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4. Meningococcal, Staphylococcal and Streptococcal Infections

4.1. [Meningococcal disease](#)

4.1.1. Overview

Among the elderly and adult persons, meningitis is often the consequence of infections with bacteria such as *Listeria monocytogenes*, particularly in pregnant women, and *Cryptococcus neoformans*, which has become a prevalent pathogen in immunocompromised patients. *Staphylococcus aureus* has been implied in community-acquired meningitis outbreaks. Other bacterial pathogens such as *Mycobacterium tuberculosis*, *Treponema pallidum* and *Borrelia burgdorferi* may also cause aseptic meningitis. *Escherichia coli* and group B streptococci are a frequent cause of meningitis in neonates. Viruses also cause meningitis, most particularly enteroviruses and coxsackieviruses and, less frequently, herpes simplex viruses and cytomegalovirus.

But most cases of meningitis occurring beyond the neonatal period are due to three bacterial species, *Haemophilus influenzae* type B (Hib), *Streptococcus pneumoniae* and *Neisseria meningitidis*. The incidence of *Streptococcus* is greatest in small infants and children less than 2 years old, that of *Haemophilus* in children from 6 months to 2 years of age, and that of *Neisseria* in children, adolescents and young adults from 1 to 29 years of age. These bacteria are characterized by their propensity to colonize the nasopharynx in a harmless way, from where they can invade the host and cause silent bacteremia or overt infection, including otitis media, pneumonia or meningitis. Most cases are acquired by person-to-person contact through aerosol droplets or contacts with respiratory secretions from the asymptomatic carriers.

The introduction of *H influenzae* type b (Hib) conjugate vaccine in routine immunization programs has nearly eliminated invasive Hib disease from many countries, leaving *S pneumoniae* and *N meningitidis* as the commonest causes of meningitis worldwide. In a few surveys done in sub-Saharan Africa, *Streptococcus* accounted for about 20% to 30% of the cases of meningitis whereas *N meningitidis* was responsible for 60% to 65% of cases. This is without taking into account the progressive implementation of the conjugated polysaccharide vaccines against pneumococcus, whose effect is seen in a decreased rate of invasive pneumococcal disease including meningitis in children and adults [1]. The first national immunization program against pneumococcal disease using a 7-valent conjugate *S pneumoniae* vaccine was launched on April 2009 in Rwanda with the aim of vaccinating all Rwandan children younger than 1 year of age on a routine basis. With the progressive generalization of such a public health measure, it is likely that *N meningitidis* (meningococcus) will eventually remain as the only major agent of meningitis worldwide.

4.1.2. Disease burden

Approximately half of the cases of meningococcal disease are acute bacterial meningitis, other syndromes including pneumonia, septic arthritis and meningococemia ('purpura fulminans'). A rash is present in the majority of cases consisting of typical petechiae on the chest, upper arms and axillae.

Bacterial meningitis remains a major threat to global health, accounting for an estimated annual 500 000 cases worldwide with at least 50 000 deaths and as many cases of neurological disability [2]. In developing countries such as The Gambia, an estimated 2% of all children will die of meningitis before they reach 5 years of age [3]. Even with sophisticated care units and antibiotic therapy, case fatality rates remain at 5% to 10% in industrialized countries and can reach up to 20% in developing countries. Between 10% and 20% of survivors develop permanent neurological sequelae such as epilepsy, mental retardation or sensorineural deafness.

Up to 5% to 10% of a population may be asymptomatic carriers of *N meningitidis*, which is a harmless commensal of the nasopharyngeal mucosa. The bacterium is transmitted by person-to-person contact through aerosol droplets or contact with respiratory secretions from asymptomatic carriers. Only a limited fraction of those who become infected will develop a clinical disease with infection of the meninges. There are approximately 3000 cases of meningococcal disease reported in the USA and 7 700 in Western Europe each year [4]. The incidence in the USA tends to range from 0.5 to 1.5 cases per 100 000 population per year [2]. Among known risk factors are concomitant upper respiratory infections, HIV infection, crowding, active and passive smoking and lower socioeconomic status.

Meningococcus serogroups that are responsible for severe meningitis belong to only 5 groups: MenA, B, C, Y and W135. A meningitis epidemic outbreak due to group X was also recently reported. Group A meningococci are characterized by their propensity to cause large scale epidemics in developing countries, as best illustrated by the epidemics of meningitis which occur in irregular cycles in the countries of the African 'meningitis belt' where they usually last for a few years, peaking in March-April at the end of the dry season and dying out during the intervening rainy season [5]. They are responsible for about 3 000 to 10 000 deaths annually according to the intensity of the epidemic. Extensive population travel such as the Hajj pilgrimage to Saudi Arabia facilitates the spread of the epidemic from country to country. The seasonality of the epidemics is linked to external environmental factors, especially the impact of Harmattan, a strong wind during the dry season blowing dust and sand particles from the Sahara [6].

MenA strains also are responsible for pandemic waves, such as that which started in China in the 1960s and spread to Russia and Scandinavian countries and eventually reached Brazil in the 1970s [7]. In the early 1980s, a second MenA pandemic wave began in China, spread through Nepal, and reached Saudi Arabia, causing an epidemic in August 1987 during the Hajj pilgrimage to Mecca with 1 841 reported cases. Pilgrims returning from Mecca introduced the strain throughout Africa where epidemics were recorded in 1988 in Chad and Sudan, in 1989 in Morocco and subsequently in most other African countries [8]. A third pandemic wave began in China in 1993, causing large epidemics in Mongolia in 1994 and Moscow in 1996. It eventually reached Africa, showing high virulence, with approximately 150 000 cases and 20 000 deaths reported in 1996-1997, and a case fatality rate of more than 10%, in spite of appropriate antibiotic treatment. The epidemic spread to many countries, including countries south of the meningitis belt such as Rwanda, Burundi, Kenya and Tanzania, finally reaching Zambia and the Central African Republic [9]. The emergence of MenW135 strains in Saudi Arabia in 2000 then in Burkina Faso in 2002 added even more complexity to the picture.

Group B meningococcus (MenB) is the most important cause of endemic meningitis in industrialized countries, accounting for 30% to 40% of the cases in North America and for up to 80% in some European countries such as Norway, The Netherlands, Germany and Denmark, with most of the remaining cases been due to group C strains. The latter are particularly prevalent in the United Kingdom, Ireland, Canada, Greece and Spain. In all countries, the incidence of group B and C disease is highest in winter in infants less than one year-old. In the USA, where MenB, C and Y occur in roughly equal proportions, an estimated 3000 cases are reported every year with a case fatality rate of 12% [10].

MenB also can cause severe, persistent epidemics, which begin slowly but may persist for 10 years or longer, as seen in the past in Norway; in Cuba, Brazil and areas of Chile; and currently in New Zealand. Persons of Pacific Islands origin and the Maoris experienced very high rates of disease, with incidences as high as 45.6 and 20.6 per 100 000 population, respectively, reaching 611 and 247 per 100 000 in <1

year old infants [11]. The global incidence of MenB disease has been estimated at between 20 000 and 80 000 cases per year, accounting for 2 000-8 000 deaths annually.

Meningococcal disease is distinct from other Gram negative bacterial infections by its propensity to release in the circulation endotoxin (lipooligosaccharide)-rich outer membrane vesicles that cause rapidly progressing cutaneous hemorrhage and skin necrosis, disseminated intravascular coagulation and shock [12]. Many meningococcal strains have reduced susceptibility to penicillins, but high levels of resistance are rarely found. Still, the antibiotic of choice for treatment of meningococcal meningitis in outbreaks in developing countries is oily chloramphenicol.

4.1.3. Bacteriology

N. meningitidis is a Gram-negative encapsulated diplococcus. At least 13 different serogroups have been defined on the basis of the immunochemistry of the capsular PS, but serogroups A, B, C, Y, and W135 account for almost all cases of disease. Meningococci are further classified into serotypes and subtypes on the basis of the immunologic reactivity of their PorB and PorA outer membrane proteins, respectively. Approaches such as multilocus enzyme electrophoresis, now replaced by multilocus sequence typing (MLST) [13] [14], have been used to monitor the global epidemiology of meningococcal disease and classify isolates as related sequence types (STs) designated as ‘clone-complexes’ [15]. The complete nucleotide sequence of the genome of isolates from serogroups A, B and C has been determined. Meningococci achieve high genetic complexity characterized by changes through horizontal gene transfer, gene conversion, phase variation, capsular switching and other antigenic variations such as clonal replacement [16].

Studies of the genetics of meningococcus indicate that most cases of invasive disease are caused by bacteria from a limited number of clone-complexes corresponding to hypervirulent lineages, whereas carriage isolates belong to many different lineages, many of which have never been associated with disease [17]. Thus the group A strains responsible for the two pandemics during the second half of the 20th century were from the lineage known as subgroup III/ST-5 complex, whereas the group B strains from the Norway epidemics belonged to the ET-5/ST-32 complex, and those responsible for the New Zealand epidemic to lineage III/ST-41/44 complex. The great majority of group A strains isolated from the meningitis belt countries between 1987 and 2003 belonged to the ST-5 complex whereas the ST-11 complex was associated to serogroup W135.

Important meningococcal virulence factors have been identified including factor H-binding protein (fHbp). Recruitment of factor H by fHbp contributes to the ability of *N meningitidis* to avoid innate immune responses by preventing complement-mediated lysis in human plasma [19], thus deregulating complement levels and rendering host cells in the vascular compartment more susceptible to complement-mediated damage, which would contribute to the hemorrhagic rash seen in meningococcal sepsis.

4.1.4. Vaccines

Vaccines against groups A, C, Y and W135 include monovalent or plurivalent polysaccharide (PS) vaccines and conjugate vaccines [20], some of which have already been combined with routinely administered vaccines to fit within the EPI regimen [21]. Meningococcus group A is well known for its ability to cause large scale epidemics of meningococcal disease in the sub-Saharan “African meningitis belt” [5] [22] where epidemic waves of meningitis occur on an almost yearly basis. As described below, an affordable monovalent MenA conjugate vaccine is in advanced development by the Serum Institute of India, Ltd, with support from the Meningitis Project, a partnership between WHO and PATH, to be made available to countries of the African meningitis belt [23].

Group B meningococcus, on the other hand, is the only meningococcal serogroup whose infection cannot be prevented by a PS vaccine. MenB vaccines based on bacterial outer membrane vesicles (OMVs) or proteins such as PorA, PorB or NspA have been used with success to fight epidemics in

Norway, Cuba or more recently New Zealand, but these vaccines are narrowly strain-specific. Newer vaccines based on broadly cross-reacting “genome-derived neisserial antigens “(GNA) identified by “reverse vaccinology” [25] are at an early stage of development and will be described below.

4.1.4.1. Vaccines against groups A, C, Y, and W135 meningococci

Groups A, C Y and W135 meningococcal diseases can readily be prevented by vaccines based on high molecular weight capsular PS [26], which were licensed in the late 1960s. Monovalent MenA or MenC and bivalent MenA/C PS vaccines are available and have been used for years for vaccination of children more than 2 years of age and adults. The recent emergence of serogroup W135 prompted the development of a trivalent A/C/W135 vaccine (GSK) and of a tetravalent A/C/Y/W135 vaccine (Sanofi Pasteur). PS vaccines, however, do not induce T cell-dependent immunity, are poorly immunogenic in infants and children less than 2 years old, who are the major group at risk for these infections [27], and fail to elicit immunological memory. Moreover, studies of MenA and MenC PS vaccines as well as those of the 23-valent pneumococcal PS vaccine in adults and children have shown that a state of immune tolerance, or hyporesponsiveness, can develop to repeated PS vaccine exposures [28].

Extrapolating from the experience gained with Hib vaccines, a series of conjugate meningococcal vaccines were developed using diphtheria or tetanus toxoid as a carrier. Thus, MenC conjugate vaccines were introduced in 1999 into the UK as an addition to routine infant immunization at 2, 3 and 4 months of age combined with a catch-up campaign among 1-18 years old children and adolescents. The vaccine program had a tremendous impact on the incidence of the disease, resulting in a more than 90% decrease in the number of deaths and clinical cases and a 66% decrease in asymptomatic carriage [30] and also decreasing by 70% the number of cases in non-vaccinated people, a substantial benefit due to induction of herd immunity [32]. The vaccine, which is now administered in two IM injections 2 months apart followed by a booster injection at 12-15 months, has been included into the routine infant vaccination programs in the UK, the Netherlands and Canada, showing 87% to 98% efficacy in various studies [33]. Vaccination of adolescents was also highly immunogenic [34].

Multivalent conjugate vaccines have since been developed, including a tetravalent (MCV4) MenA/C/Y/W135 conjugate vaccine (Sanofi Pasteur) which has been licensed in the USA and in Canada for 2-55 years old children and adults and was shown to elicit significantly higher and more persistent serum bactericidal antibody responses than the PS vaccine [35], as well as a combined Hib/MenC conjugate vaccine [36] and a heptavalent DTPw-HBV/Hib-MenA/C conjugate vaccine (GSK) [37].

4.1.4.2. The Meningitis Vaccine Project (Men A)

Epidemic group A meningococcal meningitis continues to be a major problem in countries of the sub-Saharan meningitis belt, where the use of PS MenA vaccines have not been very successful, in part because of high price. The Meningitis Vaccine Project (MVP), which was started in 2001 as a partnership between the WHO and PATH, after the terrible epidemic in 1996-97 when more than 250 000 African people fell ill and the death toll soared well above 25 000, is developing with the support of the Bill and Melinda Gates Foundation a MenA conjugate vaccine that will be made available at a cost of US \$0.40 to the countries in the African meningitis belt [38]. The vaccine is been produced by the Serum Institute of India, Ltd, in Pune, using tetanus toxoid as a carrier, MenA polysaccharide provided by SynCoBio Partners in Amsterdam and a coupling technology provided by the US Food and Drug Administration [39]. The vaccine has successfully been tested in a Phase I trial in India then a Phase II trial on 600 healthy toddlers aged 12 to 23 months in The Gambia and Mali, inducing 4-fold increases in anti-MenA bactericidal antibody titers in 73% to 85% of vaccine recipients, with GMTs much higher than those elicited by non-conjugated PS vaccines [41]. A Phase II/III clinical trial is undergoing in 2-29 year-olds in several sites in India and Africa, including sites in Bamako and in Dakar, while a study of different dose schedules in infants is taking place in Ghana. MVP plans to license the vaccine if possible starting end of 2009 to be used in single-dose mass vaccination

campaigns in 1-29 years olds in the countries of the African belt, a target population of about 250 million people.

4.1.4.3. Vaccines against group B meningococci

Group B *N meningitidis*, which is responsible for about 50% of cases of meningococcal disease worldwide, is the only serogroup against which capsular PS vaccines cannot be developed, due to antigenic mimicry with PS in human neurologic tissues [2] [42]. Consequently, vaccine research against MenB has focused on outer membrane protein (OMP) antigens such as PorA, PorB or FetA. The PorA vaccines that were developed in Norway (The Norway Institute of Public Health and Chiron-Novartis), in Cuba (The Finlay Institute and GSK) or in The Netherlands (RIVM and GSK) were successfully used to fight the MenB epidemics in these countries. The Norwegian MenB outer membrane vesicle (OMV) vaccine, Menvac™, showed good immunogenicity as judged from the fact that 65% of vaccinees had a protective bactericidal antibody titer after three doses. Ten months later, however, this proportion had declined to 28%, but a fourth dose induced a rise of antibodies to protective titers in 93% of subjects [43]. Similarly, a vaccine containing the PorA and Por B proteins from the New Zealand strain NZ together with LPS was developed by Chiron-Novartis and the University of Auckland and shown in a series of clinical trials to elicit strain-specific bactericidal antibodies in 70% of infants and 90% of teenagers. The vaccine, MenZB™ [45], has now been introduced nationwide in the under 20 years-old population of New Zealand [48].

Similarly also, the Cuban meningococcal vaccine VA-MENINGOC-BC™ is based on a combination of MenB OMV that contain membrane proteins such as PorA, PorB, Opal, Opt, NspA and others, added with purified capsular PS from a MenC strain. The vaccine, which showed 83% efficacy in a Phase III trial on 106 251 10-16 years old schoolchildren, was introduced as a nationwide vaccination campaign in 1989-90 on 3 million infants, children and adolescents aged 3 months to 24 years. It is currently administered in a routine 2-dose vaccination schedule at 3 months and 5 months of age, which resulted in a sharp and sustained decline in the incidence of the disease [49]. The vaccine has also been extensively tested in several other countries including Brazil, Columbia, Chile, Iceland, Ukraine and Russia [52].

OMV vaccines elicit strain-specific complement-mediated bactericidal activity directed against the strain used to prepare the vaccine and against strains with narrowly related PorA molecules. An alternative has been to use recombinant membrane proteins or improved OMV preparations [53]. A hexavalent PorA-based candidate vaccine was thus developed by the Netherlands Vaccine Institute using OMVs prepared from two *N meningitidis* strains that had been engineered to each express three different PorA antigens [56]. The vaccine was successfully evaluated in adults and children but showed only modest immunogenicity in infants [57]. A nine-valent vaccine has now been prepared using three *N meningitidis* strains engineered to express three different Por A molecules each.

An attempt was made at using OMVs from *N lactamica* that shares a number of antigens with *N meningitidis* but lacks an antigenically-related PorA molecule [59]. This vaccine however was found not to induce bactericidal antibodies, although it did protect animals from a lethal challenge with MenB.

Truly successful development of a broad specificity MenB vaccine is expected to come from a “reverse vaccinology” approach, or ‘genome mining’, i.e. starting from the whole genome sequence of the bacterium and attempting to identify genes of potential interest [24] [25] [63]. Thus, starting from a library of 600 candidate MenB genes, 350 genes were cloned and expressed and the resulting gene products were tested for the induction of bactericidal antibodies in mice. This labour-intensive approach successfully allowed the identification of 28 novel MenB proteins referred to as “genome-derived Neisserial antigens” (GNAs) which appear to be well conserved among the various MenB strains studied and seem to be the target of bactericidal antibodies [66]. Among the most promising candidate vaccine antigens are GNA1870 [68], GNA2132, a lipoprotein related to transferrin-binding protein [69] and NadA protein [71]. GNA 1870, a surface exposed lipoprotein which recently was renamed factor H-binding protein (fHbp) to reflect the fact that it binds factor H, an important component of complement regulation, can be found as three variants, variant 1 accounting for 83% of the strains [18] [73]. The

feasibility of engineering a recombinant chimeric protein expressing critical epitopes from all three variant groups has been demonstrated [74]. The 5 Component Vaccine against Meningococcus B (5CVMB), a MenB subunit vaccine containing 5 GNA antigens including fHbp, induced bactericidal antibodies against 78% of a panel of 85 meningococcal strains and is being developed by Novartis [75]. The theoretical coverage of the 5CVMB antigens and the feasibility to use them in a broad-range MenB vaccine are promising [76].

The introduction of conjugated vaccines into pediatric vaccination schedules has led to a drastic decrease in the incidence of invasive diseases caused by Hib, *N meningitidis* serogroup C or pneumococcus, a major public health success. These vaccines as well as the conjugate vaccines which target MenA, Y or W135 offer the potential for safe and effective control of the corresponding bacterial diseases. A definitive solution to the prevention of meningococcal disease worldwide will not however be possible until a serogroup B meningococcal vaccine is also available [16] [77].

4.2. Staphylococcal infection

4.2.1. *Disease burden*

Staphylococcus aureus is an opportunistic bacterial pathogen associated with asymptomatic colonization of the skin and mucosal surfaces of normal humans. However, it also is the cause of wound infections and has the potential to induce osteomyelitis, endocarditis and bacteremia, leading to infections in any of the major organs of the body. It also is responsible for many serious community- and nosocomially- acquired infections, being the most frequently isolated bacterial pathogen from patients with hospital-acquired infections, especially patients with implants or prosthetic devices [78]. Although there are considerable knowledge gaps in staphylococcal disease burden in the tropics, recent data from Thailand show similar clinical manifestations and endocarditis prevalence, with higher mortality than industrialized countries [79].

Asymptomatic *S. aureus* colonization occurs intermittently in children and adults, most commonly in the anterior nasal vestibule, and occasionally on the skin, hair, nails, axillae, perineum, and vagina. Invasive infections of the skin occur in previously healthy individuals, ranging from impetigo to abscess formation, cellulitis and lymphadenitis. Ocular infections include conjunctivitis and endophthalmitis. *S. aureus* is a frequent cause of endocarditis with possible complications of pericarditis, respiratory tract infections, osteomyelitis and septic arthritis. According to a current estimate, *S. aureus* is responsible for 25% to 35% of endocarditis cases [80]. Most often, *S. aureus* infections are associated with medical insertion of foreign metal, plastic or Gore-Tex devices such as those used for hemodialysis, venous catheterization, or artificial prostheses. Postoperation staphylococcal disease is a constant threat to the convalescence of hospitalized surgical patients, especially in view of the increased use of prosthetic devices and indwelling catheters.

S. aureus also is the cause of a number of toxinoses, including toxic shock syndrome (TSS), food poisoning, scalded skin syndrome and necrotizing pneumonia. Staphylococcal food intoxication is the result of the presence and multiplication of *S. aureus* in food, most often transmitted by the hands of food-handlers. Several enterotoxins are involved including the heat-stable staphylococcal enterotoxin. TSS is caused by strains of *S. aureus* which produce the toxic shock syndrome toxin 1. It frequently is associated with menses, most of the times with the use of vaginal tampons, vaginal contraception sponges or diaphragms.

Before the introduction of antimicrobials in the 1940s, the mortality rate of *S. aureus* invasive infection was about 90%. The initial success of antibiotherapy was rapidly countered by the successive emergence of penicillin-resistant, then methicillin-resistant *S. aureus* (MRSA) strains [82], and since 2002 by that of vancomycin-resistant strains [83]. MRSA isolates most often are multidrug resistant. In view of its importance as a cause of community-acquired infections [84], the development of antibiotic resistance in *S. aureus* is a strong incentive that spurs vaccine development. The same is true in

veterinary medicine, where *S aureus* is an important cause of infections in animals, especially of mastitis in dairy cattle [85].

4.2.2. Bacteriology

The virulence of *S. aureus* is due to a combination of numerous virulence factors, which include surface-associated proteins that allow the bacterium to adhere to eukaryotic cell membranes, a capsular polysaccharide (CP) that protects it from opsonophagocytosis, and several exotoxins among which α -hemolysin, which lyses erythrocytes, necroses skin, and causes the release of cytokines that may produce shock; toxins A and B, which cause the sloughing of skin that characterizes the scalded skin syndrome; the toxic shock syndrome toxin-1 (TSST-1) which is responsible for most TSS cases, especially those associated with menses; and enterotoxins that cause vomiting and diarrhoeas when ingested and are responsible for food poisoning. Enterotoxins and TSST-1 have superantigen activity, which results in a massive release of cytokines that is responsible for the clinical picture of TSS. Case fatality rates in some *S. aureus* infections today still can reach 30%.

Serotyping studies of staphylococcal isolates helped reveal at least eight putative capsular serotypes, with types 5 and 8 (CP5 and CP8) accounting for ~25% and 50% of isolates recovered from humans, respectively [86] [87]. The same isolates also were recovered from poultry, cows, horses and pigs [88] [89]. Expression of CP is highly sensitive to environmental factors, such as bacterial growth conditions. Thus, staphylococci harvested in the log phase of growth express little or no CP [90]. Paradoxically, CP production attenuated staphylococcal virulence in a rat model of catheter-induced endocarditis [91], suggesting that the *S aureus* binding domain for endothelial cells may be masked by the presence of CP [87].

Numerous staphylococcal virulence factors have been described, including hemolysins alpha [92] beta, gamma and delta, which hemolyse erythrocytes, necrose skin and cause the release of cytokines that can produce a shock syndrome, coagulase, which binds to host prothrombin, activates thrombin and results in the formation of fibrin from fibrinogen [93], and clumping factors ClfA and ClfB, which bind fibrinogen [94].

4.2.3. Vaccines

Substantial controversy exists as to whether *S. aureus* infections may be prevented by a vaccination approach, and, if so, which antigens should be selected and which patients should be targeted for vaccination. An early attempt at using a killed whole-cell vaccine combined with an α -hemolysin toxoid was a failure, as no protection could be observed against peritonitis, catheter-associated infection or asymptomatic carriage in patients undergoing continuous ambulatory peritoneal dialysis.

Attention then shifted to the *S. aureus* capsular polysaccharide (CP). Type 5 and 8 CP conjugated to a detoxified recombinant *Pseudomonas aeruginosa* exotoxin A carrier were shown to be highly immunogenic and protective in a mouse model [95] [96] and passive transfer of the CP5-specific antibodies from the immunized animals induced protection against systemic infection in mice [97] and against endocarditis in rats challenged with a serotype 5 *S aureus* [98]. A bivalent CP5 and CP8 conjugate vaccine (StaphVAX, Nabi Biopharmaceutical) was developed that provided 75% protection in mice against *S. aureus* challenge. The vaccine was tested on humans in a double blind, Phase III clinical trial on 1850 end-stage renal disease (ESRD) patients in hemodialysis: a 60% efficacy was observed for up to 10 months following vaccination but the figure dropped to 26% at 1 year, mirroring the decline in antibody levels [99] [100] [101]. A second Phase III trial was started on end-stage ESRD patients on hemodialysis who were vaccinated twice 8 months apart, but the vaccine was found not to be effective in that confirmatory trial [102].

Immunization with poly-N-acetylglucosamine [103] or poly-N-succinyl glucosamine (PNSG) [104], both *S aureus* surface carbohydrates, or with staphylococcal surface proteins such as clumping factor A (ClfA) [106], clumping factor B (ClfB) [107], iron-regulated surface determinant B (IsdB) [108] or

fibronectin-binding protein (FnBP) together with ClfA [109] was shown to generate at least partial protection against *S aureus* challenge in experimental animal models. An IsdB-based vaccine is now in Phase II clinical trials.

The reverse vaccinology approach [111] which led to the identification of multiple conserved surface proteins in group B meningococci [75] (see above) and their eventual combination into a broad-spectrum vaccine candidate, was also used to identify surface proteins of *S aureus* and test their capacity to generate protective immune responses against invasive staphylococcal disease [112]. Surface proteins ClfA, IsdA, IsdB, SdrD and SdrE were identified as generating the highest levels of protection against staphylococcal renal infection in mice. IsdA, IsdB, SdrD and SdrE were combined together into a candidate vaccine that induced high level opsonophagocytic antibodies in rabbits and generated protective immunity against lethal challenge infections with a variety of *S aureus* clinical isolates in mice [112]. This hopefully will pave the way to the development of a successful, multi-antigen vaccine that can protect humans at high risk for invasive *S aureus* infection.

Attempts were also made at developing immunotherapy products for passive anti-staphylococcal immunization (Nabi, Inhibitex and Biosynexus).

4.3. [Group A Streptococcus](#)

4.3.1. *Disease burden*

Streptococcus pyogenes (group A streptococcus, GAS) is an important species of Gram-positive extracellular bacterial pathogen which colonizes the throat or skin and is responsible for a broad spectrum of diseases that range from simple and uncomplicated pharyngitis and skin infections (impetigo, erysipelas, and cellulitis) to scarlet fever and life-threatening invasive illnesses including pneumonia, bacteremia, necrotizing fasciitis, streptococcal toxic shock syndrome (TSS), and nonsuppurative sequelae such as acute rheumatic fever, reactive arthritis and glomerulonephritis [114]. Streptococcal pharyngitis continues to be one of the most common childhood illnesses throughout the world, with an estimated 7.3 million outpatient physician visits each year among children in the USA, 15% to 36% of which are due to GAS [116]. Rheumatic fever (RF) is a delayed sequel to GAS pharyngitis. The disease seems to be of an autoimmune nature, resulting from the production of autoreactive T cells and antibodies that recognize myosin, tropomyosin, keratin and N-acetylglucosamine as well as streptococcal M protein and streptococcal membranes [118]. The incidence of RF has declined in industrialized countries since the 1950s to reach today an annual figure of around 0.5 cases per 100 000 school age children. In contrast, it remains an endemic disease in developing countries with annual incidence rates ranging from 100 to 200 cases per 100 000 school-age children. It also is a major cause of cardiovascular mortality in these countries [119]. The WHO has estimated that 12 million people worldwide have rheumatic heart disease, of whom 400 000 die every year [120]. Australia's aboriginal population suffers the highest incidence worldwide [122].

GAS also is an important cause of severe infection such as streptococcal TSS and necrotizing fasciitis. Approximately 9700 cases of invasive disease and 1300 deaths are attributed to GAS each year in the USA [124]. Rates of severe GAS infection reach 2.5 to 3/100 000 population in the northern European countries [126]. It has recently been estimated that there currently are more than 18 million cases of severe group A streptococcal disease such as rheumatic heart disease in the world, with more than 500,000 deaths each year. Considerable overlap has been observed between GAS strains that cause pharyngitis in children and those associated with invasive disease in the community, suggesting that school-age children serve as a reservoir of infection for the community [128]. Prospective, longitudinal studies are clearly needed to better understand the epidemiology of streptococcal infections in developing countries and implement more effective public health prevention programs.

4.3.2. Bacteriology

Group A streptococci are gram-positive bacteria covered with an outer hyaluronic acid capsule and a layer of group A carbohydrate, a polymer of rhamnose with N-acetylglucosamine side chains. In addition, molecules of M protein attached to the bacterial membrane extend from the cell surface as coiled-coil fibers that appear as fibrils on the surface of the bacterium [129]. More than 60 years ago, Lancefield and Dole described serotyping of GAS based on a trypsin-sensitive surface antigen, the M protein, and a variable trypsin-resistant antigen, the T antigen, which turned out to be pilus structures made of adhesion proteins [131] that promote pharyngeal cell adhesion and biofilm formation [132]. Some 20 T serotypes have been identified.

The M protein is a major surface protein of GAS, with more than 130 distinct serotypes identified [133]. The cloning and sequencing of the corresponding *emm* genes revealed repeating sequence motifs in the N-ter region of the protein, called the A region, which confers serotype specificity on the bacterial strain and induces strain-specific protective immunity against GAS infection. GAS clinical isolates *emm* typing in various settings did not show significant association between *emm* type and throat or skin isolates [136]. The M protein also is a virulence factor as it binds complement regulatory protein factor H and inhibits phagocytosis.

Another important virulence factor found at the surface of GAS is the C5a peptidase, an endopeptidase that cleaves the complement-derived chemotaxin C5a, inhibiting the recruitment of phagocytic cells to the site of infection [138]. Serum opacity factor (SOF) is yet another virulence factor expressed at the surface of *S. pyogenes*. It binds apolipoprotein A1 and disrupts the structure of high density lipoproteins. It also binds fibronectin and fibrinogen [139]. Among other surface proteins are the fibronectin-binding proteins Sfb1, FBP54 and R28, which, together with the M protein and the hyaluronic acid capsule, allow the bacterium to adhere to, colonize and invade human skin and mucus membranes [143].

Attachment of GAS to pharyngeal or dermal epithelial cells is the key initial step in colonization of the host. The attachment process actually involves multiple GAS proteins [144] including lipoteichoic acid (LTA), which binds fibronectin, the M protein, which binds CD46 on keratinocytes, the fibronectin-binding protein (FBP54), the F protein (SfBI), the serum opacity factor, and any number of other factors. The M protein and SfBI also are described as invasins, as they help intracellular invasion of epithelial cells by the bacteria. Bacterial invasion and movement through normal tissue barriers also involves binding of host plasminogen /plasmin by surface proteins such as glyceraldehyde-3-phosphate dehydrogenase, enolase, and strepto kinase, a fibrinolytic plasminogen activator that has been associated with the pathogenesis of acute poststreptococcal glomerulonephritis.

Several virulence factors of GAS have been identified which play a major role in the pathogenesis of scarlet fever, TSS, invasion of soft tissues and skin and necrotizing fasciitis. These are the extracellular pyrogenic exotoxins A, B, and C as well as exotoxin F and streptococcal superantigen SSA. All these toxins trigger massive nonspecific activation of T cells and production of inflammatory interleukins and cytokines (review in [113]). GAS also secrete a variety of proteins that play a major role in tissue invasion, including hydrolases that degrade proteins and nucleic acids, and esterases such as carboxylic esterase (Sse) [145].

4.3.3. Vaccines

Group A streptococcal vaccine development faces substantial obstacles. Firstly, the widespread diversity of circulating GAS strains and M protein types is a major obstacle [146]. Opsonizing antibodies directed against the M protein are serotype-specific and there are more than 130 identified M serotypes. Secondly, immunological cross-reactivity has been demonstrated between epitopes in the M protein and several human tissues, including heart, kidney, and cartilage. Although the pathogenesis of RF is not yet fully understood, increasing evidence indicates the existence of an autoimmune process. And, thirdly, as humans are the only hosts for group A streptococci, no really relevant animal model is available.

Numerous experimental M protein-based candidate vaccines ranging from crude cell walls to highly purified M proteins were evaluated in the 1960s and 1970s but these approaches were limited by the observation of serologic cross-reactivity between epitopes in the M protein and human tissues including the heart, joints and brain [113]. Clinical studies led to a multi-decade setback in the use of M protein-based vaccines [147]. The eventual discovery that type-specific, N-terminal regions of the M protein elicited strong bactericidal immune responses and were devoid of potentially harmful cross-reactive epitopes led to the reopening of the development of multivalent M protein-based GAS vaccines.

Recent vaccine strategies have targeted either the type specific N-terminal region of the M protein or the highly conserved C-terminal region of the molecule. Immunization with the N-ter type-specific region induced protective bactericidal and opsonic antibody against the specific GAS serotype, whereas immunization with the C-ter region of the M protein protected against multiple serotypes and prevented colonization at mucosal surfaces [148]. To develop a suitable vaccine candidate, noncross-reactive, serotype-specific M protein epitopes were selected and linked to a conserved 14 amino acid-long epitope in the C-terminal half of the protein, J14, which is shared by about 70% of isolates. Prototype vaccine constructs have demonstrated excellent immunogenicity and protection in mice [151] and tolerance in human volunteers, including a hexavalent, a heptavalent and a 26-valent M protein vaccine [154]. The 26-valent M peptide vaccine was recently evaluated in a Phase II clinical trial in Canada.

In parallel, other attempts were made at developing M protein-based subunit GAS vaccines [155]. One such approach was based on the use of a lipid core peptide (LCP) system that was chemically linked to N-ter and/or C-ter peptides from the M protein and which induced long-lasting protection against GAS infection in mice, based on the induction of opsonic antibody [146] [159]. The candidate vaccine was also effective in mice when administered by the intra-nasal route in the presence of cholera toxin B subunit [160]. Another candidate vaccine was based on the J14 peptide from the M protein incorporated into a lipoprotein construct with a universal T cell epitope and a self-adjuvanting lipid moiety, Pam(2)Cys [151].

Again, the major problem faced with M protein-based vaccines is the very high diversity of *S pyogenes* strains in the field [162]. To bypass this obstacle, several attempts are being made at developing vaccines based on other bacterial proteins. Active and passive intranasal immunization with GAS surface protein C5a peptidase (SCPA) was shown to prevent infection of nasal mucosa-associated lymphoid tissue in mice [163]. The C5a peptidase is 95% to 98% identical among different GAS serotypes. A strong immune response to SCPA was observed in serum samples from children with GAS pharyngitis [164]. Similar observations were made with the streptococcal esterase Sse [145]. Other candidate vaccine antigens include the fibronectin-binding proteins [167], exotoxins SPE A and SPE C that are involved in TSS and scarlet fever [169], streptococcal immunoglobulin-binding protein Sib 35 [170], group A carbohydrate-protein conjugates [171] and extracellular lipoproteins [172]. Other surface proteins have recently been identified through a reverse vaccinology approach [173], including the Spy 1325 protein [174] and the serine protease Spy 0416 [175].

Efficacy and long-term safety trials of group A streptococcal vaccines are expected to be difficult, as they will require both time and large sample sizes, especially if efficacy endpoints are clinical endpoints. WHO is currently developing standard protocols for the clinical evaluation of group A streptococcal vaccines.

4.4. [Group B Streptococcus](#)

4.4.1. *Disease burden*

Infection with group B streptococcus (GBS), also known as *Streptococcus agalactiae*, is one of the most important infectious causes of neonatal morbidity and mortality, causing meningitis, pneumonia and septicemia in the newborns and their mothers. Women vaginally or rectally colonized with GBS during pregnancy are at increased risk of transmitting the bacteria to their newborn infant during labor and delivery. Vaginal GBS colonization has been reported to occur in about 12%-27% of women

worldwide. In the newborn, early onset of the disease presents as pneumonia and bacteremia within the first 7 days of life, and shows a case fatality rate of 4% to 29%, whereas late onset disease primarily occurs in the form of meningitis between 7 and 90 days of age, and shows a case fatality rate of 2% to 6% only. However, late onset disease often is followed by up to 30% permanent sequelae including hearing loss, retardation, and cerebral palsy. During the 1990s, the increased use of intrapartum antibiotic prophylaxis led to an 80% reduction in the incidence rate of early-onset disease [178] but the rate of late onset disease was little affected. An estimated 1 300 cases of late-onset GBS occur annually in the USA, most often in infants born before 37 weeks of gestation [179]. Invasive GBS disease also has been frequently reported in adults with diabetes, neurological impairment, breast cancer and cirrhosis. Its manifestations include soft tissue infections, bone and joint infections and pneumonia, or, more rarely, endocarditis and meningitis. Adults over 65 years of age are at the highest risk of death from invasive GBS disease [183].

4.4.2. Bacteriology

GBS (*Streptococcus agalactiae*) are aerobic Gram-positive diplococci that can be divided into 9 serotypes (Ia, Ib, II through VIII) based on the antigenicity of their capsular polysaccharide (CP) [184]. CP serotype distribution varies with geographic areas. Some serotypes, for example CP III, seem to be associated with more severe disease, particularly neonatal sepsis. This might have to do with the sialic acid residues incorporated into the CP primary structure. Well characterized virulence factors are involved in attachment of GBS and tissue invasion, including the capsular polysaccharide, lipoteichoic acid, beta hemolysins that lyse epithelial and endothelial cells leading to tissue damage and spread of infection through host tissues, and the C protein that allows epithelial cell adherence.

4.4.3. Vaccines

Active immunization of mothers during the third trimester of pregnancy to elicit an antibody response and passively immunize the newborns represents an attractive strategy to protect the neonates from GBS infection [185]. The capsular polysaccharides (CP) from all 9 currently identified GBS serotypes were found to elicit serotype-specific protective immunity in animal models, but showed low immunogenicity if not conjugated to a protein carrier [186]. Conjugate vaccines were developed using a variety of protein carriers including tetanus and diphtheria toxoids, as well as a recombinant cholera toxin B subunit (CTB). Most of the resulting formulations were tested in mice [189]. Those few which were tested in Phase I and II clinical trials in elderly human adults and nonpregnant women [190] were well-tolerated and elicited a dose-dependent antibody response that correlated with *in vitro* opsonophagocytosis [181] [190][193]. A type III CP-TT conjugate vaccine administered to third trimester-pregnant women was well tolerated and induced CP-specific antibodies that were efficiently transported to the infant and could be detected through 2 months of life [195]. A standardized, high-throughput opsonophagocytosis assay that uses differentiated human HL-60 effector cells has recently been developed to measure the functional activity of antibodies induced by conjugate CP vaccines [196].

A more recent approach at developing GBS vaccines stemmed from the observation that low levels of maternal and neonatal antibodies to GBS surface proteins were associated with invasive neonatal GBS disease [197]. The search for GBS surface proteins allowed the discovery of many surface proteins that induced protective antibodies in animals, including the alpha and beta C proteins [199], the alpha-like protein 3 (Alp3), the Rib protein [201], the surface immunogenic protein (Sip) [202], and the C5a peptidase [204]. Encapsulated C5a peptidase administered by the intranasal route induced both IgG and IgA in the vaginal tract of mice that protected both mothers and pups against GBS challenge. Purified GBS surface proteins could be used as effective carriers for conjugate CP vaccines while simultaneously inducing protective immunity against GBS. An improved Beta C protein carrier was recently developed which significantly improved the protective efficacy of a CP conjugate vaccine in

mouse pups [205]. Microscience (USA) and Intercell (Austria) are developing vaccines based on novel surface protein candidates.

By sequence analysis of the GBS genome [206], several putative surface-exposed, highly conserved proteins have also been found, including the SAP protein with pullulanase activity which induces functional cross-reactive antibodies interfering with both GAS and GBS infections [207]. Still another approach was recently explored by Novartis in Siena which described the presence of 3 pilus variants in all GBS strains tested [210]. A combination of the 3 pilus components provided protection in animal models against all GBS challenge strains that were tested, paving the way for the development of a pilus-based vaccine [211].

A major difficulty in developing Group B streptococcal vaccines is the existence of a multiplicity of serotypes with different geographical distributions [183]. A vaccine suitable for Asian or European populations may not be suitable for African populations. Another difficulty, similar to that encountered with Group A streptococcal vaccines, is the implementation of efficacy trials. A Phase III evaluation of candidate vaccines in women before pregnancy will require large sample sizes and take a long time. Administration of the vaccine to pregnant women may be difficult because of fear of risks of birth defects and subsequent liability.