers (9/11 centers) reported the correct intended serotype for all three reference strains (EQA2-9 to EQA2-11). Two strains (EQA2-5 and EQA2-13) gave discrepant results among centers. Only 5 of 11 centers identified the intended serotype for strain EQA2-5 (nontypeable [NT]) using the standard Strep-B-Latex method; however, this strain was reported as serotype II by four centers and serotype V by two centers. Incidentally, four of these centers (centers 2 to 5) produced discrepant results using the same batch of latex reagents (lot La-1). Similarly, strain EQA2-13 was identified correctly as serotype II by 7 out of 11 centers. The remaining four centers, three of which used the same batch of reagents, reported this strain as serotype IX.

A multiplex PCR assay (24) was performed by eight participating centers, five of which reported the correct genotyping results for the majority of strains (14/15). Two other centers reported results on only 4/5 and 3/5 strains, as the remaining strains either were difficult to amplify or were not tested. Only one center misidentified one strain (EQA2-9; intended genotype, Ia) as genotype III. This discrepancy was presumably due to a "mix-up" of isolates or labeling problems during sample processing. The typeability value of this PCR method decreased from 93% to 88% in the second EQA study (Table 2). Interestingly, one center reported the correct genotype for the entire panel using a novel multiplex PCR method for GBS capsular gene typing (15). This method uses a single PCR mixture containing specific primers for the amplification of the genes, including all 10 capsular gene types (Ia to IX).

Third EQA distribution. All eight laboratories achieved fully concordant results for the entire panel using the standardized Strep-B-Latex method with the same batch of reagents (lot La-1-4). All centers except one used either one or both multiplex PCR methods (15, 24) to test the entire panel. Four of five centers reported the correct genotype for the entire panel, using the novel multiplex PCR method (15); only one center misidentified one strain (EQA3-5) as genotype Ia. The intended capsular gene type for this strain was IV. Similarly, three of four centers determined the correct genotype for the majority of strains (11/12) that were tested using the previous multiplex PCR assay (24).

Performance between participating centers. Overall, the performance (typeability) score from the 14 centers varied from 60 to 100% using any of the molecular and serological typing methods (Table 3); half of the centers (7/14 centers) achieved a typeability score of >93%. Generally, the overall performance scores improved on each successive EQA distribution, with the exception of four DEVANI laboratories (centers 1, 3, 7, and 8). Of the 11 centers that used the standardized Strep-B-Latex method, only two centers achieved a maximum score of 100% in the second EQA study; however, the overall performance scores improved significantly in the third EQA study as a result of using the same batch of validated reagents (La-1-4); all eight participating centers achieved a maximum score of 100%. Furthermore, four of five centers that used the novel multiplex PCR assay obtained a maximum score of 100% for correctly identifying the entire panel.

DISCUSSION

We have described the first international multicenter EQA studies for molecular and serological typing of GBS strains. Our purpose was to determine the current laboratory procedures for serological and molecular capsular typing of GBS strains and to test the abilities of participating centers to correctly type GBS strains using these methods. The results from these EQA schemes enabled us to compare, harmonize, and standardize serological and molecular typing methods for enhanced surveillance of neonatal GBS infections in Europe.

For the first EQA distribution, we asked participating centers to examine a GBS panel using their current GBS-typing methods. Of the many typing methods, serotyping based on the commercially available Strep-B-Latex agglutination method was the most widely used, with a typeability value of >90%. The study showed that many European laboratories use this method, as it is rapid, easy to perform, and reproducible, provided that the antiserum reagents from the manufacturer have been validated against all 10 GBS serotype reference strains in order to avoid cross-reactivity between some antiserum reagents. Furthermore, this method could be a useful adjunct for vaccine development and surveillance, as it identifies GBS strains that are serologically nontypeable. Nontypeable strains often have CPS genes but are low-level producers or may have modified capsular structures that do not react to antisera from any of the 10 known serotypes (25). Another serotyping method, used by 2 of 14 participating centers, included immunodiffusion with in-house antisera. While the typeability value of this method was higher in the second EQA study (90%), it was apparent that these in-house serotyping tests are often difficult to interpret and expensive, and limited reagents are available for only a subset of serotypes. A modified method based on Strep-B-Latex, developed by the coordinating center, was approved by the DEVANI beneficiaries after completion of the first EQA distribution. It was agreed by

TABLE 3. Performance between participating centers: accuracy of serotyping and molecular typing of GBS strains from three EQA distributions

Typing method	% (No./total) for center[e]:													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
First EQA distribution														
Serology														
Strep-B-Latex	100 (14/14)	76 (11/14)		93 (13/14)	86 (12/14)				79 (11/14)		100 (14/14)			93 (13/14)
Standard Strep-B-Latex			86 (12/14)			100 (14/14)	93 (13/14)	100 (14/14)						
In-house assays[a]									83 (10/12)			93 (13/14)		
Molecular typing														
Multiplex PCR assay[b]						93 (13/14)			93 (13/14)					
Genotype ID by PCR													93 (13/14)	
Capsular gene subtyping							93 (13/14)							
Second EQA distribution														
Serology														
Standard Strep-B-Latex	93 (14/15)	93 (14/15)[c]	67 (10/15)[c]	93 (14/15)[c]	87 (13/15)[c]	100 (15/15)	87 (13/15)	67 (10/15)	93 (14/15)		100 (15/15)			93 (14/15)
In-house assays[a]									87 (13/15)			93 (14/15)		
Molecular typing														
Multiplex PCR assay[b]	60 (3/5)	93 (14/15)	80 (4/5)		93 (14/15)	93 (14/15)	80 (12/15)		93 (14/15)	93 (14/15)				
Novel multiplex PCR assay							100 (15/15)							
Genotype ID by PCR													93 (14/15)	
Third EQA distribution														
Serology														
Standard Strep-B-Latex	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]	100 (12/12)[d]						
Molecular typing														
Multiplex PCR assay[b]		92 (11/12)	67 (2/3)			92 (11/12)		92 (11/12)						
Novel multiplex PCR assay	100 (12/12)		92 (11/12)		100 (12/12)	100 (12/12)	100 (12/12)							

[a] Ouchterlony immunodiffusion with in-house-prepared antisera.
[b] The multiplex PCR assay detected 9/10 GBS capsular gene types (24).
[c] These centers used the same batch of Strep-B-Latex reagents (lot La-1).
[d] These centers used the same batch of Strep-B-Latex reagents (lot La-1-4).
[e] Scores ≤87% are shaded.

the DEVANI participants that this modified method should be adopted as the standard method for serological typing of GBS strains.

A multiplex PCR assay for GBS capsular typing described by Poyart et al. (24) was also used by 2 of 14 participating centers and achieved a typeability score of 93%. Although the assay was shown to be robust, widely applicable, and highly discriminatory in comparison to the other methods applied throughout the first EQA study, it had some limitations: it used a two-multiplex-PCR mixture that detected only 9/10 GBS capsular gene types and it did not detect the most recently identified capsular gene type (IX), and hence, GBS strains with genotype IX could not be identified.

For the second EQA distribution, participants were asked to test another panel of GBS strains, using the standardized Strep-B-Latex method (described in this study), as well as the Poyart assay (24), if feasible. While the majority of participating centers (11/14) followed the serotyping method based on the standardized Strep-B-Latex protocol, the typeability value of this method had in fact decreased from 95% to 88% in the second EQA distribution. Serotyping results from this distribution showed that discrepancies between participating centers were due to (i) cross-reactivity between latex reagents, which in some cases were from the same batch (lot La-1); (ii) "mix-up" of isolates; (iii) labeling problems during sample processing; or (iv) transcription error when reporting results. The results from this study demonstrated the importance of utilizing a validated batch of Strep-B-Latex reagents. The GBS capsular typing results from the second EQA distribution underlined the fact that it was vital to continue monitoring laboratory performance in order to facilitate improvements, highlight technical problems, and identify training needs. Furthermore, to improve awareness and technical performance, we held a training course on "microbiological procedures for group B streptococcal diagnosis, screening, and typing" after the second EQA study in May 2009, under the auspices of DEVANI.

During the second EQA study, a novel, unpublished multiplex PCR assay was being developed and evaluated by one DEVANI center. Using this method, the center achieved a maximum score of 100% for identifying the correct intended genotype for the entire panel, including strain EQA2-7 (capsular gene type IX). Therefore, this new assay was included in the next EQA study, as it had the potential to become the standard molecular typing method for GBS capsular gene typing.

For the third EQA distribution, all DEVANI participants used the standardized Strep-B-Latex method, this time with the same batch of latex reagents (lot La-1-4) that was previously validated against all the GBS serotype reference strains, and also the novel multiplex PCR method as described by Imperi et al. (15). The performance of laboratories was excellent for the third EQA study; all eight DEVANI centers achieved fully concordant results for the entire panel using the standardized Strep-B-Latex method with the same batch of reagents (lot La-1-4). Improvements in the performance of participating centers could also have been due to the fact that these laboratories had received training prior to the third EQA study. The recently developed multiplex PCR assay for GBS capsular gene typing was shown to be an easy, robust, and highly discriminatory method, as it used a single PCR and could differentiate GBS strains that were serologically nontypeable into 10 known GBS capsular gene types (Ia to IX). This method was later published (15) and adopted by DEVANI as the standard molecular method for GBS capsular gene typing.

The multicenter EQA studies described here have enabled us to produce standardized protocols for serological and molecular typing of GBS strains based on the Strep-B-Latex agglutination method using the same batch of validated reagents (lot La-1-4) and a novel multiplex PCR assay for GBS capsular gene typing. These standardized methods are currently being used by DEVANI laboratories that are participating in a multicenter GBS clinical study as part of the DEVANI program. As the use of these standardized methods increases, it is imperative to continuously improve and assess laboratory performance and offer training to any laboratories that have technical difficulties.

Currently, there are limited data in Europe on GBS disease and its preventive strategies. The questionnaire-based surveillance undertaken at the beginning of this study determined the current laboratory capabilities and status for GBS diagnosis and typing, as well as GBS screening procedures, within 12 European countries and Canada. Guidelines on prevention of GBS infection have varied, depending on the country-specific professional body. There are currently three approaches to prevention of neonatal GBS infection: universal antenatal screening of all pregnant women for GBS colonization, along with intrapartum antibiotics for all women with positive results; universal GBS screening of all pregnant women and intrapartum antibiotics only for those with positive results, as well as other risk factors for neonatal transmission; and intrapartum antibiotics for all women with risk factors for neonatal GBS transmission without prior screening.

Many European countries, apart from Bulgaria, Denmark, Greece, Norway, and the United Kingdom, offer universal antenatal screening for GBS by following the current U.S. guidelines recommended by the CDC, where all pregnant women are tested by bacterial culture for vaginal GBS colonization between 35 and 37 weeks of gestation and culture-positive women are offered intrapartum antibiotic prophylaxis (IAP) during labor (28). In the United Kingdom, clinicians have not generally adopted guidelines from the United States, Australia, and Canada that encourage GBS screening (28, 32). Current policy based on advice from the United Kingdom National Screening Committee (UKNSC), the Royal College of Obstetricians and Gynaecologists (RCOG), and the National Institute for Health and Clinical Excellence (NICE) is not to offer routine antenatal screening for GBS to all pregnant women because there is insufficient evidence to demonstrate that the benefits of doing so would outweigh the harm (27). However, according to United Kingdom local protocols, pregnant women judged clinically to be at "high risk" for the developments of GBS infection may be investigated for carriage, and those who are found to be GBS culture positive are treated with the appropriate IAP during labor and delivery.

The true burden of GBS disease could be significantly higher than that reported in some European studies. More efforts should be made to improve epidemiological information across Europe. The application of these harmonized microbiological

procedures for GBS characterization and the existing recommendations for GBS screening within European countries should ensure that GBS disease incidence and serotype distribution are assessed more rapidly and accurately and that the best preventive strategy is adopted across Europe and should also accurately inform current and future vaccine strategies.

ACKNOWLEDGMENTS

This study was supported by the European Commission Seventh Framework (grant agreement number 200481) as part of the DEVANI program.

We thank Z. Korenman (Israel) and G. Tyrrell and D. Hurteau (Canada) for their input to this study.

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