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Review

Group B streptococcal epidemiology and vaccine needs in developed countries

Pierrette Melin[a,*], Androulla Efstratiou[b,1]

[a] National Reference Centre for Group B Streptococci, Medical Microbiology Department, University Hospital of Liege, Sart Tilman, B-23, 4000 Liege, Belgium
[b] WHO Global Collaborating Centre for Diphtheria and Streptococcal Infections, Microbiology Services Division, Public Health England, 61 Colindale Avenue, London NW9 5EQ, United Kingdom

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ABSTRACT

Development of a group B streptococcal vaccine (GBS) vaccine is the most promising approach for the prevention of GBS infections in babies, given the potential adverse effects of intrapartum antibiotic prophylaxis as well as the need for effective prevention of both adult and late perinatal disease. There are numerous prevention strategies at this time but none are 100% effective in the eradication of neonatal early onset GBS disease and there are no preventative strategies for late onset disease. The need for a GBS vaccine is therefore, of utmost importance. Efforts applying genomics to GBS vaccine development have led to the identification of novel vaccine candidates. The publication of GBS whole genomes coupled with new technologies including multigenome screening and bioinformatics has also allowed researchers to overcome the serotype limitation of earlier vaccine preparations in the search of a universal effective vaccine against GBS. This review brings together the key arguments concerning the potential need of a GBS vaccine in developed countries and describes the current status with GBS epidemiology and microbiology in these countries.

Contents

* Corresponding author. Tel.: +32 4 366 24 38/39; fax: +32 4 366 24 40.
E-mail addresses: Pierrette.Melin@chu.ulg.ac.be (P. Melin), Androulla.efstratiou@phe.gov.uk (A. Efstratiou).
[1] Tel.: +44 20 8327 7270; fax: +44 20 8205 6528.

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1. Introduction

Emerging abruptly in the 1970s as an important life-threatening pathogen in neonates causing severe invasive bacterial infections, *Streptococcus agalactiae*, also referred to as Lancefield group B streptococcus (GBS) has become a notable global problem [1]. GBS has therefore, remained as a leading cause of neonatal morbidity and mortality in North America, Australia and Europe, affecting 0.5–2.0 neonates per 1000 live births [2–9]. Two distinct clinical syndromes are identified amongst infants according to their age at onset: early onset disease (EOD) presenting with mainly sepsis during the first week of life (0–6 days), and late onset disease (LOD) affecting infants between one week and three months old (7–90 days), with bacteremia and/or meningitis [1–5,10]. In EOD, GBS is transmitted from colonized mothers to the neonate during or just before birth. In industrialized countries, the rate of GBS early onset sepsis reached 3 per 1000 live births, with a mortality rate of 40% from the late 1970s to mid 1990s. From the 1990s to the present, where guidelines for prevention of perinatal GBS disease have been widely implemented, the incidence of neonatal EOD has dramatically decreased to <0.5 cases per 1000 live births but has not been eradicated and continues to be an important cause of neonatal sepsis and meningitis [4,8,9,11–16]. Because many babies with GBS EOD are already septicaemic at birth and thus limiting the opportunity for timely interventions, disease prevention rather than treatment has been the focus of attempts to reduce neonatal GBS infections and disease burden. Selective intravenous antimicrobial prophylaxis with β-lactams administered during labour and delivery to women who are colonized by GBS appears to be the most practical and effective mode of prevention of GBS EOD at this time. The main goal is to reduce or eliminate vertical transmission of GBS to the infant and the risk of perinatal sepsis [2,4,6,8,12]. Since their implementation and evolution, specific policies for intrapartum antimicrobial prophylaxis have significantly influenced the dramatic decrease of the overall GBS EOD incidence [4,9,11–13]. However, prevention of EOD is still subject to much controversy; there has been no consensus amongst European countries, and despite considerable efforts and economic resources spent on prevention of GBS-EOD, cases continue to occur in industrialized countries [1,3,5,6,11,17–19]. Furthermore, none of the strategies of intrapartum antimicrobial prophylaxis for "at risk" pregnant women have any effect on GBS LOD [8,20–22]. New improvements for the current prevention for GBS EOD are urgently required and an alternative strategy for prevention of both GBS early and late onset diseases is still long-awaited.

At the end of the 1970s, Baker and Kasper reported upon the existing correlation of maternal antibody deficiency leading to increased susceptibility to neonatal GBS infection [23]. Therefore, vaccination represented a practical, attractive alternative, targeting women of childbearing age to subsequently protect neonates against GBS EOD or LOD. In 2013, this perspective, which is finally approaching, raises numerous questions. What vaccine type will meet the expectations, a capsule based 3-valent or 5-valent vaccine conjugated or not to specific proteins such as pili? Will a future vaccine replace the current standard of care prophylaxis? What specific surveillance studies are required to establish the pre and post introduction of GBS vaccination for evaluating the impact on colonization, on potential serotype(s) replacement and on GBS resistance to antimicrobial agents? Are there other target populations for such a vaccine as GBS diseases are not restricted to neonates? Indeed, GBS are also common pathogens in pregnant women and are recognized as an ever-growing cause of severe invasive infections in non-pregnant adults, especially amongst the elderly and patients with underlying medical disorders [1,2,4,8,24].

The following review brings together the key arguments concerning the potential need of a GBS vaccine in developed countries.

2. Description of the bacteria and virulence factors

GBS, Gram-positive encapsulated cocci occurring in pairs or short chains, share a common antigen, the Lancefield group B polysaccharide antigen and are further distinguished on the basis of their type-specific capsular polysaccharides (CPS) into ten antigenically unique types (Ia, Ib, II-IX) [2]. The capsule represents one of the major GBS virulence factors, which helps bacterial evasion by interfering with phagocytic clearance except in the presence of type specific opsonophagocytic antibodies [2,10]. A small proportion of "non-typable" strains have also been isolated, currently accounting for 1% of isolates from invasive neonatal infection to 8% of colonizing isolates [25]. On blood agar, GBS colonies are surrounded by a narrow zone of β-hemolysis, however 1–3% of isolates are non-hemolytic. GBS β-hemolysin causes damage to lung microvascular endothelial cells and may contribute to the pathogenesis in EO GBS pneumonia [10,26]. This process may allow the bacteria to gain entry into the bloodstream [10]. All β-hemolytic isolates produce a red-orange pigment, granadaene when cultured under certain conditions [27]. Further differentiation is based upon the presence of surface proteins designated as C, R, X and Rib protein. Another important protein is the surface immunogenic protein (Sip) which is shared by all GBS isolates [28]. Recently, three variant cell surface-exposed filamentous proteins constituting pilus-like structures have been described [29]. GBS pili are composed of three subunits: a backbone pilin protein and two ancillary proteins, a pilus-associated adhesin and a component that anchors the pili to the cell wall. These are encoded by two loci in different regions of the genome, designated pilus islands 1 and 2 (PI-1 and PI-2), the latter presenting two distinct variants, PI-2a and PI-2b. Margarit and co-workers found that all strains of GBS carried at least one or a combination of the three pilus components [30]. These pili are presumed to be important virulence factors, as they appear to play a key role in the specific adherence of GBS to the host epithelial cells and promote transepithelial migration [31,32]. GBS produce other identified factors that interfere with host defenses, such as C5a peptidase that inactivates human complement component C5a, an important neutrophil chemoattractant [10]. Furthermore, cell wall components, such as lipoproteins anchored in the cytoplasmic membrane and lipoteichoic acid, trigger the host's inflammatory response that can induce a sepsis syndrome by activating immune cells via Toll-like receptor TLR2 [10,33].

3. Overview of GBS global epidemiology and disease burden

3.1. Carriage and transmission

GBS is a human commensal, the gastrointestinal tract being the natural reservoir and more likely the source for vaginal colonization [2,4]. At any given time, 10–40% of healthy adults are commonly colonized by GBS in the gastrointestinal and genital tract but remain asymptomatic. Vaginal colonization is unusual in childhood but becomes more common in late adolescence [3]. Among pregnant women, the GBS carriage rate in the vaginal and rectal microbiota ranges from 10% to 37% and is similar in both developing and developed countries [12,17,34,35]. Large variations in colonization rates can be observed and can relate to ethnicity, body sites sampled, microbiological procedures performed and population studied. The site chosen for culture is critical (1): the distal vagina yields GBS more frequently than the cervix, and the collection of both vaginal and rectal swabs results in the optimal detection of carriers. Colonization with GBS can be transient, intermittent or persistent [4,12] and, as it is commonly asymptomatic, the identification of carriers must be performed by bacteriological screening. Irrespective of the preventive strategy for GBS-EOD, the GBS colonization rate among pregnant women usually remains stable over time, and no marked changes have been observed in the distribution of serotypes [12].

During delivery, vertical transmission to the infants occurs in 30–70% of cases. Detection of colonization depends on the timing, the number and specimen sites sampled. Acquisition by the infant occurs either *in utero* by the ascending route through the rupture of membranes, although GBS can also invade through intact membranes, or by contact with the bacteria in the birth canal during parturition [36,37]. Vertical transmission is related to the density of the inoculum present in the mother's genital tract and is more likely to occur with heavy GBS colonization [38]. Recovery of GBS from cultures performed shortly after birth indicates contact of the infant with GBS positive maternal secretions rather than true colonization of the infant. Many GBS "colonized" newborns are asymptomatic and never become infected. Nosocomial transmission or community acquisition also occurs but somewhat infrequently [2].

3.2. Invasive neonatal GBS diseases and risk factors

The clinical manifestations of GBS neonatal infection mainly include bacteraemia, septicaemia, pneumonia, meningitis and skin infections. GBS infections present a bimodal distribution with two distinct syndromes that differ in their clinical presentation, mortality and morbidity, epidemiologic characteristics and pathogenesis [2]. Among meningitis survivors, 15–50% present with late sequelae, which include severe disability, mental retardation, spastic quadriplegia, blindness and deafness [2].

3.2.1. Early-onset GBS disease

Neonatal infections typically occurring within the first six days of birth, are termed early-onset diseases, but in Japan or in Australia, GBS EOD is designated as invasive infection occurring within 72 hours of birth [39,40]. Nearly 90% of infants with GBS EOD present with signs of systemic infection at birth or within the first 24 hours thereafter: fulminant pneumonia, sepsis or, less commonly, meningitis [2,4,10,41]. Without prompt therapy, rapid clinical deterioration typically characterizes EOD. Maternal genital colonization with GBS is the primary risk factor for the disease. Among transiently colonized infants, 1–3% develop a severe disease [2,4,8,12,18,41]. Besides the mother's vaginal colonization with GBS during labour, additional maternal and obstetrical factors described below increase the risk for EOD [2,3,11,19]. The first surveillance studies conducted in North America identified the "classical fives", these are maternal risk factors that are still acknowledged worldwide: (a) GBS bacteriuria at any time during the current pregnancy (2), (b) intrapartum maternal fever, (c) premature rupture of membranes or rupture of membranes 18 or more hours before delivery [3,12,42], (d) preterm labour and delivery prior to 37 weeks of gestation, and (e) a previous sibling with invasive GBS disease [5,43–45]. However, a substantial proportion, up to 50%, of GBS EOD develops in neonates born to mothers who do not demonstrate any of the "classical fives" risk factors [6,34,45].

3.2.2. Late-onset GBS disease

Cases within the second peak of incidence (LOD) occur around one month after birth in infants; with most infections evident between seven and 90 days of age [1–5,10]. Infants with GBS-LOD usually present as bacteremic without any focus and often (nearly 25% of cases) develop meningitis [2,19,46]. Cellulitis and osteoarticular infections may occur but are relatively rare [2,46]. In contrast to EOD, late-onset infection is not always acquired from the mother. Horizontal transmission during the perinatal period may occur from mother to infant or from hospital or community sources. Another reported source of infection is breastmilk [15,38,47,48].

3.2.3. Disease burden and incidence

The case definition for invasive perinatal GBS disease in the majority of developed countries is the laboratory isolation of *Streptococcus agalactiae* from a normally sterile site in infants aged 0–90 days with any signs of clinical disease, for example, sepsis, pneumonia or meningitis. There is however, no official case definition apart from within those very few countries such as Belgium, Canada, Ireland and Spain that have invasive GBS disease listed as a statutory notifiable infection.

Before the introduction of any preventive strategy, the natural burden of GBS EOD comprised 70–80% of neonatal cases and 20–30% of LOD [2,18]. The natural incidence of GBS EOD, before preventive intervention, ranged from 0.5 to >4 cases per 1000 live births with substantial geographical variations.

A systematic review of 74 studies undertaken between 2002–2011 from developed countries in Europe; Czech Republic, Denmark, France, Germany, Italy, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, UK), the Americas (mainly USA), Africa, Eastern Mediterranean (Iraq, Kuwait, Saudi Arabia, Tunisia), Western Pacific (Australia, New Zealand, South Korea, Singapore) and South East Asia by Edmond et al. showed that the mean incidence of GBS in infants aged 0–89 days was 0.53 per 1000 livebirths (95% CI 0.44–0.62) and the mean case fatality ratio was 9.6% (95% CI 7.5–11.8) [49]. The incidence of GBS EOD (0.43 per 1000 livebirths [95% CI 0.37–0.49]) and case fatality (12.1%, [6 2–18 3]) were two-times higher than LOD. A comparison of incidence rates between Europe, the Americas and Western Pacific Regions was 0.57 (CI 0.44 to 0.71), 0.67 (CI 0.54 to 0.80), and 0.15 (CI 0.04 to 0.27) respectively.

The disease incidence varied amongst European countries from 0.00 in Denmark (based on 64,153 live births); 0.21 in Germany (1,454,520 live births) to 2.60 in Slovakia (based upon 6358 live births). The case fatality also varied across Europe with the highest being documented in Norway at 0.33 [CI 0.16–0.55]. Studies that reported use of any intrapartum antibiotic prophylaxis were associated with lower incidence of early-onset group B streptococcus (0.23 per 1000 livebirths [95% CI 0.13–0.59]) than studies in which patients did not use prophylaxis (0.75 per 1000 livebirths [0.58–0.89]).

Surveillance of invasive neonatal infections was undertaken during 2008 –2010 in eight countries (Belgium, Bulgaria, Czech Republic, Denmark, Germany, Spain, Italy, UK) under the auspices of a Pan-European study 'DEVANI' (Design of a vaccine to immunize neonates against GBS infections through a durable maternal immune response) [25,50]. During the study more than 25,000 pregnancies were followed and 188 cases of neonatal disease

Table 1
Global distribution of GBS capsular serotypes in neonatal disease (no separate data for EOD and LOD by Edmonds) [25,49].

Reference		Cases	GBS serotypes (%)									
			Ia	Ib	II	III	IV	V	VI	VII	VIII	IX
DEVANI (2009–2011) [25]	EOD	76	18.4	1.3	5.3	50	2.6	18.4	0	0	0	3.9
	LOD	72	12.5	0	4.2	80.6	0	1.4	0	0	0	0
	Total	148	15.5	0.7	4.7	64.9	1.4	10.1	0	0	0	2
Edmond et al. (2012) based on 19 global studies 1980–2011 [49]	Total	5683	23	7	6	49	1	9	1	<1	<1	

documented; 61% comprised EOD cases and 39% LOD. This was a unique study that provided significant information on GBS epidemiology that impacted on the facilitation of vaccine development and contributed towards a European consensus on GBS neonatal disease prevention policies.

In the Americas, the US Centers for Disease Control and Prevention (CDC) Active Bacterial Surveillance (ABC) report estimated the total disease burden from invasive GBS disease as 24,700 cases in 2010 with an estimated incidence of 8.0 per 100,000 population [13,51].

Reported neonatal case fatality rates for invasive GBS disease are reported to be between 4 and 6% in the US [5] and approximately 10% in the UK [52].

Incidence and case fatality rates were much higher amongst infants who had GBS during 0–6 days in comparison with late infancy (7–90 days). GBS disease can progress quite rapidly and many cases are said to be 'missed' because of difficulties in rapidly obtaining specimens from the babies. This is particularly prevalent in developing countries such as Africa where case fatality rates are high. Incidence and fatality rates varies among countries on the same continent and even between different studies within the same country. Preterm infants are at a 3- to 30-fold greater risk of developing EOD compared to full-term infants, with the highest risk during the lower gestational ages [4,23]. Whilst preterm newborns have a much greater risk of GBS-EOD, about 75% of cases occur among full term infants [6,23].

Further studies are therefore, needed to accurately define the burden of GBS in a wider spectrum of developed countries and also more importantly in developing countries. The global incidence estimated by Edmond and colleagues is probably an underestimate but very relevant as it also correlates with the use of IAP in high-income countries, with the exception of a few such as the UK, Denmark and Bulgaria. IAP has substantially reduced the incidence of EOD since its introduction in the 1990s [13,53].

3.3. Surveillance of GBS in Europe, USA and Australasia

Surveillance data on invasive GBS infections in babies' 0–90 days are collected by many European countries, the USA, Canada, Australia and New Zealand through their national surveillance systems. In the UK for example, data is submitted via a case report to the National Surveillance Centre and or referral of the GBS isolate from a sterile site to the National Reference Laboratory for typing. This is the system used in some European countries but with differing criteria, for example in Belgium reporting is voluntary. In the USA, data are collected through the Active Bacterial surveillance (ABC), which is part of the Emerging Infections Program Network, a collaboration between CDC and the state health departments and universities [51]. Therefore, it is essential particularly with the introduction of potential vaccines that standardized surveillance using a standard case definition is undertaken in countries. It is also essential that invasive group B streptococcal infection in neonates is notifiable. Thus, a Pan-European or global approach would be very beneficial. The DEVANI programme has laid the foundation for standard surveillance within the DEVANI network of countries; this should be maintained and expanded to include other countries within Europe and beyond. Perhaps a pilot network should be introduced comprising key countries that could be involved with the vaccine(s) developments and efficacy trials. This is of course subject to further discussion and subject to appropriate funding being made available.

3.3.1. Serotype distributions and molecular characterization

Whilst in some geographic regions, notably the USA, the distribution of GBS serotypes among currently circulating strains is well studied and even under active surveillance, consistent data on serotypes causing invasive disease that are circulating in some European countries are still not well defined. The DEVANI study however, contributed significantly towards the characterization of invasive disease isolates from the eight countries. As expected, serotype III predominated amongst all neonatal infections. This was also the situation in several other European countries such as Finland [54], Poland [55], Portugal [56], Sweden [57] and France [58].

The overall global serotype estimation undertaken by Edmond and colleagues in their systematic review showed an overall prevalence of five serotypes, Ia, Ib, II, III and V; these accounted for more than 85% of serotypes in all global regions with serotype data (Americas 96%, Europe 93%, Western Pacific 89%). Serotype distribution by disease documented 37% of EOD serotypes were type III in contrast to 53% of LOD serotypes. Overall, the global distribution of serotype III accounted for 48% of isolates in the 1980s, 49% of isolates in the 1990s and 50% of the isolates in from 2000–2010. There were no significant changes in the serotype distributions during the 2002–2011 review periods [49] (Table 1).

Further characterization of GBS strains based upon molecular technologies has enhanced epidemiological studies even further, for example, in recent years multilocus sequence typing has been used for population based epidemiological studies and more recently microarrays and high throughput sequencing. These methods clearly demonstrated the emergence of a specific clone, the clonal complex CC17, which is strongly associated with neonatal meningitis and hence designated the 'hypervirulent clone' amongst GBS of serotype III [59,60]. The capsular polysaccharide (CPS) is a major virulence factor amongst GBS and of course a vaccine target. However, population based studies showed no definitive correlation between CPS type and MLST cluster, with the unique exception of the hypervirulent GBS clone CC17, which were all until very recently type III. MLST and also pulsed field gel electrophoresis on a collection of invasive neonatal strains isolated in France demonstrated for the first time, capsular switching from CPS type III to IV within the homogenous clonal complex CC17. Further characterization revealed that the switch was due to the exchange of a 35.5 kb DNA fragment containing the entire cps operon. This therefore, showed that CC17 hypervirulent strains have switched one of their main vaccine targets. This is highly relevant not only for vaccine development but also for monitoring vaccine efficacy and type distributions during the introduction of a vaccine into the population [60]. Data from the DEVANI study showed that the GBS population in pregnant women (carriage) was much more heterogeneous than neonatal infections using MLST thus demonstrating epidemiological differences between invasive strains and

colonization. A longitudinal study was undertaken on strains over an 18-year period in Spain (1992–2009) to document changes in the prevalence of serotypes, antimicrobial resistance, and genetic lineages and to evaluate their associations with either EOD or LOD. Despite the introduction of prophylaxis, which resulted in a significant decrease in EOD, the study revealed a very stable clonal structure of GBS causing neonatal infections in Spain during the 18-year period [56].

Other important virulence associated structures such as pilus proteins have also been used to characterize GBS strains from neonatal infections by several groups. Findings by Margarit et al. (2009) showed that all GBS isolates contained at least one and often two islands which confirmed the main role of pili in the pathogenesis of GBS, thus making these proteins highly desirable vaccine candidates [30]. Identification of the pilus gene profiles and expression of the proteins was determined amongst a subset of strains from the DEVANI collection. Isolates were examined by PCR and FACs analysis to evaluate gene presence and surface exposure of pili. All isolates contained at least one gene encoding for pili [61]. Also, CPS serotypes were genetically linked to pilus islands and the same allele is shared by different capsular serotypes. This suggested that CPS serotypes most likely evolved after pilus divergence.

Martins and co-workers assembled a collection of 898 isolates associated with carriage in pregnant women, noninvasive disease among nonpregnant adults, and invasive disease from neonates and adults [62]. All isolates were characterized by serotyping and PFGE, and half the collection including representatives of all main PFGE-based genetic lineages, was characterized by MLST. In addition, all isolates were tested for the presence of alpha and alpha-like surface protein genes (*alp*) and genes encoding the pilus proteins (PI-1, PI-2a, and PI-2b loci). At least one PI was present in all isolates. The results indicated that a vaccine including components from PI-1 and PI-2a could provide potential coverage against 99.4% of isolates, supporting their use in a future vaccine as previously suggested.

3.3.2. Serological studies

There had been no systematic studies on the prevalence of serum antibodies against capsular polysaccharide in women in Europe prior to DEVANI, although two large studies were reported on women in the USA in recent years. From these studies, the amount of maternal antibody that correlated with protection of the newborn was defined for serotypes Ia, III and V. Baker and colleagues described the immune response during the third trimester of pregnancy using a type CPS glycoconjugate vaccine and demonstrated placental transfer of antibodies in concentrations sufficient to protect neonates and young infants from invasive disease [63]. Studies in the USA on adults showed that they responded to immunization and retained peak antibody concentrations of at least 40–60% for 18–24 months after immunization [64].

The DEVANI programme collected sera from pregnant women (women with healthy babies and those with infected babies) for determination of antibodies against CPS and the pilus proteins. A definitive correlation between high titres of maternal anti-CPS antibodies and reduced risk of neonatal disease was demonstrated for three serotypes (Ia, Ib and III). No statistically significant correlation for antibodies to pilus proteins was detected. There was a clear statistical and significant difference between the serum titres of mothers with infected babies in comparison to mothers of healthy babies for serotypes Ia and III. This was also documented from the antibody responses to the pilus proteins (DEVANI) in these women.

3.3.3. Antimicrobial resistance

Penicillins including penicillin G are the first line drugs of choice for intrapartum antibiotic prophylaxis and for treatment of *S. agalactiae* infections either in infants or adults since all GBS isolates are considered to be uniformly susceptible to all β-lactams. Globally, GBS clinical isolates remain fully susceptible to penicillin as well as to most β-lactams, with the exception of the emergence of very rare isolates with a decreased susceptibility to penicillin as recently reported in Japan and USA [65]. All these GBS strains with reduced penicillin susceptibility, exhibiting increased penicillin MICs (0.25 to 1 mg/L), have been characterized genetically by altered *pbp2X* genes subsequently to multiple mutations [66]. Even if still uncommon, this phenomenon raises concern for the future with the risk of increased prevalence of GBS isolates with reduced susceptibility to beta-lactams as for *Streptococcus pneumoniae* which occurred during the 1970s and spread globally, followed by increased levels of resistance involving stepwise accumulations of alteration in three penicillin binding proteins (PBPs).

Currently of more concern is the resistance to macrolides and lincosamides which has increased worldwide amongst GBS over the last two decades: from <5% to a common resistance of 20% to 35% or even 54% for erythromycin and 43% for clindamycin in a New York hospital as recently published [13,67,68]. In Belgium, as determined by the National Reference Centre for GBS, macrolide resistance increased from 10.4% in the early 2000s to 33% among invasive strains isolated from 2008 to 2011 [67]. These figures are consistent with similar reports from Europe [21,69–72], North America [15,68,73–76], and Asia [77,78], except some surveillance studies in Sweden reported <10% [79], thus showing some geographical differences. Different and known mechanisms account for the acquired resistance to macrolides in streptococci [80]. The most prevalent of these is target site modification by 23S rRNA methylases, commonly encoded by the *erm*B and *erm*A subclass *erm*TR genes. The Erm enzymes confer resistance to macrolides and inducible or constitutive resistance to lincosamides and streptogramin B, so-called MLS_B phenotype. Another mechanism involving active drug efflux, is encoded by the *mef*A and *mef*E genes (which are 90% identical); the Mef pump only affects 14- and 15- membered ring macrolides but not 16-membered macrolides, neither lincosamides nor streptogramin B (M phenotype). This increased resistance to macrolides has been reported amongst all *Streptococcus species* [80] and is not specific to GBS strains. Apart from this worrying resistance, a further phenotype involving low-level clindamycin resistance (with erythromycin remaining susceptible) in GBS isolates has recently been reported in the USA, Canada, New Zealand, Asia and Argentina: the L phenotype encoded by *lnu* (formerly *lin*) genes [73,74,77,81–85]. These genes encode for the nucleotidyl-transferase. Macrolide and lincosamide resistances are unevenly distributed among the different capsular serotypes of GBS strains [15,21,70,77]. For instance, erythromycin resistance is more likely to occur in serotype V and the efflux resistance mechanism shows a significant association with serotype Ia [69,70,76,86]. This increase of macrolide and lincosamide resistance rates stresses the importance of performing susceptibility testing for GBS strains isolated from antenatal screening specimens.

Most human GBS isolates are considered to be highly resistant to tetracyclines. The tetracycline resistance mechanisms involve protection of the ribosomal target. The *tetM* gene encoding ribosomal protection proteins is predominant amongst human isolates whilst the *tetO* gene is predominant among bovine isolates [87].

In recent years, the emergence of fluoroquinolone resistance has also been reported in Asia [88,89]. In 2012, two reports of concern from Japan described high frequency fluoroquinolone and macrolide resistance among clinically relevant GBS with a reduced penicillin susceptibility [90] and, the nosocomial spread of multidrug resistant GBS strains with reduced penicillin susceptibility in a general hospital [91].

Therefore, the threat of spread of these different emerging and resistant strains should trigger awareness of the appropriate therapeutic strategy for dealing with severe GBS infections and the strategy for intrapartum prophylaxis for GBS carriers. These strains

may present future public health challenges. Resistance surveillance is mandatory to guide prophylaxis and treatment of serious GBS infections but also to identify newly acquired resistance mechanisms.

3.4. Genomic approaches to surveillance

Whole genome sequencing (WGS) of GBS strains has concluded that the classic serotyping scheme based upon the CPS does not reflect their genetic diversity. Strains that belong to different serotypes can be more closely related than strains of the same serotype. This information will surely impact upon vaccine development and a larger collection of strains from carriage and infected neonates need to be examined, this is work that is currently underway between key research groups; Public Health England; The Sanger Centre; Novartis and the 'DEVANI network' (Pers comm.A.Efstratiou). The publication of the genome sequence for a GBS strain representing ST17 isolated from a colonized woman at eight weeks post partum was an invaluable addition to the published genomes and has promoted comparative genomic studies on GBS [92].

4. Prevention strategies of GBS neonatal infection by chemoprophylaxis

Since the late 1980s, huge efforts have been made to prevent and treat GBS disease in infants and pregnant women. Two approaches have been suggested for prevention of neonatal infection: chemoprophylaxis to reduce maternal GBS colonization and thus prevent transmission to neonates [41,93–95], and immunoprophylaxis to induce protective immunity in mothers and infants [2,3].

4.1. Intrapartum antibiotic prophylaxis (IAP)

Intravenous IAP has been proposed and has evolved after the demonstration by clinical trials performed in the late 1980s that GBS-EOD might be prevented by administering antibacterial prophylaxis during labour and delivery to women who are colonized by GBS [4,41]. Selective intravenous antimicrobial treatment with β-lactams turned out to be the most practical and effective mode of prevention [3,4,6,41]. In 1996, the first consensus guidelines were issued and further revised in the United States [2,6,13]. Very similar national guidelines were also issued in some European countries, Australia, New Zealand and Japan [34,39,96–103]. Whilst the value of IAP in at-risk women is widely agreed, the best means of targeting these women is less clear. There are two options to targeting prophylaxis, identification of carriers by late antenatal cultures or treating by identification of clinical risk factors.

In general, the screening approach recommends that all pregnant women should be screened between 35 and 37 week's gestation for GBS vaginal and rectal colonization. At the time of labour or rupture of membranes as early as possible, intrapartum antibiotics should be given to all women tested positive for GBS colonization or with GBS isolated from the urine at any time during the current pregnancy or with a previous sibling with invasive GBS disease. For women with a negative GBS result, no prophylactic antibiotic is recommended. If any screening result is available at the time of labour and delivery, IAP should be given to women who are <37 week's gestation, have a duration of membrane rupture $\geq$18 h, or have a temperature of $\geq$38 °C. In the risk-based approach, intrapartum antibiotic prophylaxis is given according to the presence of any of the above-mentioned five risk factors. Both strategies have their advantages and drawbacks. The risk-based approach avoids routine screening, is easier and potentially less expensive, and is particularly useful in settings in which women might receive little or no prenatal care. But up to 50% of women with risk factors are not all colonized with GBS leading to unnecessary treatment [104] and many infants with GBS EOD are born to mothers who had not showed any of the recognized risk factors leading to missed opportunities [34,46]. In 2002 in a large retrospective cohort study, the screening-based approach was found to be the most effective appropriate option and was greater than 50% more effective [5], but concerns about its complexity are a barrier to its wider implementation. One of these concerns is the speed and accuracy of prenatal screening culture, which has to be performed 3–5 weeks before the expected delivery. Even with optimized methods, such antenatal screening cannot identify all GBS carriers at time of delivery due to intermittent colonization. The potential area for improvement would be use of a rapid sensitive and accurate test, performed at the bedside of women admitted in labour and for delivery. They would allow a better management of colonized mothers and their infants as the targeted IAP would then be limited to GBS carriers, reducing unnecessary treatment. Currently, there are GBS-specific real-time PCR assays that provide this potential alternative for rapid GBS detection [105,106]. However use of these more expensive rapid tests is not currently widespread.

In the late 1990s, when prophylaxis was offered on the basis of risk factors, very few practitioners used all risk factors appropriately [5,107,108] suggesting that a significant proportion of health providers were unaware of the epidemiolology of GBS EOD or do not perceive GBS EOD as an important clinical problem.

Other diverse strategies to reduce maternal colonization and vertical transmission have been studied; but, none could prove effective for prevention of GBS EOD [13,109]. Currently, intravenous administration of penicillin G, being the first choice, or ampicillin, at least 4 hours before delivery, have proven effective for prevention of GBS vertical transmission and are recommended [2,13,41,110], but shorter duration might still provide some protection [111,112]. The dosages of penicillin or ampicillin used for IAP are aimed at achieving adequate levels in the foetal circulation and amniotic fluid rapidly [113,114]. Alternatives for penicillin allergic patients include cefazolin, clindamycin and vancomycin according to the type of allergy and to susceptibility results.

4.2. Different guidelines for GBS neonatal prevention

Today, most developed countries have guidelines for the prevention of GBS perinatal disease. According to countries, these are issued by public health authorities or by professional societies potentially resulting in different levels of implementation. In the United States for instance, the last updated version of the guidelines issued by CDC in 2010 have been endorsed by several societies including the American College of Obstetricians and Gynaecologists, the American Academy of Paediatrics and the American Society of Microbiology. They recommend universal antenatal screening at 35–37 weeks' gestation and use of intrapartum antibiotic prophylaxis. The screening based approach, with some variations, is also recommended in Italy (1996), Germany (1996, 2008), Spain (1998, 2003, 2012), France (2001), Belgium (2003, 2013), Switzerland (2007), Japan (2008, 2011) and Poland (2008) [34,96–98,103]. A risk-based approach is recommended in guidelines from the Netherlands (1999, 2008), Australia, Denmark, United Kingdom and New Zealand [50,99–101].

4.3. Antenatal screening culture, variability and predictive value

To improve the screening-based strategy, increased sensitivity and high predictive values for GBS vaginal colonization at delivery is mandatory. The crucial criteria to control are the specimen, time of collection, transport conditions and microbiological procedure for detection. Currently, pregnant women should have antenatal

follow ups and the screening result must be available to the obstetric team at the time of delivery.

4.3.1. Specimen

As vaginal colonization originates in the proximity of the recognized and presumed reservoir, the rectum; numerous studies have demonstrated that predictive values obtained with a low vagino-rectal swab substantially increase the yield of culture compared to a single genital swab [6,13,115,116]. Since the first guidelines were launched by CDC in 1996, a vagino-rectal swab has always been recommended; collection from the rectum has been much more difficult to implement in some European countries and Australia even if recommended, as ten years ago in Belgium for instance, the collection of a rectal swab was regarded as a "taboo" topic (Ref., personal data). In Australia, as reported in a survey set in the late 1990s, only one of 84 hospitals collected from both sites [107]. It has been suggested that Australian women would find collection of anal swabs unacceptable [107]. For other countries for example, France, there is still disbelief concerning the added value of vagino-rectal collection for screening when compared to a single vaginal collection, therefore only a vaginal swab is currently recommended for antenatal screening [97].

4.3.2. Transport conditions

For support of GBS viability during the transportation of specimens to laboratory, Amies or Stuart media have been widely recommended by the various guidelines worldwide. But the criteria differs between countries, particularly the accepted duration for storage and transport which varies from one day [96], to 24–48 h [34] and up to 1–4 days [13] at 4 °C. Several studies showed the negative impact of the length of time that has elapsed between collection and inoculation to the recovery of GBS [117]. The elapsed time before processing the swab is crucial upon GBS viability, hence the sensitivity of a positive culture decreases significantly for specimens not inoculated within 24 h of collection and could explain the false negative results especially for specimens collected from women with a low degree of colonization.

4.3.3. Timing

GBS carriage using vagino-rectal cultures could be detected as early as 26 to 28 weeks gestation but the stability of carriage is questionable. Early detection could be advantageous for prevention of GBS EOD disease in premature deliveries [41]. Since GBS colonization is dynamic, its status can change during pregnancy and the observed predictive values of antenatal screening cultures performed too early have been shown to be very low. Therefore, the closer to delivery that bacteriological screening is undertaken, the greater its utility, as sensitivity and specificity are both increased [3,118]. Based on Yancey's study and also to improve predictive values, collection for antenatal screening was further recommended between 35 and 37 weeks and has been used as a surrogate marker for intrapartum GBS colonization [2,34,96,102,119]. In France, the ANAES recommends an extended period for screening, between 34 and 38 weeks' gestation [97] and in Japan the recommendation is between 33 and 37 weeks' gestation [103]. The 35–37 weeks' gestation period for screening has increased predictive values but is still not perfect. A substantial number of positive mothers for GBS at delivery, (up to 30%), are not identified as carriers and up to 25% of women identified as GBS carriers are no longer positive at the time of delivery [5,120].

4.3.4. Specimen processing

Since the first studies, for GBS carriage, inoculation of a selective enrichment broth incubated overnight and further subculture was shown to be superior to culture performed by direct plating of the specimen. In the 1996 CDC guidelines, the screening-based strategy for prevention of GBS perinatal disease recommended the inoculation of a selective enrichment broth such as Lim or Transvag broth (Todd-Hewitt broth supplemented either with colistin and nalidixic acid or with gentamicin and nalidixic acid) and further subculture onto sheep blood agar with/without colistin and nalidixic acid [3,6,13,34,102], however, many other countries do not recommend the selective enrichment step [97]. Since then, several selective differential media for GBS have been developed and recommended in Europe [34,96]. The first differential agar is the Granada medium formulated by Manuel de la Rosa from Spain [121]. This medium, incubated in an anaerobic atmosphere, allows the selective growth of GBS and its differentiation by enhancing the production of the orange pigment specific to β-hemolytic GBS. All orange colonies growing on this medium are GBS, but non-hemolytic colonies of GBS appear greyish-white and cannot be differentiated from other commensal bacteria like enterococci. Nevertheless, use of this medium has significantly improved culture for GBS screening when compared either to direct plating or subculture onto blood agar. Granada like agar has been used and recommended for more than 10 years at least within the Spanish and Belgian guidelines [34,96]. In 2005 and 2007, two chromogenic media, StreptoB ID (bioMérieux, France) and StreptoB select (Biorad, France), became available for the selective differential growth of GBS [122,123]. They compare well to Granada agar and are superior to blood agar with or without nalidixic acid; StreptoB Select has shown to be slightly more sensitive and both are less specific than Granada agar [123]. The identification of all presumptive GBS on chromogenic media as on blood agar must be confirmed. Chromogenic media have been used in Europe for the last few years. In the USA, the revision of the guidelines launched in 2010 by CDC recommended the use of Granada like media as well as chromogenic agar for sub-culture from incubated selective enrichment broth [13]. The methods for GBS screening have been improved but nonetheless cases still occur due to "false" negative screening. These false negative predictive values for GBS vaginal colonization at the time of delivery can be due to either new acquisition of GBS after screening or to false negative culture. Failure to culture GBS, unrelated to culture process, can be associated with the length of transportation or to oral antibiotherapy taken before screening or to feminine hygiene. To a great extent these false negatives could explain a substantial number of the cases; on the other hand, the false positives or changes in GBS colonization status in women before delivery can promote unnecessary intrapartum antibioprophylaxis.

4.4. Non-culture methods for antenatal screening

To improve both positive and negative predictive values for screening, several rapid tests have been developed aimed at real time assessment of intrapartum vaginal GBS colonization. Several studies have also confirmed the expected benefits of using a reliable, highly sensitive rapid test resulting in an increase of targeted IAP whilst reducing the number of unnecessary IAP. In addition to high sensitivity and specificity and to allow for timely IAP, a short turnaround time which should not exceed one hour from ward to bench should be included and the test should be available 24 h a day, seven days a week. During the last two decades, rapid tests were developed with minimal success and uptake until very recently. The first generations included optical immunoassays and enzyme immunoassays. Although they had good specificity, they showed disappointing performance with low sensitivity, which only increased with heavy colonization; hence a negative test could not rule out GBS colonization [124].

With the advances in the polymerase chain reaction (PCR) and fluorescence labelling technologies providing new detection

platforms for bacterial identification [105], a new generation of tests have now become available. Currently, real-time PCR-based tests (or NAAT–Nucleic Acid Amplification Test) can equal or surpass the sensitivity of antenatal culture at 35–37 weeks' gestation and compare favourably with the gold standard of enrichment followed by subculture for the detection of GBS colonization at presentation for delivery [13,17,105,125,126]. As far as the use of available NAAT for GBS after enrichment step increases sensitivity up to 92–100%, they could be used for antenatal screening at 35–37 weeks' gestation. Currently, despite the availability of NAAT for GBS through real-time PCR, their utility to assess intrapartum GBS colonization and thus to avoid antenatal screening remains limited. At least, they offer the potential for GBS detection among women without antenatal care or among those in whom no antenatal culture was performed. Among the available tests approved by the Food and Drug Administration (USA), the Xpert™ GBS (Cepheid), can yield results in 30 to 45 minutes and is characterized by an extremely low workload. It is simple enough for even inexperienced technicians to perform. However, use of this relatively new and more expensive technology is not currently widespread among European hospitals and must still be assessed for its use in the screening-based strategy. Haberland & Benitz's cost analysis suggested that rapid simple NAAT benefits exceed their costs in Europe, one of the first experiences in a clinical setting has showed its superiority for prevention of perinatal GBS early onset disease at a neutral cost when compared to the vaginal antenatal cultures performed at 35–37 weeks gestation and has been implemented as unique screening in term deliveries [106]. Further studies using real-time NAAT performed in intrapartum setting are needed to identify targeted population and settings where the test will be most useful. To avoid one important drawback of these tests, an expected improvement would be the combined detection of GBS and of markers for resistance to clindamycin, in order to guide the appropriate IAP for penicillin-allergic women at high risk of anaphylaxis.

4.5. Drawbacks of use of antibiotics and unintended consequences

With the successful implementation of IAP strategies, concerns arise regarding the increased use of antimicrobials among pregnant women in labour resulting in the exposure of a large number of women and infants to possible adverse effects without benefit [127]. Maternal anaphylaxis associated with IAP occurs but is very rare; since the issue of the first guidelines in the United States in 1996, only four reports of non fatal cases have been published [13]. There is no risk of anaphylaxis resulting from IAP in the foetus or newborn as they are unlikely to have had previous exposure to the antibiotic and because maternal IgE antibodies are not transmitted across the placenta [13]. Therefore, might there be adverse effects such as increased incidence of Gram negative or drug–resistant neonatal sepsis? Even though limited studies have been controversial, global surveillance has not confirmed these fears and these studies are not of sufficient magnitude to outweigh the benefits of IAP to prevent GBS EOD [5,9,11–13,18,128,129]. As reported by Tanaka, infants' antibiotic exposure through maternal IAP might influence the development of neonatal intestinal microbiota [130]. Continued surveillance of neonatal sepsis is needed to monitor changes in neonatal pathogens, antibiotic resistance profiles and antibiotic use.

Another cause of anxiety is how IAP might affect the clinical presentation of GBS-EOD and could prevention efforts impair the ability to diagnose the disease? Several studies have found that the pattern of GBS EOD was not affected by exposure to IAP [131].

The widespread use of GBS IAP raises concern about the development of antibiotic resistance among GBS isolates. GBS continues to be susceptible to β-lactams and the exceptions of the few cases reported in Japan and in the United States [13]. However, the clinical significance of the elevated MICs to penicillin observed in those strains remains unclear [13]. The increasing resistance to clindamycin is more worrying for choosing an alternative agent for IAP in penicillin allergic patient but is not associated to IAP. Thus, antimicrobial susceptibility testing of GBS isolates is crucial for selection of the appropriate antimicrobial agent for intrapartum prophylaxis in penicillin allergic women at high risk for anaphylaxis.

5. Immunoprophylaxis to prevent GBS neonatal disease

Vaccination represents the most attractive strategy for GBS disease prevention. As anti-CPS IgG concentrations in infants are inversely correlated with their risk of developing GBS EOD and LOD [23], a long awaited alternative to chemoprophylaxis is immunization, targeting women of childbearing age to subsequently protect neonates against GBS infection. Furthermore GBS vaccine might prevent a broad range of GBS associated diseases such as EOD, LOD, miscarriage, stillbirth and maternal infection [2,4,12,13,18,19,23,30,132]. During the last two decades, the first generations of vaccine targeting serotype CPS have been extensively studied. Phase 1 and phase 2 clinical trials among healthy adults have demonstrated safety and immunogenicity of monovalent or multivalent, uncoupled CPS or conjugated vaccines against GBS types Ia, Ib, II, III and V [2,51,63,64,131–138]. Today, CPS-based vaccines are in advanced stage of development but are not yet licensed. The development of such effective GBS vaccine with global relevance has been hampered by changes in the distribution of GBS serotypes of clinical strains in different continents and time periods [139]. Meanwhile, aiming to overcome the type-specificity, efforts have been directed towards the identification of highly protective protein antigens. A new generation of vaccines targeting conserved surface proteins are considered the best candidates against GBS infection. Antibodies directed against these surface antigens can interfere with streptococcal virulence factors. Before 2005, among known surface proteins, only C5a peptidase and Sip were expressed at the surface of all GBS isolates and were identified as potential vaccine candidates [28,140,141]. Thanks to reverse vaccinology, a new approach built on genome-based antigen discovery using integration of several techniques such as genomics, bioinformatics and molecular biology [142], has allowed the discovery of pilus–like structures on GBS surface [143]. Studies by Maione et al., Rosini et al. and Margarit et al. confirmed that GBS pili protein components, a combination of three proteins, could represent valid candidates for the development of the most interesting serotype independent vaccine. Furthermore a pilus-based vaccine could also be useful in preventing GBS colonization, by interfering with bacterial adhesion to host cells and by inhibiting GBS biofilm formation [144,145].

The most promising route towards a universal GBS vaccine would be a combination of glycoconjugates representing the most diffused serotypes combined with a mix of highly protective pilus proteins.

6. Future perspectives and needs

Development of a GBS vaccine is the most promising approach for the prevention of GBS infections in babies, given the potential adverse effects of intrapartum antimicrobial prophylaxis as well as the need for effective prevention of both adult and late perinatal disease. There are numerous prevention strategies at this time (IAP; screening; or 'do nothing') but none are effective in the eradication of neonatal EOD GBS disease and there are no preventative strategies for LOD. The need for a GBS vaccine is therefore, of

utmost importance, but numerous questions arise, particularly for developed countries. Will the vaccine completely replace standard of care prophylaxis? Which vaccine formula/structure would be most effective i.e.: the 3-valent or the 5-valent conjugate vaccine. The efficacy of the 3-valent vaccine will be established in the forthcoming Phase III clinical trials. The optimal strategy is to ensure that protective levels of antibody are present at delivery. Efforts applying genomics to GBS vaccine development have led to the identification of novel, highly conserved protein antigens that are expressed on the bacterial cell surface, for example the pilus-like structures on the surface of GBS are also potential and important candidates. The publication of GBS whole genomes coupled with new technologies including multigenome screening and bioinformatics has allowed researchers to overcome the serotype limitation of earlier vaccine preparations in the search of a universal effective vaccine against GBS [143].

For the introduction of any GBS vaccine there are urgent needs for pre and post vaccine enhanced surveillance studies in countries. A Pan-European surveillance would be recommended but this may prove difficult as GBS invasive disease is not notifiable in the majority of developed countries. Perhaps a system similar to the ABC undertaken in the USA would suit the majority of countries in addition to awareness campaigns which are all at a huge economic cost to the health services within each country. It is difficult to predict the impact of a CPS or protein vaccine on the GBS population. This therefore strengthens the case for a robust and standardized global surveillance of GBS in particular during the phase 3 or 4 clinical trials.

7. Conclusions

The data in the literature from developed countries strongly indicate that a conjugate vaccine incorporating five serotypes (Ia, Ib, II, II, V) could prevent more than 85% of global GBS disease in infants ≥90 days. The type distributions in Europe, the Americas, Western Pacific and even some parts of Africa and the Eastern Mediterranean have not changed within the last 30 years. This is in contrast with the regional variation observed with other vaccine preventable diseases [146,147]. Standardized surveillance, improvements in GBS diagnostic techniques, treatment and management coupled with widespread implementation of a GBS vaccine would substantially reduce the global burden and would also contribute towards a reduction in prematurity and still births.

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