

Baharak Afshar¹, Androulla Efstratiou¹ and members of the DEVANI project

¹ Respiratory and Systemic Infections Department, HPA Centre for Infections, London, UK Baharak.Afshar@HPA.org.uk

INTRODUCTION

Group B streptococcus (GBS, *Streptococcus agalactiae*) is the major cause of sepsis, meningitis and pneumonia in neonates and of invasive disease in pregnant women, non-pregnant women, immunocompromised adults, and the elderly¹. An estimated 20-30% of pregnant women are GBS-colonised, approximately 50% of babies of carriers become colonised perinatally, of which 1-2% become infected². Case-fatality rates from GBS disease are estimated at between 4-10%^{2,3}. Early-onset disease (EOD; onset at <7 days of life) accounts for over 80% of GBS disease during infancy and the remaining 20% of cases accounts for late-onset disease (LOD; onset at 8-90 days of life). The incidence of GBS neonatal infection ranges from 0.5-2 newborns/1000 live births in Europe⁴.

An effective vaccine is likely to prevent the majority of infant disease (both early and late onset), as well as group B streptococcus-related stillbirths and prematurity, to avoid the current real and theoretical limitations of intrapartum antibiotic prophylaxis, and to be cost effective.

DEVANI (DEsign of a Vaccine Against Neonatal Infections), launched on 1st January 2008, is a three year pan-European programme funded through the European Commission Seventh Framework. This project is coordinated by Novartis Vaccines & Diagnostics, Siena, Italy, and includes Public Health Institutes and Universities from eight European countries (Table 1). The overall objective of this study is to design and develop a new vaccine for the prevention of neonatal GBS disease through the transfer of antibody from the mothers to the foetus during the last weeks of pregnancy. The programme is divided into eight specific work packages, one of which is the work package for Method Standardisation and External Quality Assessment (EQA) schemes (work package 6). One of the first objectives of this particular work package was to undertake a questionnaire-based surveillance amongst all DEVANI participating countries to assess microbiological procedures for GBS screening, diagnosis and typing.

METHODS

A questionnaire (Figure 1) was sent to eight DEVANI participating centres (beneficiary number 2 to 9, Table 1) on the 28th April 2008. All centres completed and returned their questionnaire to the HPA Centre for Infections, UK (coordinating centre for this work package) by 16th May 2008.

Figure 1: The questionnaire for the assessment of microbiological procedures for group B streptococcal diagnosis, screening and typing.

Countries within the DEVANI consortium

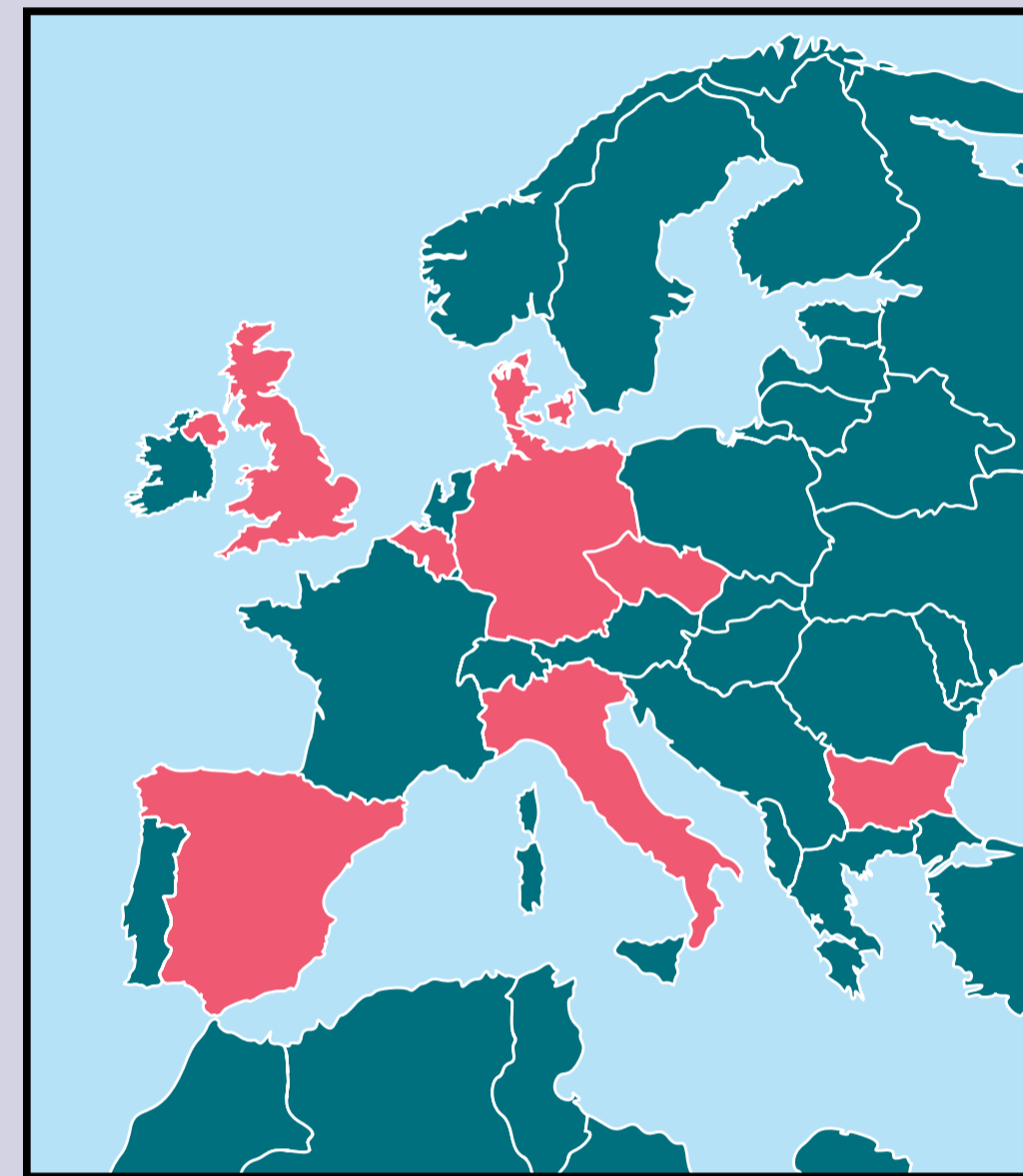
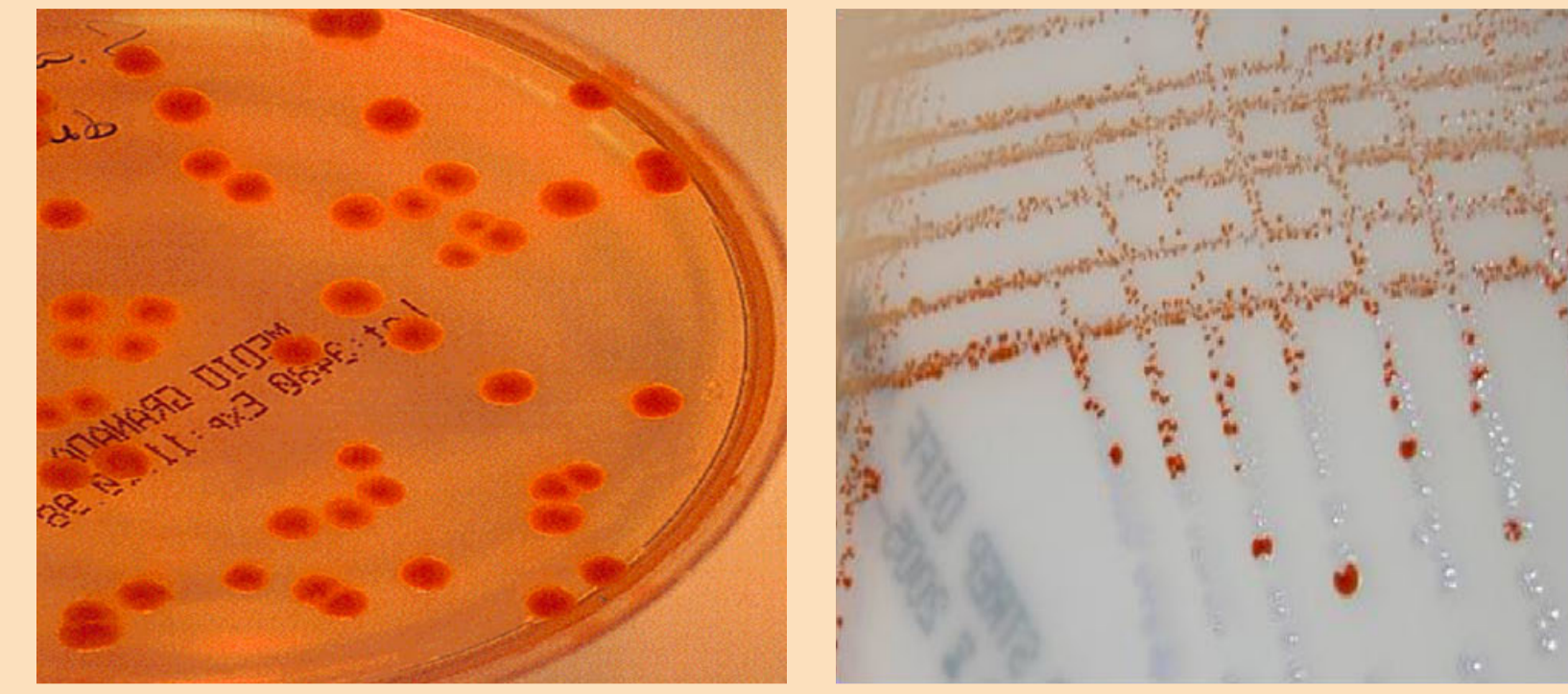


Figure 3: GRANADA Media (BioMérieux)



This selective media enables easy and rapid detection and identification of GBS in clinical samples. The media contains Granadaene, a polyenic red pigment which differentiates GBS from other bacteria.

Table 3: Non-culture based methods used by DEVANI centres for GBS screening and confirmation.

Country	Real-time PCR	Other methods
Italy (ISS)	-	-
UK (HPACf)	Based on <i>cyfB</i> gene	-
Belgium (CHULg)	Not in routine use	-
Spain (SAS)	-	-
Czech Republic (NIPH)	-	Serogrouping, CAMP test, Biochemical identification
Germany (UKL-FR)	-	-
Bulgaria (NCIPD)	-	CAMP test, latex agglutination
Denmark (AU)	-	Gram stain, CAMP test, latex agglutination

Table 4: Serotyping methods used for GBS typing.

Country	In-house reagents	Commercial kit
Italy (ISS)	-	Strep-B Latex (SSI)
UK (HPACf)	Antiseria c, R, X	Strep-B Latex (SSI)*
Belgium (CHULg)	-	Strep-B Latex (SSI)
Spain (SAS)	-	Strep-B Latex (SSI)
Czech Republic (NIPH)	Antiseria c, R, X	Strep-B Latex (SSI)
Germany (UKL-FR)	-	Strep-B Latex (SSI)
Bulgaria (NCIPD)	-	-
Denmark (AU)	-	Strep-B Latex (SSI)

SSI, Statens Serum Institut, Denmark; *, This centre uses a modified protocol.

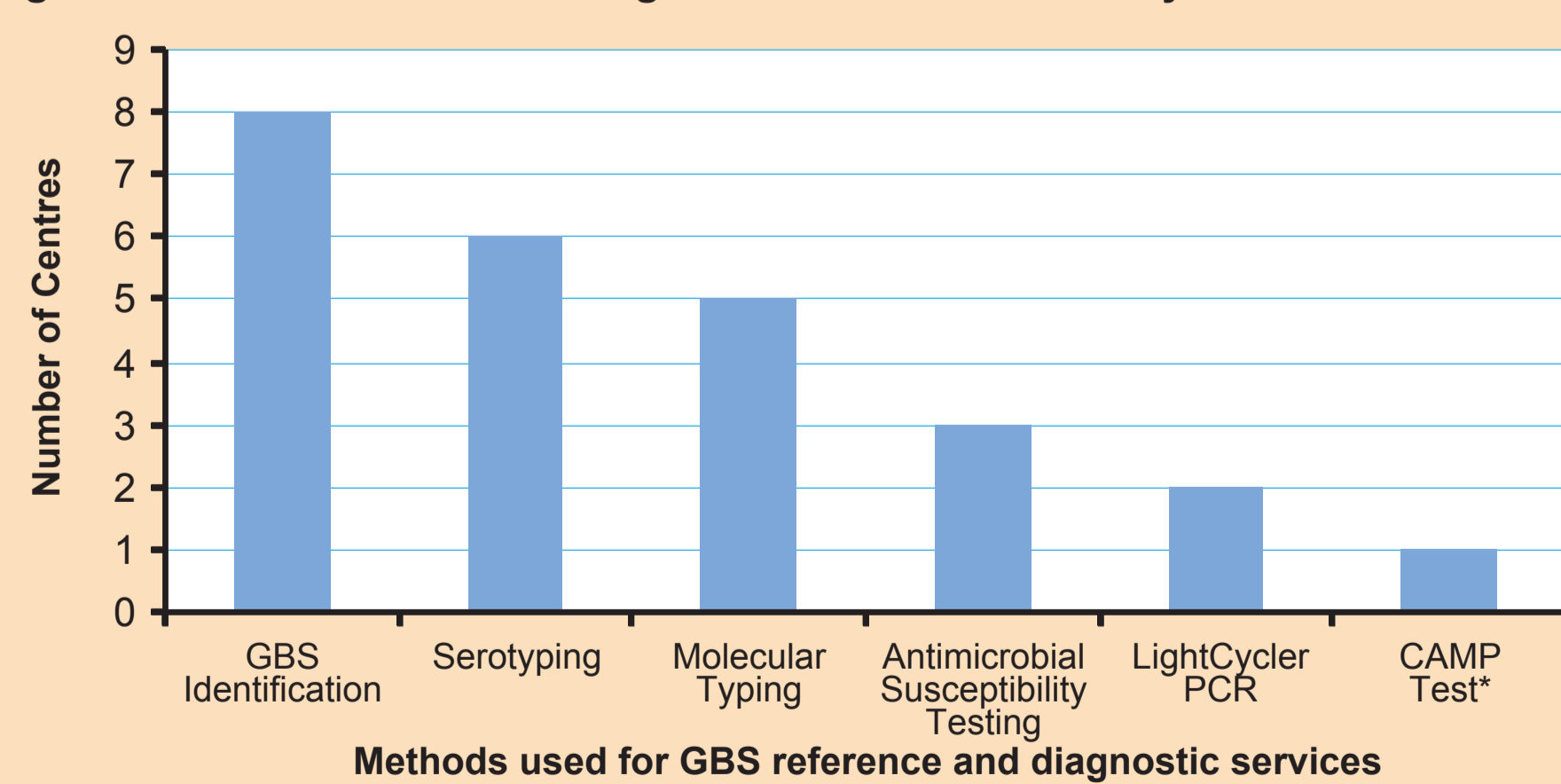
Table 1: List of DEVANI beneficiaries

Beneficiary Number	Beneficiary Name	Beneficiary Institute Name	City/Country
1 (Co-ordinator)	Dr John Telford	Novartis Vaccines & Diagnostics (Novartis VD)	Siena, Italy
2	Dr Lucilla Baldassarri	Istituto Superiore di Sanità (ISS)	Rome, Italy
3	Dr Androulla Efstratiou	Health Protection Agency, Centre for Infections (HPACf)	London, UK
4	Dr Pierrette Melin	Centre Hospitalier Universitaire de Liege (CHULg)	Liege, Belgium
5	Dr Manuel de la Rosa Fraile	Servicio Andaluz de Salud (SAS)	Granada, Spain
6	Dr Pavla Kriz	National Institute of Public Health (NIPH)	Prague, Czech Republic
7	Prof Dr Reinhard Berner	University Hospital Freiburg (UKL-FR)	Freiburg, Germany
8	Dr Antoaneta Detcheva	National Centre of Infectious and Parasitic Diseases (NCIPD)	Sofia, Bulgaria
9	Professor Mogens Kilian	Aarhus University (AU)	Aarhus, Denmark
10 (Project management)	Dr Antonella Chiuicchiuni & Graziella Orefici	ALTA Ricerca e sviluppo in Biotecnologie S.r.l	Siena, Italy

RESULTS

- Currently European countries within the DEVANI consortium that do not offer a national screening programme for GBS are the United Kingdom, Bulgaria and Denmark. Countries that do offer routine screening for GBS also provided national guidelines on screening policies for diagnosis, prevention and management of neonatal GBS disease.
- 6/8 centres provide a streptococcal reference service for their entire country. Of these 6 centres, three (including centre from the UK, Czech Republic and Denmark) can also provide reference services for streptococcal cultures referred from other countries.
- 7/8 centres offer routine national surveillance scheme for GBS disease in their country.
- GBS reference and diagnostic services are offered by all eight centres. The methods which are currently being used by the DEVANI centres are primarily GBS identification, serotyping and molecular typing which are offered by 8/8, 6/8 and 5/8 centres, respectively (Figure 2). Other types of method used include antimicrobial susceptibility testing, LightCycler PCR assays and CAMP test.
- The majority of centres (7/8) tend to use culture based methods for GBS screening and confirmation (Table 2; Figure 3). Non-culture based methods (e.g. PCRs, latex agglutination, CAMP test, etc) are also used by 5/8 centres (Table 3).
- Almost all centres (7/8) are able to serotype GBS isolates using the Strep-B Latex kit (Statens Serum Institut, Denmark) (Table 4), however, only 5 centres reported data on number of GBS isolates serotyped (Figure 4).
- 5/8 centres can use molecular typing methods such as: Multiplex PCR Assay, Multilocus Sequence Typing (MLST) and Pulse Field Gel Electrophoresis (PFGE) (Table 5).

Figure 2: GBS reference and diagnostic services offered by DEVANI centres.



* Identifies Group B β -streptococci based on their formation of a substance (CAMP factor) that enlarges the area of hemolysis formed by streptococcal β -hemolysin.

Table 2: Culture based methods used by DEVANI centres for GBS screening and confirmation.

Country	Primary isolation media	Selective media
Italy (ISS)*	-	-
UK (HPACf)	Columbia Blood Agar	Granada (see Figure 3)
Belgium (CHULg)	Lim broth	Granada & Strep B Select
Spain (SAS)	-	Granada
Czech Republic (NIPH)	Columbia Blood Agar/broth	Columbia Blood Agar/broth with CNA supplement
Germany (UKL-FR)	-	Blood agar + colistin & oxolinic
Bulgaria (NCIPD)	Blood Agar	-
Denmark (AU)	Blood Agar (5% horse blood)	Todd-Hewitt broth + NA + GS

CNA, colistin and nalidixic acid; NA, nalidixic acid; GS, gentamycin sulphate
*, Reference laboratory-clinical samples are not handled.

Figure 4: Total number of GBS isolates serotyped by each centre (from 2004-2007). The GBS isolates (invasive and non-invasive isolates) are from various sources and age groups.

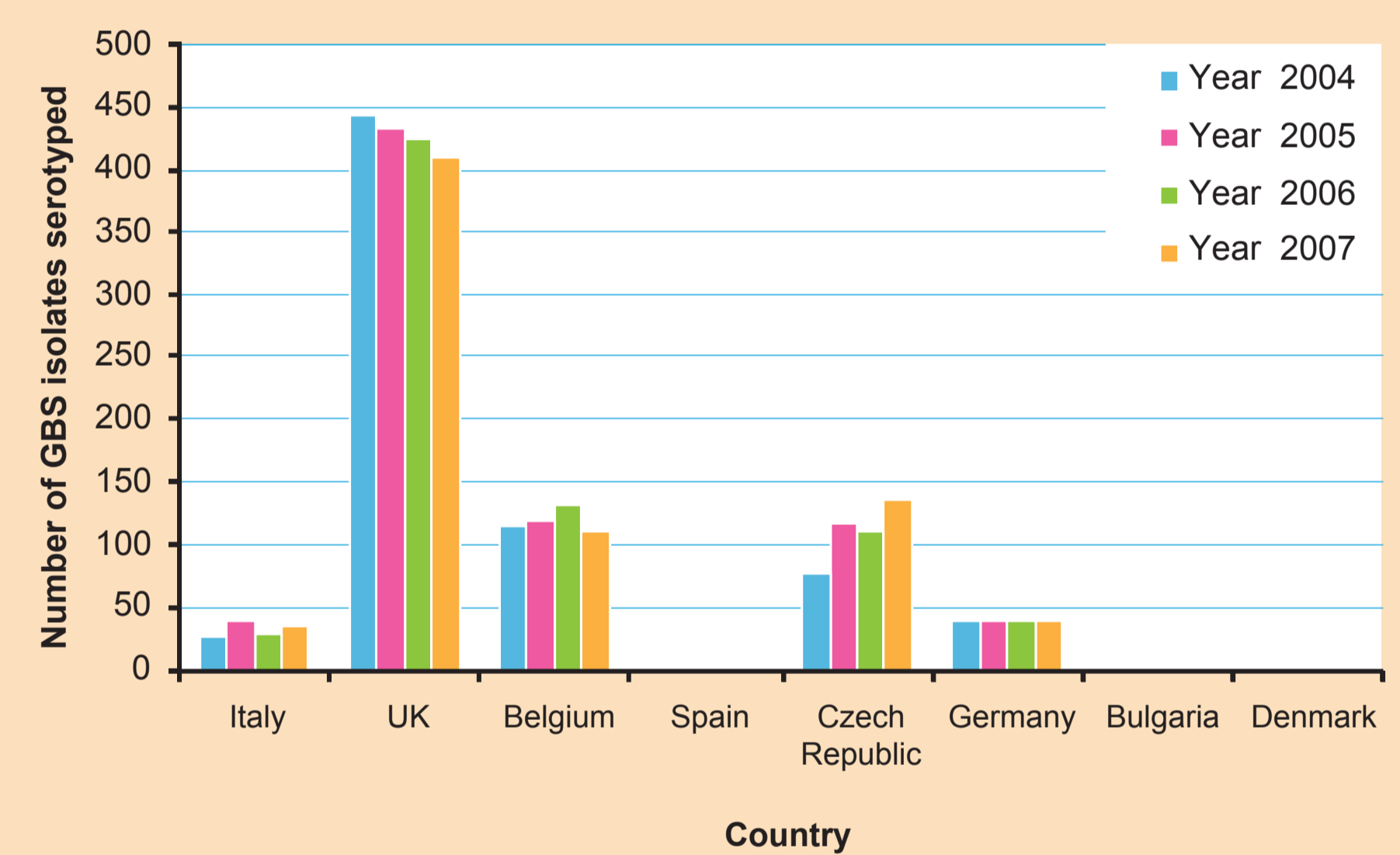


Table 5: Molecular typing methods used for GBS typing

Country	Multiplex PCR assay	MLST	PFGE	Other methods
Italy (ISS)	✓	✓	-	-
UK (HPACf)	✓	✓	✓	VNTR*
Belgium (CHULg)	-	-	-	-
Spain (SAS)	-	-	-	-
Czech Republic (NIPH)	-	✓	-	-
Germany (UKL-FR)	-	✓	✓	-
Bulgaria (NCIPD)	-	-	-	-
Denmark (AU)	-	✓	✓	Molecular Capsular Typing

MLST, Multilocus Sequence Typing; PFGE, Pulse Field Gel Electrophoresis; VNTR, Variable Number Tandem Repeat; *, This method is currently under development.

CONCLUSIONS

There are limited data in Europe on GBS disease and its preventative strategies. In the USA, current guidelines⁵ recommend that all pregnant women should be tested by bacterial culture for vaginal colonization by GBS at week 35-37 of gestation and that culture positive women should be offered antibiotic prophylaxis. Since these guidelines were introduced the rate of EOD has decreased significantly from 1.71/1000 livebirths to 0.34/1000 livebirths (70% reduction)³. In Europe, antibiotic prophylaxis is not widely recommended due to an increase in antibiotic resistance and allergies to penicillin, also recent evidence suggests that the use of penicillin during delivery may cause respiratory distress in the newborn⁶. Therefore, an alternative approach would be to develop a novel conjugate vaccine which would be given to women of child bearing age.

The true burden of GBS disease could be significantly higher than that reported in some European studies. Efforts should be made to improve epidemiological information, and to harmonise microbiological procedures for GBS diagnosis, screening and typing within European countries, in order to ensure that GBS disease incidence and serotype distribution are assessed more accurately and that the best preventative strategy is adopted across Europe.

The questionnaire-based surveillance described here has determined the current laboratory capabilities and status for GBS characterisation as well as potential GBS screening procedures within the DEVANI consortium.

ACKNOWLEDGEMENTS

This project is supported by funding to Baharak Afshar from the European Commission Seventh Framework (grant agreement number 200481). We would like to thank the DEVANI centres for completing and returning the questionnaire.

REFERENCES

- Schuchat, A. (1999) Group B streptococcus. *Lancet*, 353:51-56.
- Heath, PT, Balfour G, Weisner, AM, et al. (2004) Group B streptococcal disease in UK and Irish infants younger than 90 days. *Lancet*, 363:292-294.
- Schrag, SJ, Zywicki S, Farley, MM, et al. (2000) Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med*, 342:15-20.
- Trijals-Smeulders, MA, Kollee, LA, Adriaanse, AH, et al. (2004) Neonatal group B streptococcal infection: incidence and strategies for prevention in Europe. *Pediatr Infect Dis J*, 23:172-173.
- Centres for Disease Control and Prevention. (1996) Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR Recomm Rep*, 45:1-24.
- Moore MR, Schrag, SJ, Schuchat, A. (2003) Effects of intrapartum antimicrobial prophylaxis for prevention of group B streptococcal disease on the incidence and ecology of early-onset neonatal sepsis. *Lancet Infect Dis*, 3:201-213.