ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Review Article

Group B *Streptococcus*: Vaccine Development Art

Abeba Mengist*

Department of Medical Laboratory Science, College of Health Science and Medical Sciences, Debre Markos University, Debre Markos, Ethiopia

***Corresponding Author:** Abeba Mengist, Department of Medical Laboratory Science, College of Health Science and School of Medicine, Debre Markos University, Debre Markos, Ethiopia.

Received: December 07, 2017; **Published:** February 17, 2018

Abstract

Group B *Streptococcus* (GBS) is a cause of neonatal invasive diseases as well as of severe infections in the elderly and immune-compromised patients. Despite significant advances in the prevention and treatment of neonatal disease, sepsis and meningitis caused by GBS still represent a significant public health care concern globally and additional prevention and therapeutic strategies against infection are highly desirable. The introduction of national recommended guidelines in several countries to screen pregnant women for GBS carriage and the use of antibiotics during delivery significantly reduced early-onset disease, but it has had no effect on the late-onset diseases occurring after the first week and is not feasible in most countries. In addition, there is no current strategy for preventing GBS disease among elderly and immunocompromised, non-pregnant adults. The development of GBS vaccines with efficacy across serotypes may address many of the clinical gaps left by GBS intrapartum antibiotic prophylaxis. New technologies and innovative approaches are being used to identify GBS antigens that overcome serotype-specificity and that could form the basis of a globally effective vaccine against this opportunistic pathogen. This review provides an overview of the burden of invasive disease caused by GBS in infants and adults and highlights the strategies for the development of an effective vaccine against GBS infections.

Keywords: *Genomic and Gene-Expression Approaches; Group B Streptococcus; Proteomic Approach*

Abbreviations

ALP: Alpha-Like Proteins; CDC: Centers for Disease Control and Prevention; CPS: Capsular Polysaccharides; CSP: Cell Surfaces Protease; EOD: Early Onset Disease; GBS: Group B *Streptococcus*; IAP: Intrapartum Antibiotic Prophylaxis; IGG: Immunoglobulin G; IGM: Immunoglobulin M; LOD: Late Onset Disease; LRRG: Leucine-Rich Repeat Protein; OCT: Ornithine Carbamoyl Transferase; PGK: Phosphoglycerate Kinase; PGN: Peptidoglycan; SIA: Sialic Acid; SIP: Surface Immunogenic Protein; ST17: Sequence Type 17; TT: Tetanus Toxoid; WHO: World Health Organization

Introduction

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is the most frequent pathogen isolated from neonates with invasive bacterial disease and responsible for serious infections in newborns such as pneumonia, septicemia and meningitis [1,2]. GBS is also associated with significant maternal peripartal disease, including bacteremia, endocarditis, chorioamnionitis, endometritis, urinary tract infections, arthritis, and responsible for serious bacterial illness and deaths in non-pregnant women with underlying diseases and in elderly adults [3,4]. GBS can also pass through the cervix without causing serious cervicitis, and cross-intact amniotic membrane causing amnionitis thereby infecting the foetus within the uterus [3].

However, GBS is also a commensal organism able to primarily colonize the urogenital and gastrointestinal tracts of more than 30% of the healthy population and, in particular, 25 - 40% of healthy women are asymptomatically colonized. It was estimated that, if 20 - 30% of pregnant women are colonized with GBS, approximately 50% of their infants become colonized and approximately 1% of these infants develop disease [5,6].

Multiple trials have demonstrated that the use of intrapartum penicillin or ampicillin significantly reduces the rate of neonatal colonization with GBS and the incidence of GBS early-onset disease (EOD). Since 1996, the Centers for Disease Control and Prevention (CDC) has endorsed the use of intrapartum antibiotic prophylaxis (IAP) for the prevention of neonatal GBS EOD, based on the presence of specific risk factors and/or on the results of antepartum screening of pregnant women for GBS colonization [7]. The CDC revised recommendations for GBS IAP in 2002 and again in 2010, and used multistate surveillance to assess the impact of GBS recommendations. Although these two approaches, often used in combination in clinical practice, have significantly reduced the incidence of neonatal GBS sepsis and meningitis, invasive disease due to GBS infection remains the leading cause of neonatal morbidity and mortality [8,9]. In addition, these measures are unlikely to prevent late-onset disease (LOD), prematurity, and stillbirths related to GBS, while obviously not addressing GBS disease in non-pregnant adults. Indeed, GBS is also a frequent cause of infections in pregnant women and in clinically ill and older adults, such as those suffering from diabetes, cirrhosis, malignancies, and immunodeficiency [10] with a reported increase in macrolide resistance in clinical isolates of GBS, efforts have shifted toward vaccination as an attractive alternative for disease prevention [8]. Therefore, the best long-term solution to prevent GBS disease is the development of an effective vaccine that could be administered to all adults, a strategy that would alleviate the limitations of IAP [11]. This review discusses the microbiologic and immunologic considerations regarding GBS vaccine development, reviews clinical trials completed to date, and summarizes the challenges that remain before GBS vaccines can be brought to clinical care.

Microbiology of GBS

GBS is a Gram-positive encapsulated bacterium and appear in pairs or short chains and share a common antigen, the Lancefield group B polysaccharide antigen, and are distinguished on the basis of their type specific capsular polysaccharides (CPS), which the microorganism expresses at high levels on its surface, into 10 structurally and antigenically unique types (Ia, Ib, II, III, IV, V, VI, VII, VIII, IX) [12-14]. The capsule surely represents the major virulence factor, which helps GBS evade host defence mechanisms by interfering with phagocytic clearance [15], and the level of capsule expression correlates with virulence in animal models of GBS infection [16-20]. Capsular GBS mutants are nearly avirulent in animal models of GBS infection [16]. GBS also contains surface proteins, cell membrane phospholipids, and secreted toxins that further mediate bacterial virulence [20,21]. Two-component regulatory systems and eukaryotic-like serine/threonine kinases and phosphatases also contribute to GBS virulence by coordinating the bacterial responses to changing environmental conditions, including the response to cell-wall active antibiotics [22-24].

Group B Streptococcus Disease

Infection in Pregnancy

Pregnant women are at a higher risk for disease because GBS vaginal colonization is a risk factor of maternal peripartum infection [25]. In pregnant women, GBS can cause clinical infections, but most women have no symptoms associated with genital tract colonization [26].

The clinical presentations of disease among pregnant women are diverse and include urinary tract infection (usually asymptomatic bacteriuria), endometritis, intra-amniotic infection (chorioamnionitis), wound infections associated with caesarean delivery or episiotomy, and, less frequently, puerperal sepsis and meningitis [25]. Colonization with GBS was significantly associated with prolonged labour, premature rupture of membranes and preterm delivery. GBS colonization may play a causal role in the occurrence of intrauterine deaths, late abortions and low birth weight infants [27]. Fatalities among women with pregnancy-associated GBS disease are extremely rare [26].

However, infection of urinary tracts or genital colonization may lead to preterm delivery and low birth weight of infants, especially those pregnant women heavily colonized with GBS. One study in Canada revealed that the threat from GBS may lie in the possibility of prematurity instead of the infection. There was a significant difference in the rate of preterm labor between colonized women and non-colonized women (5.7% and 1.75 respectively). The risk of developing EOD in infant from heavily colonized women is 2.5 times more likely than those born in lightly colonized women [28].

Neonatal Infection

GBS is a leading agent of severe, invasive bacterial infection in human newborns [29]. The majority of infections in newborns occurs in the first week of life and is designated EOD. LOD occurs in infants aged > 1 week, with most infections evident in the first 3 months of life [30].

The maternal carriage is a major risk factor of neonatal GBS disease, which is influenced by the degree of bacterial colonization; women with heavy colonization are more likely to have symptomatically infected infants and heavily colonized infants are more likely to develop invasive disease [25]. In addition, various obstetric factors associated with an increased risk of infection of the newborn, such as prematurity (< 37 weeks) or premature rupture of the membranes was prolonged (> 18 hours), presence of intrapartum fever (> 380C), history of children infected with GBS and presence of bacteriuria during pregnancy caused by this organism [31], young maternal age (under 20 years) belonging to the black race, the child's birth weight below 2500 gram, high surface colonization of *Streptococcus* newborn during childbirth, lack of or low titer of antibodies against capsular antigens *S. agalactiae* in the mother [20,32].

EOD and LOD can differ in clinical presentation, mode of transmission and risk factors for disease [25,26]. Early-onset group B streptococcal infection typically presents with sepsis (69% of cases), pneumonia (26% of cases), respiratory distress (13% of cases) and rarely, meningitis (11% of cases). About 90% of cases present within 24 hours of birth [25,27]. EOD is typically related to maternal carriage of GBS in the genital tract, with vertical transmission occurring prior to or during labor and delivery [33]. Even though perinatal transmission can occur across intact membranes, both premature and prolonged rupture of membranes increases the risk of GBS acquisition [25]. Once the infected amniotic fluid is aspirated or swallowed by the fetus, pathogens may penetrate through immature mucosal barriers, resulting in pneumonia or bacteremia, and may penetrate the blood-brain barrier, leading to meningitis [34].

The second peak of disease incidence, LOD, occurs to 1 month after birth to infants with most infections evident between the seventh day and the third month, after which infections are rare [35]. An infant with GBS-LOD usually presents bacteremia and is complicated in 40% to 60% of cases by bacterial penetration of the blood-brain barrier to produce meningitis. Mortality in late-onset GBS infections is lower than for EOD; however, 20% to 40% of infants with meningitis are left with permanent neurological sequelae including cerebral palsy, cognitive deficits, deafness, blindness, or seizures [36]. In contrast to EOD, late onset infection is not always acquired from the mother but is transmitted both vertically and horizontally from nosocomial and community sources [37]. Another reported source of infection is breastfeeding [35].

Infection in Adults

There is a substantial decline in the incidence of GBS infection in newborns due to prevention by use of IAP. However, GBS has been considered as an unusual pathogen in non-pregnant adults, and the rate of invasive GBS disease in adults continues to climb [38] especially those who are elderly and those who have serious underlying diseases such as alcoholism, diabetes mellitus, neoplasias, and HIV infection [39,40]. Colonization rates of up to 31% and 34% have been documented in young men and non-pregnant women, respectively [14] with the incidence of 4.1 to 7.2 cases per 100,000 non-pregnant adults [33]. Whereas a rate of 22% has been observed in individuals over 65 years of age [14]. Other factors including ever engaging in sexual activity appear to be a common behavior associated with GBS colonization, the presence of an intrauterine device, time since last menses, tampon use, milk consumption, infrequent hand washing and use of yeast medication. Further, those adults residing in a nursing home facility are 4 times more likely to have GBS disease when compared to age-matched individuals living in the community [20,33].

A clinical manifestation of invasive *S. agalactiae* infection varies widely depending on the sites of infection [26]. Among non-pregnant adults, skin and soft-tissue infections and bacteremia of the uncertain source are the most common manifestations of invasive disease. The clinical spectrum also includes urosepsis, pneumonia, peritonitis, meningitis, septic arthritis, and endocarditis. Despite the nearly universal sensitivity of the organism to penicillin, approximately 20% of cases of adult group B streptococcal disease are fatal [42]. The possible emergence of GBS as a respiratory pathogen associated with cystic fibrosis has also been proposed in a recent report [26]. Recurrent GBS infection was identified in adults, and suggested to being mostly caused by relapse, but an association of relapse with persistent carriage or poor clinical management of primary infection was not determined. The nosocomial disease is also raising concerns as more than 20% of patients with GBS invasive infection are thought to have acquired the bacteria from hospital settings [42].

Targets of Protective Immunity

The knowledge of the pathogenesis of many microorganisms, the identification of the main virulence factors, and the characterization of the immune response after infection have been fundamental for the design of new vaccines based on highly purified antigenic components, on genetically detoxified toxins, and on polysaccharides or oligosaccharides conjugated to proteins [43]. Acquired immunity to natural group B streptococcal infection in human neonates and to experimental infection in mice and in neonatal rats has been associated with antibody to the type-specific polysaccharides or proteins of these organisms [44].

GBS Capsular Polysaccharide Antigens

Surface-associated polysaccharides are common features of both gram-positive and gram-negative bacteria. It is thought that microorganisms may have evolved extracellular polysaccharides for protection against both environmental and host factors that may be detrimental to their survival [45]. The type-specific CPS expressed on the surface of GBS is considered protective antigens and is the target of protective antibodies [46]. These extracellular polysaccharides may allow the organism to survive within the host by masking antigenic determinants associated with the bacterial surface, by mimicking host antigens or by interfering with complement- mediated killing [45].

Most GBS isolate can be classified into ten serotypes (Ia, Ib, II to IX) by different antigenicity of the CPS. All of which are associated with human infection, but 4 to 7% are non-typeable. However, the numbers of non-typeable isolate have been an increasing problem [47]. The most prevalent GBS serotype causing neonatal GBS diseases in the world, including the United States, Germany, and Malawi, are type Ia (14% to 30%) and type III (50% to 68%), (accounting for 80% of all invasive isolates). Serotype V has emerged as the most common serotype (30%) isolated from non-pregnant adults, and serotypes VI and VIII are prevalent colonizers of pregnant women in Japan [40].

GBS express two distinct carbohydrate entities, i.e., a type-specific and a group-specific polysaccharide [45]. The CPS of all serotype composed of repeating subunits of four monosaccharides, i.e., glucose, galactose, N-acetylglucosamine, and N-acetylneuraminic acid, polymerized in serotype-specific configurations. Although the physical structure of the CPS of each GBS serotype is unique, all GBS serotypes invariably share a terminal 2, 3-linked N-acetylneuraminic acid, which is identical to the predominant sialic acid (SIA) found on human cells. The SIA residues have been shown to play an important role in the immune determinant structure of the polysaccharide with the desialylated or core polysaccharides forming immunologically incomplete antigens [48].

It has been well established that the polysaccharide contributes to GBS virulence by interfering with C3 opsonization through inhibition of the alternative complement pathway to the absence of type-specific capsule antibodies [49]. Furthermore, type-specific polysaccharide contributes to immune evasion by host structure mimicry via the SIA residue. A role of type-specific polysaccharide as a positive inflammatory agent of the innate immune system has repeatedly been proposed both *in vitro* and *in vivo* [50].

In contrast to type-specific polysaccharide, the group-specific polysaccharide from GBS has been found to be a more potent inflammatory stimulus. In particular, the dimeric adhesion molecule CD11b/ CD18 has been implicated in recognition of this carbohydrate [48]. The type- and group-specific polysaccharides are covalently linked to peptidoglycan and, possibly, other cell wall components of GBS. Therefore; it is difficult to ascertain the role of the polysaccharide alone in the absence of other potentially inflammatory stimuli [51].

GBS Protein Antigens

The ability of GBS to avoid opsonophagocytosis is enhanced by surface proteins that can act in concert with CPS. The expression of cell surface receptors determines adhesive properties of streptococci, which include binding to eukaryotic extracellular matrix proteins, epithelial cells, and endothelial cells, as well as to other bacteria [52]. Among the surface proteins of GBS that confer immunity, the first to be identified were two molecules designated the alpha and beta C proteins. In addition, the R protein, Rib protein, and alpha-like proteins (ALP) purified from type III GBS (ALP2, ALP3, ALP4, and epsilon/ALP) and from type V GBS were identified [46].

The C antigen (also designated Ibc) has been found in many strains (i.e. Present in approximately 50% of all clinical isolates and in approximately 70% of non-type III GBS isolates [46] but not in the clinically important type III strains. Characterization of this antigen subsequently showed that it is composed of two unrelated protein components, the trypsin-resistant α-protein and the trypsin-sensitive β-protein. Of note, a strain that is reported to express C antigen may express either or both of the α and β-proteins. Because α and β together constituted the C antigen, the designations alpha C and beta C, have also been used for these proteins [53].

Serotype II strains displaying both components of the C protein antigen are more resistant to phagocytic killing than are serotype II strains lacking C protein. The β antigen of C protein binds human IgA, and IgA deposited nonspecifically on the bacterial surface probably inhibits interactions with complement or IgG. The alpha antigen is resistant to the protease trypsin and does not bind immunoglobulins [54]. Active immunization with these proteins has been shown to induce protection against invasive disease. Furthermore, antigenic variation by variable expression of tandems repeats in these proteins might be an immune evasion mechanism of GBS. On the other hand, expression of the alpha C protein on the surface of GBS mediates entry into cervical epithelial cells involving an interaction of the alpha C protein N-terminal domain and the host cell glycosaminoglycan [54].

Another interesting GBS surface protein that inhibits opsonophagocytosis is cell surfaces protease (CSPA). CSPA, targets host fibrinogen, producing adherent fibrin-like cleavage products that coat the bacterial surface and interfere with opsonophagocytic clearance [8]. A host of other surface proteins important for GBS virulence, the C5a peptidase, is a cell-surface-localized serine protease that inactivates human C5a. C5a is a neutrophil chemo attractant produced during complement activation. This streptococcal enzyme is thought to contribute to virulence by interfering with neutrophil recruitment and may contribute to the poor inflammatory response observed in infected tissues [53].

Laminin is a major component of the basement membrane. The adherence of bacteria to laminin may be a crucial step in the development of invasive GBS infection. Translocation of GBS into the bloodstream as well as entry of the bacteria into the cerebrospinal fluid, which occurs in the case of meningitis, requires the passage of bacteria through the basement membranes. The interaction of bacterial surface proteins with laminin could be important to this context [52].

Another important virulence factor of GBS, is the hemolysin/cytolysin, pore-forming membrane-associated toxin. GBS is capable of forming pores in a variety of eukaryotic cell membranes [55] and exert its effect on direct tissue injury or activation of the host inflammatory response [56]. Studies also demonstrate a role of β-hemolysin/cytolysin during adherence of lung epithelium and induction of neutrophil chemo attractant cytokine interleukin-8 [57]. On occasion, GBS can cross intact placental membranes, which may lead to severely ill neonates at times of delivery or even to stillbirth [35].

Strategies for Prevention

The CDC with the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics issued consensus guidelines in 1996 for the prevention of early-onset GBS infections through maternal IAP. This risk-based strategy called for the routine administration of intravenous penicillin or ampicillin to parturient women who are colonized with GBS or those who have risk factors associated with invasive GBS disease in neonates (i.e. delivery of < 37 weeks gestation, an intrapartum maternal temperature of 100.4 F (38 de), or the rupture of membranes 18 hours) [7].

The second strategy is the screen based strategy, promoted universal screening for all pregnant women between the 35 - 37 weeks' gestation with vaginal and rectal swabs for GBS colonization, with colonized women receiving antibiotics intrapartum. Under both strategies, women with GBS bacteriuria or a history of a previous infant with EOD were also to receive intrapartum antibiotic therapy [8].

Although these two approaches, often used of combination in clinical practice, have significantly reduced the incidence of neonatal GBS sepsis and meningitis, invasive disease due to GBS infection remains the leading cause of neonatal morbidity and mortality [8]. In

addition, these measures are unlikely to prevent late-onset infections, prematurity, and stillbirths related to GBS, while obviously not addressing GBS disease in non-pregnant adults. Indeed, GBS is also a frequent cause of infections in pregnant women and in clinically ill and older adults, such as those suffering from diabetes, cirrhosis, malignancies, and immune deficiencies [10] with a reported increase in macrolide resistance in clinical isolates of GBS, efforts have shifted toward vaccination as an attractive alternative for disease prevention [8]. Therefore, the best long-term solution to prevent GBS disease is the development of an effective vaccine that could be administered to all adults, a strategy that would alleviate the limitations of IAP [11].

Conventional Approaches of GBS Vaccine Development

The rationale for GBS vaccine development is supported by the observation that the risk of neonatal infection is inversely proportional to the maternal amounts of specific antibodies to the CPS antigen that surrounds GBS, the implication being that protective immunoglobulin G (IgG) antibodies are transferred from the mother to the baby through the placenta [58]. Already, Baker and Kasper [59], have demonstrated a correlation between maternal antibody deficiency in delivery and susceptibility to neonatal GBS infection. That finding to suggest that vaccination of pregnant women could become a very efficient prophylactic strategy to prevent GBS infection in neonates since it could stimulate transplacental transfers of GBS specific antibodies from the mother to the fetus, thus considerably increasing the level of protective antibodies present at the time of delivery [10].

Like group A *Streptococcus*, there is no commercial vaccine available to prevent GBS infection. Whilst several vaccine candidates, both CPS and protein-based, are currently under investigation. The principal difficulty in developing globally effective GBS vaccines are the existence of several serotypes with different geographical distributions and the heterogeneous cross-reactivity between serotypes-a vaccine suitable for Asian or European populations might not be suitable for African populations [60].

Other problems include the need to serotype GBS isolates prevalent from different developing countries because of fear of risks of birth defects and the potential for subsequent liability administration of the vaccine to pregnant women is also a problem. In addition, there is considerable discussion of the experts on this field without consensus regarding the most appropriate section of the population to immunize non-pregnant adolescents or pregnant women in the third trimester [61]. Finally, given the current use of antibiotics both in prophylaxis and in the treatment of neonatal GBS disease, it is not feasible to design a placebo-controlled, double-blind, randomized clinical trial to determine the efficacy of a GBS vaccine. One possible solution to this problem is to use functional antibody measurements as a correlates with immunity and protective levels for each serotype, calculated from paired umbilical-cord blood from case-control samples [61].

Capsular Polysaccharide-Based Vaccines

Immunologic strategies proposed to the prevention of GBS disease include treatment of newborns with intravenous immunoglobulin preparations and active immunization of women with a vaccine against GBS. The goal of both approaches is to supply the newborn with protective levels of IgG specific to the GBS CPS antigen, because antibodies to this cell surface component are protective [62].

Vaccine strategies have focused on several surface-associated GBS molecules. Interest in CPS is based on the observation that low maternal anti-capsular antibody concentration is correlated with neonatal susceptibility to infection [17,18]. The first evidence of the protective nature of CPS-specific antibodies was in the 1930s when Rebecca Lancefield demonstrated that, using CPS-specific polyclonal rabbit serum; mice could be protected against GBS infections. Studies done in the 1976s demonstrated an unambiguous association with low levels of maternal antibody to type III CPS and susceptibility of the newborn to GBS EOD or LOD. This study also showed that CPS-specific antibody was transferred from mother to newborn, a finding that provided additional rationale for developing a vaccine based on the CPS antigen expressed by GBS. The association with maternal antibody levels against serotype Ia and III and infant susceptibility was later confirmed by subsequent multicenter seroepidemiology studies [63-65].

Current progress of the development of an effective GBS vaccine has primarily focused on the use of the bacterial capsule as a primary immunogen, with ten serotypes having been identified so far [8]. In the 1980s the first human clinical trials were conducted with purified native CPS from GBS. These trials demonstrated the safety of the antigen but also highlighted the need to improve the immunogenicity of the CPS, as only Ia- 40%, II- 88%, III- 60% of the recipients showed significant IgG responses [58].

Polysaccharide-protein Conjugate Vaccines

Since bacterial polysaccharides have properties of thymus-independent type 2 antigens, namely, restricted IgG subclass responses, relatively poor generation of memory B cells, and failure to stimulate antibody responses in neonates and in xid mice [66]. Coupling a polysaccharide or component oligosaccharide to a carrier protein to produce a conjugate molecule may result in immunogenic properties more like those of thymus-dependent antigens, including stimulation of higher levels of IgG antibodies, enhanced memory responses, and immunogenicity in infants. However, immunogenicity has varied widely from polysaccharide-protein conjugates, even for conjugates based on the same polysaccharide, an observation that suggests that specific physical-chemical properties of the conjugate affect the polysaccharide-specific immune responses [66].

The first GBS conjugate vaccines were prepared with serotype III coupled to tetanus toxoid (TT). Coupling of type III CPS to a carrier protein, such as TT, enhanced the immunogenicity of type III CPS and resulted in antibody levels that were less variable [46]. TT is a component of several other vaccines, and the most adults have high levels of preexisting antibody to it. Since TT is not relevant to GBS infection, it might be advantageous to incorporate a GBS protein into the vaccine for the purpose of enhancing protection and expanding the number of strains covered by the vaccine [46].

Vaccination of healthy non-pregnant women with a type III CPS-TT (IIITT) conjugated vaccine elicited a 50-fold increase in type III CPS-specific antibody levels, while vaccination with an unconjugated types III CPS vaccine elicited a 7-fold increase. Even at lower doses, 90% of recipients reacted to the III-TT vaccine with a fourfold increase in type III CPS-specific antibody concentrations, as opposed to only 50% of recipients of the unconjugated type III CPS vaccine [46].

Despite the positive results collected during this trial, some aspects of the effect on pregnant women and their babies still needed to be investigated. To this purpose, a subsequent trial comparing III-TT with unconjugated types III CPS in pregnant women showed that, after glycoconjugate vaccination, titers of protective IgG to type III CPS were elevated in cord blood, persisted for at least 2 months in the neonates, and correlated with levels of type III CPS-specific antibody in maternal serum [67,68]. Monovalent conjugated vaccines representing the most frequent disease-causing serotypes (Ia, Ib, II, III and V) have been prepared coupled to TT and tested in phase I and II clinical trials in healthy women. For each vaccine, an improved immunogenicity was demonstrated against the isotypic unconjugated polysaccharide, which was dose-dependent and more consistent with a memory response. Moreover, glycoconjugate vaccines were able to induce functionally active serotype specific IgG [except for type V CPS-TT conjugate, which primarily elicited immunoglobulin M (IgM) antibodies] which, in the presence of complement, were able to opsonize and induce killing of GBS by human peripheral blood leukocytes in *in vitro* assays [69-72]. Altogether these studies have demonstrated that CPS-TT vaccines were technically feasible and could be administered safely to women early in the third trimester of pregnancy, providing important health benefits to both mother and infant.

Vaccine serotype coverage is a critical issue if GBS vaccines are to replace, rather than augment, GBS IAP for the prevention of neonatal disease in the international settings. Recent population studies of serotype distributions suggest that a CPS vaccine would need to contain capsule types Ia, Ib, II, III, V, VI, VIII and IX in order to prevent the majority of GBS infections [73]. Conjugate vaccines based on all nine currently identified GBS serotypes have been prepared and tested pre-clinically, although it is little or no to cross protection against serotypes. As such, capsular conjugate vaccines of this type will need to be multivalent in order to provide sufficient coverage against the prevalent serotypes [46]. In a murine model of infection, up to four TT-conjugated serotypes (Ia, Ib, II and III) were successfully combined

[62]. Additionally, Clinical phases 1 and phase 2 trials of conjugating vaccines prepared for CPS from GBS types Ia, Ib, II, III, and V. These vaccines are likely to provide coverage against the majority of GBS serotypes that currently cause disease in the United States, they do not offer protection against pathogenic serotypes that are more prevalent in other parts of the world (e.g., serotypes VI and VIII, which predominate among GBS isolates from Japanese and Type I in Ethiopian women) [74-76].

Because, bacterial CPSs are T-cell-independent antigens, and experiments carried out with both animal and human subjects have shown that immune responses to purified bacterial CPSs fail to induce immunological memory [73]. Furthermore, sialic acid constituents of the GBS CPS mimic the human Lewis X antigen, thus making GBS CPS poor immunogenic. Moreover, other potential drawbacks of CPS vaccines include geographical variations in serotype prevalence and the possibility that these vaccines will ultimately select for increased prevalence of serotypes not included in the vaccines [73], interest has shifted towards GBS proteins as vaccine antigens or as carrier proteins for serotype-specific GBS polysaccharides [77].

Protein-based Vaccines

The incorporation of GBS proteins into a glycoconjugate vaccine could provide additional efficacy across serotypes and improve the overall protection from GBS disease. Several GBS surface proteins have been investigated as potential vaccine candidates; these include Rib, the alpha and beta subunits of the C protein, surface immunogenic protein (SIP), Fbs, C5a peptidase and the R proteins [56]. These Surface antigens represent good candidates for vaccines, as antibodies directed against these targets have the potential to interfere with bacterial virulence and also to promote opsonophagocytosis through binding to Fc receptors on phagocytes. In contrast to CPSs, protein antigens have the potential to elicit protective T-cell-dependent antibody responses and long-lasting immunity without the need for conjugation to other molecules [10,73]. Furthermore, such bacterial proteins were shown for other bacterial pathogens to be present in most pathogenic strains and to induce cross-protective immunity [10].

The ability of the C-protein complex to elicit antibodies that provide passive protection in animal models indicates that these antigens are important virulence factors for human infection. Antibodies to this complex have also been detected in the sera of both mothers and their newborn infants. Further research revealed that the C-protein complex from type II GBS could be one factor contributing to bacterial resistance to opsonization [78], and GBS strains that express these proteins can resist intracellular killing by phagocytes. It was also suggested that these proteins, in combination with either type-specific CPS or oligosaccharides, could function as an adjuvant to stimulate capsular antibody response and provide additional antigenic determinants to the vaccine [79].

SIP, is a surface immunogenic protein. This protein is produced by all GBS isolates examined to date and is capable of conferring protection against experimental infection with GBS strains representing the five major disease-causing serotypes [73,80]. In addition, passive administration of rabbit anti-SIP serum to pregnant mice or immunization of female mice before pregnancy with purified recombinant SIP conferred protective immunity to their offspring against GBS infection. These results, which involved the transfer of functional antibodies from pregnant mice to their pups, suggest that SIP specific antibodies could play an important role in protection against GBS disease. Most importantly, the surface exposure of SIP is not hindered by other surface antigens. These observations further intensify the interest in SIP as a potential vaccine candidate [80].

Moreover, almost all strains of the clinically important type III express protein Rib, which elicits protective immunity. In total; 90% of GBS strains of the four classical serotypes express either α or Rib, suggesting that a combination of these two proteins may be used for the development of a protein vaccine against GBS. Theα and Rib proteins have been extensively characterized and were found to identify a family of streptococcal cell surface proteins with extremely repetitive structure. Although these two proteins show extensive residues identity, they do not cross-react immunologically [81]. But, both the alpha subunit of the C protein and Rib possesses repeating peptide sequences that show strain-to-strain variations in the numbers of repeats, something which can influence their immunogenic properties [73].

The C5a peptidase (termed SCPB or ScpB) is a conserved, cell-surface localized protein; this antigen is an attractive vaccine candidate. Immunization of mice with purified SCPB/ScpB resulted in enhanced clearance of bacteria from the lungs of mice who were inoculated intranasally with GBS. Although this study used only a single GBS strain with the uncommon serotype VI, the results indicate that SCPB/ScpB has been potential of a vaccine candidate, even if the antibodies produced did not inhibit the enzymatic activity of the surface localized protein [82].

The identification of proteins located on the outer surface of GBS cells is an approach that has been used to find new potential protein-based vaccine candidates. Lmb is an important surface-exposed protein for the laminin binding properties of S. agalactiae, thus allows the bacteria to adhere to or invade tissues. It is present at the surface of all GBS strains and has been proposed as a good vaccine candidate [52]. However, progress in the development of this protein as a vaccine candidate is unknown. Another protein, termed leucine-rich repeat protein (LRRG), cell-surface located protein antigen that is highly conserved among all serotypes of GBS indicating its potential to afford broad cross-protection that was found to induce protection against experimental GBS infection in mice. Consequently, it was proposed that this protein was a highly promising candidate antigen for potential use as a GBS vaccine [73].

New Approaches of GBS Vaccine Development

Despite the many studies that are focused on developing a GBS vaccine using conventional approaches, including the cultivation of pathogens and the identification of highly immunogenic and protective antigens using standard biochemical and microbiological techniques, little success has been achieved in terms of developing a vaccine that is globally effective. However, recent years have witnessed the welcome emergence of genomics, proteomics, gene expression and *in silico* technologies that are presenting exciting new opportunities in the hunt for an effective and globally relevant GBS vaccine [61].

Genomic and Gene-Expression Approaches to Vaccine Development

The advents of whole-genome sequencing of bacteria and advances in bioinformatics have revolutionized the study of bacterial pathogenesis, enabling the targeting of possible vaccine candidates starting from genomic information. Genomic-based technologies have many advantages compared with conventional approaches, which can be time-consuming and can only usually identify abundant antigens that are expressible under *in vitro* culture conditions. Genomics allows antigen candidates to be identified as the basis of sequence conservation in different serotypes and strains of a given pathogen, and by predicting the surface exposure of a protein [61].

Tettelin and colleagues compared the predicted protein sets of *Streptococcus agalactiae, Streptococcus pyogenes* and *Streptococcus pneumoniae*. This analysis revealed that approximately 50% of the genes are homologous, indicating substantial overlap in the virulence mechanisms used by these pathogens [83]. In terms of vaccine development, the identification of shared virulence factors and protective antigens could support a concept of combined vaccination approaches. Although, the sequence of a single genome does not reflect how genetic variability drive's pathogenesis within a bacterial species, and is a limitation regarding genome-wide screens for vaccine candidates or for antimicrobial targets, the identification of universal GBS vaccine candidates by multigenome analysis and screening has been reported [84].

In this approach, Maione., *et al*. compared and analyzed the genome sequences of eight GBS strains belonging to different serotypes of GBS. This study revealed that ~80% of each genome were shared by all strains the 'core' genome and the remaining (20%) genes were not present in every strains the 'variable' genome. Using *in silico* analysis, genes encoding putative surface-associated and secreted proteins were identified from these two subgenomes. A total of 589 proteins were identified (396 'core' genes and 193 'variable' genes), of which 312 were successfully expressed, purified and used to immunize mice. A combination of four proteins, SIP (SAG0032), present in the core subgenome, and three other putative, surface-associated proteins (SAG1408, SAG0645, SAG0649), from the variable subgenome, elicited protection in infant mice and their combination proved highly protective of a large panel of GBS strains, including all circulating serotypes. This study revealed that multistrain genome analysis and screening constituted an effective new approach to identifying vaccine candidates that can provide broad protective activity when used in combination [73]. In GBS and probably other bacterial pathogens that adopt the strategy of gene variability to escape the immune system, universal protective protein antigens are unlikely to exist. However, some protein antigens are conserved in sufficiently large subpopulations of GBS that in combination, they can be broadly protective [85].

The successful use of multistrain genome analysis and screening described here for GBS provides the basis for the potential development of universal protein-based vaccines against other important and highly variable pathogens such as *Streptococcus pyogenes* and *Streptococcus pneumonia* [58,86]. The pan-genome concept has shown that the initial strategy of sequencing one or two genomes per species is not necessarily sufficient, and that multiple strains may need to be sequenced to understand the basis of bacterial species, and to overcome the problem represented by gene variability [86].

The presence in GBS of pilus-like structures composed of antigens that confer protection in a mouse model of maternal immunization suggests that pili may play a significant role in the virulence of Gram- positive bacteria as well as in Gram-negative. Genome surveys may therefore reveal other important features of pathogens hitherto missed by classical methodologies [66]. Interestingly, the use of *Lactococcus lactis* as a heterologous host to express GBS pili was recently shown to be a promising approach for the development of multivalent live vaccines [87]. Remarkably, in the same study, vaccines based on combinations of recombinant pilus components protected mice against lethal challenge with a wide variety of GBS strains. This study paves the way for the development of simple formulations of multivalent live vaccines in which pilin elements from different streptococcal species are combined in a single recombinant clone, providing broad intra- and interspecies protection against streptococcal infections. This is particularly relevant in view of the urgent need for efficacious preventive measures in developing countries, where endemic streptococcal infections are responsible for high morbidity and a large number of fatalities [87].

Mathematical extrapolation of the GBS genomic data indicates that the gene reservoir available for inclusion in the GBS pan-genome is vast, and that unique genes will continue to be identified even after the sequencing of hundreds of genomes. Thus, the genomes of numerous, independent isolates are required to understand the global complexity of bacterial species. Analysis of multiple GBS genomes was found to be instrumental in the development of vaccines and for the functional characterization of important genetic determinants [84]. Diversity among isolates also arises by homologous recombination leading to the exchange of complete loci encoding surface proteins, or of the internal part of genes encoding putative antigens, as was first described for the α-C/ Rib family. The combination of the distinct alleles at these multiple loci allows GBS strains to express different combinations of surface proteins, a strategy used by the pathogen to evade host immune mechanisms; however, this versatility will as well have to be taken into consideration when designing a universal vaccine that is effective for GBS [88].

Proteomic Approaches to Vaccine Development

Proteomics is the systematic identification and characterization of proteins for their structure, function, activity, quantity, and molecular interactions. The subfield of quantitative proteomics seeks to provide information about both protein and modified protein expression levels [89]. Proteomics, in conjunction with genomic approaches, provides interesting insights into microbial pathogenesis at an organism level. There are several reasons for focusing on the analysis of proteins. The first reason is, the level of mRNA expression frequently does not represent the amount of 'active' protein in a cell. Secondly, the gene sequence does not give any information on post-translational modifications that could be essential for protein function and activity; and third, genome analysis does not provide information on dynamic cellular processes [90].

The application of proteomics to vaccine development provides interesting opportunities to elucidate both bacterial pathogenic mechanisms and new vaccine candidates. With the availability of genomic sequences, the progress achieved in 2D-gel electrophoresis separation techniques and advances in mass spectrometry analysis means that it is now possible to separate, identify and catalogue the proteins expressed on a cell under several conditions. The entire set of proteins encoded by the genome has been defined as "proteome" [91].

A proteomic analysis was undertaken by Hughes and colleagues to identify the most abundant surface-associated proteins of GBS. Six of these proteins, previously unidentified in GBS, were sequenced and cloned. These were ornithine carbamoyl-transferase (OCT), phosphoglycerate kinase (PGK), non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase, purine nucleoside phosphorylase, enolase, and glucose-6-phosphate isomerase. These studies demonstrate the successful application of proteomics as a technique for identifying

vaccine candidates and antisera raised against OCT and PGK of the identified proteins were capable of providing a degree of protection against a lethal challenge of GBS in a neonatal-mouse model, demonstrating both the presence of the protein on the cell surface and the utility of this proteomic strategy in identifying possible vaccine candidates [Hughes., *et al*. 2002].

Many of the anchorless surface-associated proteins identified by this proteomic approach may not have been identified by the conventional whole-genome screening strategy. However, this method identified a relatively small number of streptococcal wall-associated proteins containing cell wall anchor motifs, despite genomic predictions of 25 - 35 proteins in GBS. Although labor intensive, this approach is quantitative and identifies bona fide surface-associated proteins [92].

Future Perspectives for GBS Vaccine Development

During the past 20 years, many advances in the prevention and treatment of GBS disease have been achieved. Unfortunately, despite all the efforts, GBS is remains one of the major health problems for infants. The glycoconjugate generation vaccine has been demonstrated to be able to prevent GBS disease. At present, the licensing of GBS vaccines is difficult because of the challenge in conducting efficacy clinical trials in humans due to the low incidence of neonatal diseases. In addition, the current vaccine preparations for GBS are based on the serotypes and multilocus sequence types prevalent in the United States and Europe, and target the hyper-virulent sequence type 17 (ST17) [93]. However, these vaccine preparations are not as effective in other regions because of the prevalence of different serotypes or virulent sequence types expressing a different repertoire of surface proteins. To ensure effective vaccine development, it will be important to continually monitor the distribution pattern of the prevalent serotypes and sequence types in all regions of the world, thereby ensuring the inclusion of the most relevant components in a global GBS vaccine.

Initial efforts applying a genomics approach to GBS vaccine development have led to the identification of new, highly conserved protein antigens that are expressed on the bacterial surface. One of the positive aspects of this methodology is that each of the antigens that demonstrate protection can be produced as a soluble recombinant protein in *Escherichia coli*, a property that is a considerable asset for the commercial production of a vaccine. The reverse vaccinology approach reduces the time and cost required for the identification of suitable antigen candidates and provides new opportunities for those microbial diseases for which conventional vaccine development approaches have failed [94,95].

Conclusions

The drawback of using the genome sequence of one single strain is that it does not offer a complete picture of the genetic diversity within a species. The advantage of the combination of the two approaches is that indeed only a small pool of proteins that extend beyond the cell wall and polysaccharide capsule, thus interacting within the environment, will go through the purification step and then tested *in vivo* or *in vitro* thus reducing time and expense. The major limitation of the proteomics approach is that the bacteria are grown *in vitro* in a rich medium and harvested at a single growth phase. This situation does not necessarily reflect the *in vivo* conditions. Furthermore, this approach is unsuitable to detect protease resistant protein complexes such as bacterial pili.

In conclusion, the successful use of multigenome screening methods coupled with the application of proteomics could be a template for the development of protein-based vaccines against human pathogens, such as GBS, for which vaccines are either not available or if they do exist, are in need of significant improvement.

Bibliography

1. Artz LA., *et al*. "Rapid screening for Streptococcus agalactiae in vaginal specimens of pregnant women by fluorescent in situ hybridization". *Journal of Clinical Microbiology* 41.5 (2003): 2170-2173.

2. Motlova J., *et al*. "Vaginal and rectal carriage of Streptococcus agalactiae in the Czech Republic: incidence, serotypes distribution and susceptibility to antibiotics". *Indian Journal of Medical Research* 119 (2004): 4-87.

3. Tazi A., *et al*. "Fluoroquinolone-resistant group B streptococci in acute exacerbation of chronic bronchitis". *Emerging Infectious Diseases* 14.2 (2008): 349-350.

4. Phares CR., *et al*. "Group B streptococcal disease in the United States, 1999-2005". *Journal of the American Medical Association* 299.17 (2008): 2056-2065.

5. Jones N., *et al*. "Carriage of group B Streptococcus in pregnant women from Oxford, UK". *Journal of Clinical Pathology* 59.4 (2006): 363-366.

6. Heath P and Schuchat A. "Perinatal group B streptococcal disease". *Best Practice and Research Clinical Obstetrics and Gynaecology* 21.3 (2007): 411-424.

7. CDC. "Prevention of perinatal group B streptococcal disease: a public health perspective". *MMWR Recommendations and Reports* 45.RR-7 (1996): 1-24.

8. CDC. "Prevention of perinatal group B Streptococcal disease: a public health perspective". *Morbidity and Mortality Weekly Report* 45 (2002): 679.

9. CDC. "Prevention of perinatal group B Streptococcal disease: a public health perspective. Revised guidelines". *Morbidity and Mortality Weekly Report* 59 (2010): 1-32.

10. Brigsten AK., *et al*. "Induction of cross reactive antibodies by immunization of healthy adults with type Ia and Ib group B Streptococcal polysaccharide tetanus toxoid conjugate vaccines". *Journal of Infectious Diseases* 185.9 (2002): 1277-1284.

11. WHO. "State of the art of vaccine research and development: Initiative for Vaccine Research" (2005).

12. Kong F., *et al*. "Serotype identification of group B streptococci by PCR and sequencing". *Journal of Clinical Microbiology* 40.1 (2002): 216-226.

13. Kong F., *et al*. "Simultaneous detection and serotype identification of Streptococcus agalactiae using multiplex PCR and reverse line blot hybridization". *Journal of Medical Microbiology* 54.12 (2005): 1133-1138.

14. Slotved H., *et al*. "Serotype IX, a proposed new streptococcus agalactiae serotype". *Journal of Clinical Microbiology* 45.9 (2007): 2929-2936.

15. Avci F and Kasper D. "How bacterial carbohydrates influence the adaptive immune system". *Annual Review of Immunology* 28 (2010): 107-130.

16. Rubens CE., *et al*. "Transposon mutagenesis of type III group B streptococcus: correlation of capsule expression with virulence". *Proceedings of the National Academy of Sciences of the United States of America* 84.20 (1987): 7208-7212.

17. Wessels MR., *et al*. "Isolation and characterization of type IV group B Streptococcus capsular polysaccharide". *Infection and Immunity* 57.4 (1989): 1089-1094.

18. Wessels MR., *et al*. "Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule". *Proceedings of the National Academy of Sciences of the United States of America* 86.22 (1989): 8983-8987.

19. Rubens CE., *et al*. "Molecular analysis of two group B streptococcal virulence factors". *Seminar in Perinatology* 14 (1990): 22-29.

20. Doran KS and Nizet V. "Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy". *Molecular Microbiology* 54.1 (2004): 23-31.

21. Maisey HC., *et al*. "Recent advances in understanding the molecular basis of group B Streptococcus virulence". *Expert Reviews in Molecular Medicine* 10 (2008): e27.

22. Jiang SM., *et al*. "Regulation of virulence by a two-component system in group B streptococcus". *Journal of Bacteriology* 187.3 (2005): 1105-1113.

23. Rajagopal L., *et al*. "A eukaryotic type serine/ threonine kinase and phosphatase in Streptococcus agalactiae reversibly phosphorylate an inorganic pyrophosphatase and affect growth, cell segregation, and virulence". *Journal of Biological Chemistry* 278.16 (2003): 14429-14441.

24. Klinzing DC., *et al*. "The two component response regulator LiaR regulates cell wall stress responses, pili expression and virulence in group B Streptococcus". *Microbiology* 159.7 (2013): 1521-1534.

25. Hansan SM., *et al*. "Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants". *Journal of Clinical Microbiology* 42.1 (2004): 83-89.

26. Yang Q. "Proteomic investigation of the Group B Streptococcus". PHD Thesis (2011).

27. Doran KS., *et al*. "Perinatal/neonatal case presentation". *Journal of Perinatology* 22 (2002): 326-330.

28. Fluegge K., *et al*. "Incidence and clinical presentation of invasive neonatal group B Streptococcal infections in Germany". *Pediatrics* 117.6 (2006): e1139-e1145.

29. Bunyasontigul K., *et al*. "High morbidity associated with invasive group B Streptococcal disease among non-pregnant adults in Thailand, 1999 to 2009". *Journal of Infectious Diseases and Antimicrobial Agents* 28 (2011): 169-177.

30. Wang Y., *et al*. "Group B Streptococcal disease in nonpregnant patients: emergence of highly resistant strains of serotype Ib in Taiwan in 2006 to 2008". *Journal of Clinical Microbiology* 48.7 (2010): 2571-257.

31. Sambola A., *et al*. "Streptococcus agalactiae infective endocarditis: analysis of 30 cases and review of the literature, 1962-1998". *Clinical Infectious Diseases* 34.12 (2002): 1576-1584.

32. Milton M., *et al*. "Streptococcal genotypes gene diversity among group B selection, recombination, and virulence". *Journal of Bacteriology* 191.17 (2009): 5419-5427.

33. Farley MM. "Group B Streptococcal disease in non-pregnant adults". *Clinical Infectious Diseases* 33.4 (2001): 556-561.

34. Wernecke M., *et al*. "Evaluation of a novel real-time PCR test based on the ssrA gene for the identification of Group B Streptococci in vaginal swabs". *BMC Infectious Diseases* 9 (2009): 148-155.

35. Ruiz MC. "Anorectal and vaginal colonization by Streptococcus agalactiae in the third quarter pregnancy". PSCP (2006).

36. Jackson LA., *et al*. "Risk factors for group B Streptococcal disease in adults". *Annals of Internal Medicine* 123.6 (1995): 415-420.

37. Serruto D., *et al*. "Genome-based approaches to develop vaccines against bacterial pathogens". *Vaccine* 27.25-26 (2009): 3245-3250.

38. De-Cueninck BJ., *et al*. "Isolation, chemical composition, and molecular size of extracellular type II and type la polysaccharides of group B Streptococci". *Infection and Immunity* 41.2 (1983): 527-534.

39. Cieslewicz MJ., *et al*. "Structural and genetic diversity of group B Streptococcus capsular polysaccharides". *Infection and Immunity* 73.5 (2005): 3096-3103.

40. Gravekamp C., *et al*. "Alpha C protein as a carrier for type III capsular polysaccharide and as a protective protein in group B Streptococcal vaccines". *Infection and Immunity* 67.5 (1999): 2491-2496.

41. Yang HH., *et al*. "Recombinant group B Streptococcus alpha-like protein 3 is an effective immunogen and carrier protein". *Clinical and Vaccine Immunology* 15.7 (2008): 1035-1041.

42. Carlin AF., *et al*. "Group B Streptococcal capsular sialic acids interact with siglecs (immunoglobulin-like lectins) on human leukocytes". *Journal of Bacteriology* 189.4 (2007): 1231-1237.

43. Martin TR., *et al*. "The effect of type-specific polysaccharide capsule on the clearance of group B Streptococci from the lungs of infant and adult rats". *Journal of Infectious Diseases* 165.2 (1992): 306-314.

44. Hunolstein V., *et al*. "Soluble antigens from group B Streptococci induce cytokine production in human blood cultures". *Infection and Immunity* 65.10 (1997): 4017-4021.

45. Cuzzola M., *et al*. "Beta 2 integrins are involved in cytokine responses to whole gram-positive bacteria". *Journal of Immunology* 164.11 (2000): 5871-5876.

46. Deng L., *et al*. "Characterization of the linkage between the type III capsular polysaccharide and the bacterial cell wall of group B Streptococcus". *Journal of Biological Chemistry* 275.11 (2000): 7497-7504.

47. Spellerberg B., *et al*. "Lmb, a protein with similarities to the LraIadhesin family, mediates attachment of Streptococcus agalactiae to human laminin". *Infection and Immunity* 67.2 (1999): 871-878.

48. Lindahl G., *et al*. "Surface proteins of Streptococcus agalactiae and related proteins in other bacterial pathogens". *Clinical Microbiology Reviews* 18.1 (2005): 102-127.

49. Jerlstrom PG., *et al*. "The IgA-binding beta antigen of the C protein complex of group B Streptococci: sequence determination of its gene and detection of two binding regions". *Molecular Microbiology* 5 (1991): 843-849.

50. Harris TO., *et al*. "A novel Streptococcal surface protease promotes virulence, resistance to opsonophagocytosis, and cleavage of human fibrinogen". *Journal of Clinical Investigation* 111.1 (2003): 61-70.

51. Liu GY., *et al*. "Linked GBS-hemolysin cytolysin and carotenoid pigment function to subvert host phagocyte defense". *Proceedings of the National Academy of Sciences of the United States of America* 101.40 (2004): 14491-14496.

52. Pritzlaff CA., *et al*. "Genetic basis for the b-haemolytic/cytolytic activity of Group B Streptococcus". *Molecular Microbiology* 39.2 (2001): 236-247.

53. Kline KA., *et al*. "Immune activation and suppression by Group B Streptococcus in a murine model of urinary tract infection". *Infection and Immunity* 79.9 (2011): 3588-3595.

54. Melin P. "Neonatal Group B Streptococcal disease: from pathogenesis to preventive strategies". *Clinical Microbiology and Infection* 17.9 (2011): 1294-1303.

55. Schrag SJ., *et al*. "Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis". *New England Journal of Medicine* 342.1 (2000): 15-20.

56. Seepersaud R., *et al*. "Characterization of a novel leucin rich repeat protein antigen from group B Streptococci that elicits protective immunity". *Infection and Immunity* 73.3 (2005): 1671-1683.

57. Brodeur BR., *et al*. "Identification of group B Streptococcal sip protein, which elicits cross-protective immunity". *Infection and Immunity* 68.10 (2000): 5610-5618.

58. Paoletti LC., *et al*. "Neonatal mouse protection against infection with multiple group B Streptococcal serotypes by maternal immunization with a tetravalent GBS polysaccharide-tetanus toxoid conjugate vaccine". *Infection and Immunity* 62.8 (1994): 3236-3243.

59. Baker CJ and Kasper DL. "Correlation of maternal antibody deficiency with susceptibility to neonatal group B Streptococcal infection". *New England Journal of Medicine* 294.14 (1976): 753-756.

60. Bronze MS and Dale JB. "Progress in the development of effective vaccines to prevent selected gram positive bacterial infections". *American Journal of the Medical Sciences* 340.3 (2010): 218-225.

61. Johri AK., *et al*. "Group B Streptococcus: global incidence and vaccine development". *Nature Reviews Microbiology* 4.12 (2006): 932-942.

62. Baker CJ and Kasper DL. "Vaccination as a measure for prevention of neonatal GBS infection". *Antibiotics and Chemotherapy* 35 (1985): 281-290.

63. Lin F., *et al*. "Level of maternal antibody required to protect neonates against early onset disease caused by group B Streptococcus type Ia: a multicenter, seroepidemiology study". *Journal of Infectious Diseases* 184.8 (2001): 1022-1028.

64. Lin F., *et al*. "Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen". *Journal of Infectious Diseases* 190.5 (2004): 928-934.

65. Baker C., *et al*. "Maternal antibody at delivery protects neonates from early onset group B streptococcal disease". *Journal of Infectious Diseases* 209.5 (2014): 781-788.

66. Buccato S., *et al*. "Use of Lactococcus lactis expressing pili from group B Streptococcus as a broad-coverage vaccine against Streptococcal disease". *Journal of Infectious Diseases* 194.3 (2006): 331-340.

67. Baker C., *et al*. "Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine". *Vaccine* 21.24 (2003b): 3468-3472.

68. Baker C and Edwards M. "Group B streptococcal conjugate vaccines". *Archives of Disease in Childhood* 88.5 (2003): 375-378.

69. Baker C., *et al*. "Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib". *Journal of Infectious Diseases* 179.1 (1999): 142-150.

70. Baker C., *et al*. "Use of capsular polysaccharide-tetanus toxoid conjugate vaccine for type II group B streptococcus in healthy women". *Journal of Infectious Diseases* 182.4 (2000): 1129-1138.

71. Baker C., *et al*. "Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines". *Journal of Infectious Diseases* 189.6 (2004): 1103-1112.

72. Baker C., *et al*. "Dose-response to type V Group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine in healthy adults". *Vaccine* 25.1 (2007): 55-63.

73. Maione D., *et al*. "Identification of a universal group B Streptococcus vaccine by multiple genome screen". *Science* 309.5731 (2005): 148-150.

74. Lachenauer CS., *et al*. "Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women". *Journal of Infectious Diseases* 179.4 (1999): 1030-1033.

75. Mikamo H., *et al*. "Adherence to, invasion by, and cytokine production in response to serotype VIII group B Streptococci". *Infection and Immunity* 72.8 (2004): 4716- 4722.

76. Schmidt J., *et al*. "Colonization of pregnant women and their newborn infants with group B streptococci in the Gondar College of Medical Sciences". *Ethiopian Medical Journal* 27.3 (1989): 115-119.

77. Payne NR and Ferrieri P. "The relation of the Ibc protein antigen to the opsonization differences between strains of type II group B Streptococci". *Journal of Infectious Diseases* 151.4 (1985): 672-681.

78. Michel JL., *et al*. "Cloned α and β c-protein antigens of Group B Streptococci elicit protective immunity". *Infection and Immunity* 59.6 (1991): 2023-2028.

79. Rioux S., *et al*. "Localization of surface immunogenic protein on group B Streptococcus". *Infection and Immunity* 69.8 (2001): 5162-5165.

80. Areschoug T., *et al*. "Group B Streptococcal surface proteins as targets for protective antibodies: identification of two novel proteins in strains of serotype V". *Infection and Immunity* 67.12 (1999): 6350-6357.

81. Cheng Q., *et al*. "The group B Streptococcal C5a peptidase is both a specific protease and an invasion". *Infection and Immunity* 70.5 (2002): 2408-2413.

82. Mora MS., *et al*. "Microbial genomes and vaccine design: refinements to the classical reverse vaccinology approach". *Current Opinion in Microbiology* 9.5 (2006): 532-536.

83. Tettelin H., *et al*. "Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V Streptococcus agalactiae". *Proceedings of the National Academy of Sciences of the United States of America* 99.19 (2002): 12391-12396.

84. Tettelin H., *et al*. "Genomic analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial pan-genome". *Proceedings of the National Academy of Sciences of the United States of America* 102.39 (2005): 13950-13955.

85. Movahedi A and Hampson DJ. "New ways to identify novel bacterial antigens for vaccine development". *Veterinary Microbiology* 131.1-2 (2008): 1-13.

86. Lauer P., *et al*. "Genome analysis reveals pili in group B Streptococcus". *Science* 309.5731 (2005): 105.

87. Cole JN., *et al*. "Human pathogenic Streptococcal proteomics and vaccine development". *Proteomics Clinical Applications* 2.3 (2008): 387-410.

88. Brochet M., *et al*. "Genomic diversity and evolution within the species Streptococcus agalactiae". *Microbes and Infection* 8.5 (2006): 1227-1243.

89. Gerber SA., *et al*. "Absolute quantification of proteins and phosphorylation from cell lysates by tandem MS". *Proceedings of the National Academy of Sciences of the United States of America* 100.12 (2003): 6940-6945.

90. Humphrey-Smith I., *et al*. "Proteome research: complementarity and limitations with respect to the RNA and DNA worlds". *Electrophoresis* 18.8 (1997): 1217-1242.

91. Capecchi B., *et al*. "The genome revolution in vaccine research". *Current Issues in Molecular Biology* 6.1 (2004): 17-28.

92. Fluegge K., *et al*. "Identification and immune reactivity of proteins released from Streptococcus agalactiae". *European Journal of Clinical Microbiology and Infectious Diseases* 23 (2004): 818-824.

93. Jones N., *et al*. "Multilocus sequencing typing system for group B Streptococcus". *Journal of Clinical Microbiology* 41.6 (2003): 2530-2536.

94. Mora M *et al*. "Reverse vaccinology". *Drug Discovery Today* 8.10 (2003): 459-464.

95. Serruto D and Rappuoli R. "Post-genomic vaccine development". *FEBS Letters* 580.12 (2006): 2985-2992.

Volume 14 Issue 3 March 2018