A narrative review of invasive *Haemophilus influenzae* infections after three decades of Hib protein-conjugate vaccine use.

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SUMMARY

Haemophilus influenzae serotype b (Hib) was previously the most common cause of bacterial meningitis and an important etiologic agent of pneumonia in children aged <5years. Its major virulence factor is the polyribosyl ribitol phosphate (PRP) polysaccharide capsule. In the 1980s, PRP-protein conjugate Hib vaccines were developed and are now included in almost all national immunization programs (NIPs), achieving a sustained decline in invasive Hib infections. However, invasive Hib disease has not yet been eliminated in countries with low vaccine coverage and sporadic outbreaks of Hib infection still occur occasionally in countries with high vaccine coverage. Over the past two decades, other capsulated serotypes have been recognized increasingly as causing invasive infections. Serotype a is now a major cause of invasive infection in indigenous communities of North America, prompting a possible requirement for an Hia conjugate vaccine. Serotypes e and f are now more common than b in Europe. Significant year-on-year increases in non-typeable *H. influenzae* (NTHi) invasive infections have occurred in many regions of the world. Invasive *H. influenzae* infections are now seen predominantly in patients at the extremes of life and in those with underlying co-morbidities. This review provides a comprehensive and critical overview of the current global epidemiology of invasive *H. influenzae* infections in different geographic regions of the world. It discusses who is now at risk of invasive Hib disease, describes the emergence of other severe invasive *H. influenzae* infections and emphasizes the importance of long-term comprehensive, clinical and microbiologic surveillance to monitor a vaccine's impact.

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KEYWORDS *Haemophilus influenzae*, Hib, conjugate vaccine, immunization, epidemiology, non-typeable *H. influenzae.*

INTRODUCTION

Haemophilus species are fastidious Gram-negative coccobacilli, requiring one or both of two accessory growth factors: X factor (hemin) and V factor (nicotinamide adenine dinucleotide). *H. influenzae* is restricted to humans, colonizing the nose and throat, and to a lesser extent the conjunctivae and genital tract. It is differentiated into six capsular types (a to f) according to its capsular polysaccharide structure. Other strains do not have a capsule and are classified as non-encapsulated or non-typeable *H. influenzae* (NTHi). The most virulent serotype is *H. influenzae* type b (Hib) and the capsule, composed of polyribosyl ribitol phosphate (PRP), is the predominant virulence determinant. Studies on unimmunized individuals in Finland found that serum anti-PRP immunoglobulin-G (IgG) antibody concentrations ≥0.15µg/mL correlated with a lower incidence of Hib meningitis (1, 2). Additional studies determined that serum anti-PRP IgG antibody levels ≥0.15µg/mL provide short-term protection against invasive Hib disease, but long-term protection requires concentrations ≥1.0µg/mL(3). It is now 30 years since Hib protein-conjugate vaccines were first licensed and since then Hib immunization has been incorporated into almost all national infant immunization (NIP) programs globally. Wherever Hib protein-conjugate vaccines have been introduced and high coverage achieved, there has been a profound and a sustained decline in the incidence of invasive Hib disease. This review starts by examining the impact of Hib protein-conjugate vaccines during these three decades and the consequent changes in the worldwide epidemiology and disease burden of Hib and other invasive *H. influenzae* infections. The emergence of NTHi and other *H. influenzae* non-b

serotypes as important invasive pathogens is emphasized. The increased identification of NTHi invasive infections in vulnerable patient populations where infection is associated with high rates of morbidity and mortality, and *H. influenzae* type a (Hia) as a major cause of invasive disease in Indigenous groups in North America are highlighted. The impact of Hib conjugate vaccines on antimicrobial resistance in *H. influenzae* is reviewed. Finally, we conclude that the changing epidemiology of invasive *H. influenzae* infection emphasizes the importance of continuing surveillance and the possible need for developing new vaccines to control these emerging non-b infections.

SEARCH STRATEGY

We undertook a literature search using PubMed on 8 August 2018 employing the following terms; ((invasive) AND Haemophilus) AND influenzae) AND (non-typeable OR “nontypable” OR “NTHi” OR “serotype a” OR “serotype b” OR “serotype c” OR “serotype d”, OR “serotype e” and “serotype f”) AND (epidemiology OR “risk factor” OR “clinical” OR “outcome” OR ”antimicrobial resistance” OR “surveillance”) for papers published between 1985 and 2018. A further search was conducted on 16 November 2020 using the same search criteria.

Search Results The two literature searches identified 1482 articles These were reviewed independently by two of the authors (MPES and AWC). We searched all publications, but excluded those that were not published in English, non-human studies, carriage studies, individual case reports of Hib infections, vaccine evaluation studies, immunogenicity studies and laboratory methods. We identified 657 articles that were considered relevant. After screening the abstracts of these articles, two authors (MPES and AWC) excluded 261, leaving 396 articles. The full text articles of these 396 articles were retrieved and formed the basis for this review.

GLOBAL BURDEN OF *H. influenzae* DISEASE PRIOR TO ROUTINE Hib PROTEIN-CONJUGATE VACCINE IMMUNIZATION

Before the introduction of routine immunization with Hib protein-conjugate vaccines, Hib was the most important cause of bacterial meningitis in young children(4, 5). Furthermore, subsequent vaccine-probe studies established that both bacteremic and non-bacteremic pneumonia as the most common presentations of Hib disease (6, 7), thereby also identifying Hib as a significant cause of pneumonia in young children.

Seventy-five percent of invasive Hib infections were in children between 3 months and 3 years of age. Hib meningitis had a case fatality rate of 5% to 10% in high-income countries. Long-term sequelae following Hib meningitis was observed in 15% to 30% of survivors, manifesting as sensorineural hearing loss, blindness, spasticity, intellectual impairment, epilepsy or hydrocephalus as well as functionally important behavioral disorders, neuropsychologic impairment or auditory dysfunction adversely affecting academic performance(8, 9). While Hib was also the most common cause of acute epiglottitis, which usually presented in children aged 2 to 4 years, it was rarely seen in Indigenous children in Australia (10) or in Native Alaskan and Canadian Inuit children (11) where the greatest burden of disease was found in those aged <18 months. Other manifestations of invasive Hib infection include bacteremia without a focus of infection, orbital and periorbital cellulitis, facial cellulitis, and skeletal infections.

The incidence of invasive Hib disease varied widely between countries and in some countries between ethnic groups. The mean incidence of Hib meningitis in children aged 0 to 4 years in the United States (US) was 54/100 000 per year (range 19 to 69/100 000) 0(8). Reported rates in other parts of the world ranged from <20 to 50/100 000 per year.

However, much higher rates of Hib meningitis in this age group of 150 to 450/100 000 per year were reported in Indigenous populations residing in Alaska, Northern Canada, and Central and Northern Australia (8). In Europe, the incidence rates reported in Northern Europe were higher than those in Southern Europe (12). Patients with asplenia, sickle cell disease (SCD) and certain malignancies were also at a higher risk of developing invasive Hib infections (13).

Prior to introducing Hib protein-conjugate vaccines in the United Kingdom (UK), most cases of invasive *H. influenzae* were from Hib and affected young children, with only about 10% of all invasive Hib occurring in adults(14). Similarly, in Finland, just 6% of invasive Hib infections were in individuals aged $\geq$10 years(15). In contrast, while only approximately 10% of invasive *H. influenzae* cases in the UK were from NTHi, two-thirds of these infections involved adults, especially those >65 years of age(14).

β-lactamase resistance in *H. influenzae* increased steadily during the 1970s and 1980s. This was mainly due to TEM-type β-lactamase, or less commonly to ROB-1 β-lactamase(16). These strains are designated β-lactamase positive, ampicillin-resistant strains (BLPAR). Less commonly resistance was due to modifications in the penicillin-binding proteins (designated β-lactamase negative, ampicillin-resistant BLNAR) or both enzymatic and intrinsic resistance (β-lactamase positive, amoxicillin clavulanic acid resistant BLPACR) strains(16, 17). There was wide geographic variation globally in the rates of resistance, but overall 16.6% of all Hib strains globally were β-lactamase positive (17). After Hib immunization was introduced, the incidence of Hib infections and the rate of β-lactamase resistance in Hib isolates declined (18). However, antimicrobial resistance to a range of antibiotics in NTHi remains an important clinical challenge and is discussed later in this review.

Hib POLYSACCHARIDE VACCINE

The original Hib vaccine was a PRP plain polysaccharide vaccine. A large field trial , recruiting 100 000 children aged 3 months to 5 years in Finland demonstrated an age-dependent response to PRP, but failed to show any impact on nasopharyngeal carriage and therefore no interruption of transmission(15). The PRP vaccine did not induce protective levels of anti-PRP antibodies in children aged <18 months, but it was 90% efficacious in those aged ≥18 months. Polysaccharides are T-cell independent antigens, which lead to primarily IgM responses with little isotype switching to IgG antibody responses in infants aged <18 months. They also lack immunologic memory and fail to elicit a booster response on repeated exposure (1, 19).

Hib PROTEIN-CONJUGATE VACCINES

To address the reduced immunogenicity of polysaccharide vaccines in infants, conjugated protein-polysaccharide (glycoconjugate) vaccines were developed. (Figure 1)

Antibody responses to proteins are T-cell dependent and can be elicited from a very young age, resulting in high-affinity antibodies with isotype switching, affinity maturation and the generation of immunologic memory. Subsequent exposure to the antigen results in a significant booster response. Hib PRP protein-conjugate vaccines which activate a T-cell dependent response with high levels of protective antibodies in infants from 2 months of age were developed in the late 1980s (20). Hib nasopharyngeal carriage is reduced in those who are vaccinated, thereby interrupting transmission of *H. influenzae* serotype b to susceptible unvaccinated children and adults (i.e. herd protection)(21). Initially four different monovalent Hib protein-conjugate vaccines were produced. They used different carrier proteins, varying lengths of PRP saccharide and different protein-polysaccharide

conjugation techniques. The four protein carriers were tetanus toxoid (PRP-TT), diphtheria toxoid (PRP-D), *Neisseria meningitidis* outer membrane complex (PRP-OMP) and a non-toxic mutant *Corynebacterium diphtheriae* protein CRM_{197} (PRP-CRM). The Hib protein-conjugate vaccines had different immunologic properties, resulting in different antibody titres, avidity maturation, rapidity of response and idiotypic expression of the antibody response following vaccination (22-25). A double-blind, randomized controlled trial of the four Hib protein-conjugate vaccines administered at 2, 4 and 6 months found that only 29% of infants achieved the putative long-term protective concentration of Hib antibodies (≥1µg/mL) after three doses of PRP-D, compared with 55%, 75% and 83% of infants who had received PRP-OMP, PRP-CRM and PRP-T respectively(26). The PRP-OMP vaccine stimulated the highest antibody concentration after the first dose in infants aged as young as 2 months(27) with 57% of infants achieving an antibody concentration ≥1.0µg/mL at age 4 months. However, PRP-OMP did not produce significant increases in antibody concentration when further doses of vaccine were administered at 4 and 6 months of age. Despite this, PRP-OMP was the first vaccine used in Indigenous populations in North America and Australia where a high burden of disease existed in very young infants. Over time PRP-D was withdrawn, and Hib protein-conjugate vaccines have since been incorporated into a range of combination bivalent (Hib/MenC), pentavalent (DTaP/IPV/Hib) and hexavalent (DTaP/IPV/Hib/HepB) vaccines.

IMPACT OF Hib-PROTEIN-CONJUGATE VACCINES ON INVASIVE Hib DISEASE

The inclusion of Hib glycoconjugate vaccines in national infant immunization programs (NIP) has led to a significant and sustained decline in invasive Hib disease across all age groups, through direct and indirect (herd) protection (28, 29). Routine use of Hib protein-conjugate

vaccine has consistently reduced the incidence of invasive Hib disease by ≥90% in high-income countries(29). The inclusion of Hib protein-conjugate vaccines into the NIP of low- and middle-income countries has been generally much slower than its introduction in high-income countries. This was due to several factors, including limited local data on the burden of Hib infections as a result of high rates of antimicrobial therapy before specimen collections for culture and difficulties in growing this fastidious organism, as well as the associated vaccine costs (30). By 2004 few low-income countries had adopted Hib vaccines. The World Health Organization (WHO) and the Global Alliance for Vaccines and Immunization (GAVI) sought to address this problem in a number of ways. Vaccine probe studies(6) and the WHO Hib Rapid Assessment Tool (HibRAT) (31) provided a prompt and timely assessment of Hib disease burden in many countries. In 2005 the GAVI Alliance established the Hib Initiative to accelerate the introduction of Hib protein-conjugate vaccine into GAVI-eligible countries(32). In 2006 the WHO advocated the global introduction of Hib protein-conjugate vaccines (33), enabling GAVI-eligible countries to apply for Hib vaccine introduction without needing to generate data on local disease burden. For GAVI-eligible countries, initially free vaccine was provided through the GAVI-Alliance with co-financing guidelines offering a financial commitment to cover vaccine costs until 2015, together with a small co-payment by each country to address concerns regarding the long-term sustainability of the program. As a result of these endeavors, the number of countries implementing Hib immunization increased from 89/193 (46%) in 2004 to 158/193 (82%) in 2009 (34). As of 2020, all countries in the world, except China, have included Hib protein-conjugate vaccines in their routine NIP (see Tables 1 to 6).

A substantial reduction in Hib disease burden has been seen wherever the vaccine has been used. In 2000, it was estimated that Hib caused approximately 8.13 million (uncertainty

range [UR] 7.33 to 13.2 million) cases of serious disease and 371 000 deaths (UR 247 000 to 527 000) per year in children aged <5 years(35). Between 2000 and 2015, the estimated global burden of Hib meningitis, pneumonia and non-pneumonic bacteremia in human immunodeficiency virus (HIV) negative children aged <5 years had declined to 340 000 cases (UR, 196 000 to 669 000) and 29 800 deaths (UR,18 600 to 41 100) (36).From 2000 to 2015 Hib-related deaths declined by 90% (78%–96%). Most Hib deaths occurred in just four countries, India (15 600 deaths, UR, 9800 to 21 500), Nigeria (3600 deaths, UR, 2200 to 51 000), China (3400 deaths, UR, 2300 to 4600) and South Sudan (1000 deaths, UR, 600 to 1400).

CURRENT EPIDEMIOLOGY OF INVASIVE *H. influenzae* DISEASE

The burden of invasive Hib disease is limited currently to a small number of countries where Hib immunization is either not freely available or has only been incorporated recently into the NIP. Reducing invasive Hib disease to the lowest possible level depends on maintaining high vaccine coverage and maintaining good surveillance systems for Hib disease.

Initially, concerns were raised that other capsulated serotypes of *H. influenzae* might occupy the ecologic niche occupied formerly by Hib and emerge as significant causes of invasive disease once Hib vaccination had been introduced. In general this has not happened in many parts of the world, with an important exception of the Indigenous communities of North America where Hia is an emerging pathogen (37, 38). There has also been a slight increase in invasive *H. influenzae* serotype e (Hie) and *H. influenzae* serotype f (Hif) cases in Europe(39, 40). In contrast, the number of cases of invasive NTHi infections have increased significantly in multiple regions of the world(41, 42). In the following sections, the current epidemiology in the different WHO designated regions are examined.

REGION OF THE AMERICAS

All countries in the WHO Region of the Americas have introduced Hib vaccine. In 2020 the average coverage of three doses of Hib vaccine was 85%(43).

The data on individual countries in the Region of the Americas are shown in Table 1.

(i) North America

Hib was a major cause of invasive bacterial infection in young children in the US and Canada before the introduction of Hib vaccine. Population-based surveillance studies in the US and Canada reported 10 000-20 000 cases annually of Hib meningitis and other serious invasive infections, with a case fatality rate of 3 to 5% and long-term sequelae in as many as 25% of meningitis survivors(44). Much higher incidence rates of invasive Hib disease were observed in Alaska Native (45), White Mountain Apache (46) and Navajo Indian children(47). The annual incidence rates of invasive Hib disease in Alaska Native (11) and Navajo Indian children (47) were 700/100 000 and 214/100 000 children aged <5years respectively . In North America, Hib infection occurred at a younger age in Indigenous than non-Indigenous children. The peak incidence of Hib meningitis in Navajo children was at age 4 to 8 months (47) and in White Mountain Apache children, 4 to 7 months of age (46). In Alaska Native children 23% of cases were in infants aged <6 months(45). In North American non-Indigenous children, the peak rate of disease was 6 to 11 months of age (44). Although most cases in North American Indigenous children presented with meningitis. case fatality rates in Navajo Indian and Alaska Native children were lower than in the US general population, 4% and 3% versus 5% respectively (44, 45, 47). However, 16% of Navajo children who survived meningitis suffered neurologic sequelae (47). Carriage of Hib in Indigenous children began at

a very young age (47), with asymptomatic carriers acting as a major reservoir and source of disease transmission.

Hib immunization was initiated in Alaska in 1991 using PRP-CRM and then changed to PRP-OMP as this vaccine elicited protective antibody levels after a single dose (27). The incidence of invasive Hib disease in Alaska Native children aged <5years fell from 309.4/100 000 in 1980-1991 to 18.3/100 000 in 1992-1995 (48). The PRP-OMP vaccine was used in Alaska until 1996 when it was replaced by a combination vaccine with a PRP-CRM component. Cases of Hib disease in Alaska Native children increased over the next year and in 1997 Alaska adopted a sequential Hib vaccine policy for Alaska Native children, with an initial dose of PRP-OMP and PRP-CRM for the rest of the schedule. Alaska reintroduced a schedule using PRP-OMP alone or a combined hepatitis/PRP-OMP vaccine in 2001 (48). Although Hib disease is largely controlled, the rate of disease is still higher among rural Alaska Native children than the rate in non-Native Alaska and other non-Indigenous US children.

In 1990 Hib protein-conjugate vaccine was added to the routine infant immunization program in the US (49). The program had a dramatic impact and by 1995 invasive Hib disease incidence in children aged <5years had declined by more than 95% (44). On-going active surveillance of invasive *H. influenzae* disease has continued in the Active Bacterial Core Surveillance (ABC) sites co-ordinated by the Centers for Disease Control and Prevention (CDC). This surveillance system encompasses a population of over 42 million in five states and five metropolitan areas across the US.(50). Invasive Hib disease continued to decline, and from 2009 to 2015, only 77 cases of invasive Hib disease were reported from the ABC sites (46) with a median patient age of 49 years. Twenty three (29.9%) Hib infections occurred in children <5 years of age, nine presenting with meningitis and six with

bacteremic pneumonia. Only five (21.7%) of the children had been age-appropriately vaccinated and had none had reported underlying comorbidities. Nine of the 54 (70.1%) Hib patients >5 years of age were aged 5 to 17 years (16.7%), 28 (51.9%) were 18 to 64 years and 17 (31.5%) were ≥65 years of age.

At the same time the risk of invasive *H. influenzae* infection in adults ≥65 years increased with age, with incidences of 3.48/100 000; 4.65/100,000; 6.48/100,000; 8.56/100,000 and 13.56/100,000 in those aged ≥65 years; 70to 74 years; 75 to 79 years; 80 to 85 years and ≥85 years respectively. The majority of infections in ≥65year old patients were caused by NTHi (79.3%) or non-b serotypes (19.9%; with serotype f the most common). Only 0.08% of cases were due to Hib. Bacteremic pneumonia was the most common presentation in those aged ≥65 years (73.4%) and 74% had at least one underlying comorbidity. In ≥6 year old patients, 19.9% of infections were fatal. The overall incidence of invasive *H. influenzae* disease was 1.70/100 000 population, with a case fatality rate of 14.5%. NTHi had the highest incidence (1.22/100 000) and case fatality (16%) compared with Hib (0.03/100 000 and 4% respectively) and non-b capsulated *H. influenzae* serotypes (0.45/100 000 and 11% respectively).

The incidence of invasive *H. influenzae* disease was greatest in children <1 year of age (8.45/100 000) and adults aged ≥65 years (6.30/100 000). In all, 140/317 (44.2%) cases in infants <1year of age occurred in the first month of life, and of these 127/140 (90.7%) were in the first week of life, with 109/127 (77.9%) presenting at birth. Most who developed infection in the first month of life were pre-term and low-birthweight infants and 80.7% of these infections were from bacteremia with 98.5% caused by NTHi. The incidence in

children <5 years of age was 2.84/100 000 with a substantially higher incidence in Alaska Native and American Indian children (51).

From 2002 to 2015, the estimated overall incidence of invasive *H. influenzae* infections increased by 2% per year(51, 52). Annual incidence of NTHi and Hia invasive infections increased by 3% and 13% respectively. The incidence of all other non-b serotypes declined or remained the same. The case-fatality rates of NTHi and Hia infections were 16.1% and 8.3% respectively. Among the non-b encapsulated serotypes, Hia affected predominantly children <5years of age, Hif had the highest overall incidence (0.27/100 000) and Hie exhibited a high case fatality rate (18.4%). While Hid was detected rarely, it had the highest case fatality ratio (50.0%).

From 2017 to 2018, invasive NTHi infections in HIV-infected men who had sex with men increased in metropolitan Atlanta, Georgia (53). The incidence of invasive NTHi infection in HIV positive individuals (41.7/100 000) in 2017 to 2018 was significantly greater than in HIV positive individuals from 2008 to 2016 (9.9/100 000; $p<0.001$) and from those without HIV (1.1/100 000; $p<0.001$) from 2008 to 2018. Comparing NTHI infections in 2017 to 2018 and in 2008 to 2016, HIV positive adults aged 18 to 55 years with NTHi infection in 2017 to 2018 were significantly more likely to be male (94% versus 49%; $p<0.001$), Afro-American (100% versus 53%; $p<0.001$) and to have septic arthritis (35% versus 1%; $p<0.001$) than HIV negative adults with NTHi infection in 2008 to 2016. Whole genome sequencing confirmed two distinct, but genetically related clonal lineages of NTHi. Among 38 cases of NTHi infection in persons with HIV, all were male Afro-Americans with a median age of 34.5 years and 82% reported having sex with men. Thirty-two (84%) of the men lived in two districts in close geographic proximity.

There is an increased risk of invasive infections caused by encapsulated bacteria, including Hib, in children with sickle cell disease (SCD). In the US, antibiotic prophylaxis and Hib and pneumococcal conjugate vaccines have led to a marked decline in the overall rates of invasive infections in SCD, including Hib infections. A retrospective review of the medical records of 815 children with SCD at the Children's Hospital of Philadelphia from 2000 to 2010 did not find a single case of Hib bacteremia (54). In one study in a pediatric tertiary care center in Atlanta, Georgia there were 8 episodes of *H. influenzae* bacteremia over a 17-year period (2000 to 2017): 5 Hif, 2NTHi and 1 Hia (55). Seven of the 8 infections occurred in children aged <5years, with an incidence of 1.6/1000 person-years in this age group. Although uncommon, the possibility of non-Hib *H. influenzae* bacteremia should therefore also be considered in children with SCD.

In the pre-Hib vaccine era, Hia as a cause of invasive *H. influenzae* disease was overshadowed by Hib, and epidemiologic data on Hia disease prior to 2000 are very limited. However, during the last decade, Hia has been recognized increasingly as a clinically significant pathogen causing severe disease and high case fatality rates in certain geographic areas and populations (38). Based on animal experiments, Hia was found to be the second most virulent serotype after Hib (38). Indeed, Hia and Hib share many similarities: both serotypes cause invasive disease preferentially in young children and both pathogens are characterized by serious infections, including meningitis, bacteremic pneumonia and septic arthritis (38). Whereas at present invasive Hia disease is extremely uncommon in Europe or Asia, it has been reported consistently from certain regions of North America where Indigenous people comprise a high proportion of the population, with concerning trends towards increasing incidence rates in some regions (38, 56).

In Alaska, Hia has been recognized as an emerging pathogen; no Hia disease was identified before 2002, but since then the incidence has been increasing and outbreaks have occurred (57). The highest incidence rate of invasive Hia disease are reported in Alaska Native children (18/100 000 <5 years of age [2002 to 2011]) with most cases occurring in south-western Alaska where the incidence reached 204/100 000 in those aged <5 years in 2010 to 2011 (57). The latest report on pediatric Hia disease in Alaska (2002 to 2014) noted the severity of the infection (presenting as meningitis in 42% of cases) and a high prevalence of unfavorable outcomes, including death (11%) or neurologic sequelae (14%) (58). All, but two of 36 (94%) affected children were Alaska Natives, and 32 (89%) were <18 months of age (58).

The 10 US ABC surveillance sites, which encompass 12%–13.7% of the US population, reported the average annual incidence of Hia disease increased by 13% from 2002 to 2015, with the greatest burden of disease being observed in American Indian and Alaska Native children <5years of age (51). The estimated incidence of invasive Hia disease in these children was 16.94, 14.44, and 3.37/100 000 population aged <1 year, 1 year, and 2 to 4 years, respectively. From 2011 to 2015, the medical records of 169 patients with Hia infection were reviewed: 96% were admitted to hospital, 23.5% required ventilatory support, 47.5% needed intensive care and 6.2% died during hospitalization. In children aged <1year, meningitis was the most common clinical presentation (71.4%). Overall, 17.7% of patients had adverse sequelae following discharge from hospital, as did 17.8% 1 year after the onset of the infection. Patients with meningitis had the highest proportion of sequelae (48.5%), including deafness and developmental and/or speech delay, with all adverse outcomes documented in children aged <5 years.

In addition to the ABC areas of surveillance (59), Hia has emerged as a significant pathogen in Utah (60, 61), and North and South Dakota(62). In a study in Utah from 1998 to 2008, 28% of all invasive disease in children aged <5 years was due to Hia, and 18% due to Hib. Fifty percent of the Hia cases presented as meningitis. Interestingly, Hia and Hib did not occur predominantly in American Indian children in these states. The authors noted that Utah has the highest birth rate (21/1000 population) and largest household size in the US(60). A recent study from Utah described 51 cases of invasive Hia disease in children over the ten-year period 2007 to 2017(61). Meningitis was the most common clinical presentation (53%) with pneumonia and septic arthritis each accounting for 14% of cases; 43% of children required admission to an intensive care unit and one child died (61). In Canada, the highest incidence rates of invasive Hia are reported from the Nunavut and Nunavik territories in Northern Canada, which are populated mainly by Inuit people where Hia epidemiology is very similar to that in Alaska. In these Canadian territories, Hia affects mainly young Indigenous children, has severe clinical presentation, such as meningitis with high case fatality rates, and causes outbreaks when the incidence can reach 300/100 000 children age <1 year(56, 63). In addition, Hia disease has been reported consistently from northern parts of the Canadian provinces of Manitoba, Ontario, and Quebec, regions largely inhabited by Indigenous peoples(38, 56, 64). Thus, invasive Hia disease is now prevalent in populations of North American Indigenous people where several outbreaks have occurred since 2002, although sporadic cases have been reported from countries outside this continent. The emergence of Hia as a significant cause of invasive disease has prompted moves to develop a conjugated Hia vaccine(65), which if effective could prevent the high rates of morbidity and mortality seen with invasive Hia disease in addition to saving healthcare systems millions of dollars in both acute and long-term care (65).

(ii) Latin America and the Caribbean

Before Hib protein-conjugate vaccines were introduced, incidence rates of Hib meningitis and all invasive Hib disease in 16 countries of this region were estimated to be 35/100 000 and 60/100 000 in children aged <5years respectively (66). The first country in Latin America to introduce large scale immunization with Hib protein-conjugate vaccines was Uruguay in 1994(67). By 2006, all countries and territories in this region, except Haiti (68), had incorporated Hib vaccines into their NIPs (Table 1). Haiti subsequently introduced pentavalent vaccine (DTwP-HepB-Hib) in 2012(69) . As in other regions, cases of Hib meningitis have declined substantially with the use of Hib vaccines(70). Surveillance in four countries suggested that high vaccine coverage resulted in a low incidence of Hib meningitis and low rates of Hib nasopharyngeal carriage in countries that used a three dose primary course with (Uruguay and Argentina) or without (Colombia and Chile) a booster dose in the second-year of life(67). In Mexico, a cross-sectional study (71) undertaken in 2007 found that only 40 to 50% of 110 children aged 12 to 23 months of age had anti-PRP antibody titres >1µg/mL, the putative concentration required for long-term protection, despite 92% of these children having been fully immunized with 3 doses of Hib combination vaccine given at 2, 4 and 6 months of age. As a result, Mexico introduced a booster dose of an acellular pertussis pentavalent vaccine (DTaP-Hib-IPV) in 2007 (71).

Hia infections have also been recorded in several Caribbean and South American countries, including Columbia, Venezuela, Argentina and Cuba with most reports coming from Brazil (56). In the first-year of routine Hib immunization an increase in Hia meningitis in children <5years of age was reported in Salvador, Brazil (72), but this does not appear to have been sustained over subsequent years (73). In Paraguay, invasive Hib infections in children

<5 years of age declined following the introduction of Hib vaccine, but was an associated increase in invasive NTHi infections in older children and adults (74).

EUROPEAN REGION

Almost all nations in the WHO European Region have included Hib vaccine in their recommended immunization programs. In the Russian Federation, Hib vaccines are recommended for certain risk groups, but are not yet included in the NIP. In 2019 the average coverage of 3 doses of Hib vaccine in the WHO European Region was 85%(75).

The data on individual countries in the region are shown in Table 2.

The mean annual incidence of Hib meningitis among children <5 years of age in Europe was 23/100 000 before Hib vaccination was implemented(76). By 2005 all countries in the European Union had introduced Hib vaccine (Table 2). It was estimated that there were 11 300 (UR 6500-22 400) cases of severe Hib infection in the WHO European Region in 2015 (36). This study only reported data on invasive Hib infections and did not analyse data on infections due to NTHi or other serotypes. From 1996 to 2006 surveillance of invasive *H. influenzae* infections in western Europe was undertaken by the European Union Invasive Bacterial Infection Surveillance (EU-IBIS) network (funded by the European Commission DG SANCO)(77). In 2007 coordination of the network was transferred to the European Centre for Disease Prevention and Control (ECDC)(77). Data from this network collected from 1996 to 2006(40) and from 2007 to 2014 (78) have been reported separately. Between 1996 and 2006, 14 countries reported 10 081 cases of invasive *H. influenzae* infection, and serotype data were reported for 9117 (80.5%) of the isolates: 44% were due to NTHi, 28% were Hib and 7.9% were from other capsulated serotypes. Some isolates were “non-Hib,” but were not fully serotyped and others were not available for serotyping at the reference laboratory.

By 2006, national reference laboratories in 14 European countries with an annual denominator population of 150 million persons routinely serotyped all invasive *H. influenzae* isolates(40).

From 2007 to 2014, 12 European countries reported 10 624 cases of invasive *H. influenzae* infection (78). Serotyping data were reported for 8781 (83%) isolates. A total of 6853 (78%) were NTHi, 828 (9%) Hif, 239 (3%) Hie, and 811 (9%) Hib. By 2014 the notification rates for invasive Hib in children aged <1 year and 1 to 4 years had declined to 0.65/100 000 and 0.18/100 000 respectively (78). The notification rates for all invasive *H. influenzae* infections gradually increased from 0.27/100 000 in 1999 to 0.56/100 000 in 2014, driven by changes in the incidence of NTHi and to a lesser extent by non-b capsulated serotypes. The incidence of invasive *H. influenzae* infections in adults aged ≥65 years increased from 0.53/100 000 in 1999 to 1.55/100 000 in 2014. Invasive NTHi infections occurred in older patients than Hib infections (median age 58years versus 5years [p<0.0001]) and were associated with a higher case fatality rate (12% versus 4% [p<0.001], especially in infants (17% versus 3% [p<0.001]). Non-b serotypes were almost exclusively Hif (72%) and Hie (21%) with a case fatality rate of 9%.

In 1992 the Hib protein-conjugate vaccine was introduced in the UK, with a 2, 3 and 4 months schedule and no booster in the second year of life (79). Simultaneously, a catch-up program offered Hib vaccine to all children aged <4 years. There was a rapid and sustained decline in invasive Hib disease in children <5 years of age from 23.8/100 000 in 1991-1992 to 1.8/100 000 in 1993-1994. The decline in the vaccinated age group was soon followed by a decline in other age groups through herd protection (80). This continued until 1998 when the estimated incidence of Hib disease in children aged <5years was 0.63/100 000(80).

However, from 1999 cases of invasive Hib infection in children increased (81) with 134 cases in children aged <5years in 2002 compared with 31 cases in 1996 (Figure 3).

There were several reasons for this resurgence(79). Firstly, immunization in infancy resulted in a lower than expected vaccine effectiveness (VE), which was also lower than the VE in children who had been given one dose of Hib vaccine at an older age in the catch-up program. The VE in those immunized in infancy declined over time reaching zero after 1 year. This only became apparent after the direct and indirect protection provided by the catch-up program had waned. By 1998 all children aged <5 years had only received Hib vaccine in infancy. Next, a shortage of the combination vaccine used in the UK at that time, which had a whole cell pertussis component (DTwP-Hib) resulted in utilizing an alternative combination vaccine containing an acellular component (DTaP-Hib). This vaccine was given to approximately 50% of infants (82). The Hib component of some DTaP-Hib vaccines can be less immunogenic, particularly when administered in an early, accelerated schedule, as was the case in the UK (83). A third possible reason was the introduction of Meningococcal group C protein-conjugate vaccine (MenC) in 1999, which was co-administered with the Hib vaccine. The MenC vaccine used was mainly CRM-based and there is evidence that the MenC-CRM vaccine given with DTaP-Hib also reduces the immunogenicity of the Hib component (84).

Control of the resurgence was achieved by providing a single dose of Hib vaccine to all children aged 6 months to 4 years in 2003. In 2004, a 12 month booster dose of Hib vaccine was added to the NIP. In 2007, there was a second campaign administering a pre-school booster to children who were too old for the 12-month booster, but too young for the campaign in 2003. These actions led to a rapid decline in Hib disease(79). The UK experience

indicated protective levels of serum anti-PRP antibodies at the time of nasopharyngeal acquisition are necessary and immunologic memory alone will not prevent colonization. By giving a booster dose of vaccine in the second year of life, serum anti-PRP antibody titres are maintained above the protective threshold in children <5 years of age.

Between 2001 and 2010 there were annual increases in England and Wales of 11.0% and 7.4% in Hif and Hie infections respectively. Hif incidence was 0.09 (95% confidence interval [CI] 0.07–1.10) per 100 000 persons and Hie incidence was 0.03 (95%CI 0.02–0.04) per 100 000 persons in 2009-2010, with the majority of cases occurring in infants and older adults (39). There were 1275 invasive *H. influenzae* infections reported in 2009-2010, comprising 715 (56.1%) NTHi, 99 (7.8%) Hif, 69 (5.4%) Hib and 33 (2.6%) Hie cases. In the remaining 359 (28.2%) cases the serotype was unknown.

Of the 132 cases of Hif and Hie infection, 25 (18.9%) were in children aged <15years. Ten were in previously healthy infants aged <1 year: nine had meningitis and one infant had Hif septic arthritis. Three Hie meningitis cases died and the two survivors had long-term sequelae. All Hif cases survived without sequelae. Over half of the cases (52 Hif, 14 Hie) occurred in adults ≥65years of age. Fifty of the 52 (96.2%) cases of Hif infections had underlying comorbidities (including 11 with cancer) and the most frequent presentation was pneumonia (35/50 [70%]) followed by sepsis (9/50 [18%]). The case fatality rate in this age group was 54% (27/50). Thirteen of 14 Hie infections (93.9%) had comorbidities, and again pneumonia (10/14 [71.4%]) was the most common presentation. Eight of the Hie patients (57.1%) died. Hie and Hif invasive infections are comparable to invasive NTHi infections by generally presenting as pneumonia in older adults with underlying comorbidities. Hie causes more cases of meningitis and has a higher case fatality rate compared to Hif infections.

From 2012 to 2016, a low incidence of invasive Hib disease in all age groups was reported in England (0.05/100 000 population) with just 67 cases of Hib reported (85). In contrast, during this period there were 2451 cases of NTHi (85.0%), 274 (9.5%) Hif, and 85 (2.9%) Hie invasive disease. There were only four cases of Hia and just two cases of *H. influenzae* serotype d (Hid). Indeed, the incidence of NTHi increased 10-fold from 0.12/100 000 in 2012 to 1.2/100 000 in 2016.

More recent data from 2017-2018 from four European countries, England, Germany, Finland and Italy(86-89) also indicate the predominance of NTHi invasive infections (76.7% to84.5%). In contrast, Hif was the most prevalent capsulated serotype in England, Germany and Finland (9.8%–12.6%), whereas Hib was the most frequent capsulated serotype in Italy (11.5%). Hie was less commonly isolated (2.4%to3.4%). Hia caused only five invasive infections in England from 2008 to 2015 but caused 10 infections in the twelvemonths between December 2016 and December 2017(86).

In a detailed analysis of 4044 invasive *H. influenzae* infections in Germany, 2001 to 2016, NTHi caused 1545/1902 (81%) infections where the strain was available for typing(90). Among the capsulated strains, 69% were Hif and 17% were Hib. NTHi and Hif infections in those aged <5years and ≥60years respectively, and ampicillin-resistance in NTHi, all increased significantly.

In Portugal, 260 invasive *H. influenzae* isolates received from 2011 to 2018 were characterized (91): NTHi 206 (79.2%); Hib 35 (13.5%); Hif 8 (3.1%); Hia 7 (2.7%); and Hie 4 (1.5%). The NTHi strains were mainly isolated from adults (161/260 [78.2%]), especially among those ≥65 years of age (103/161 [64.0%]), whereas 56.3% of *H. influenzae* infections in children were due to NTHi. Most of the capsulated strains causing infection were from

pre-school aged children (35/54 [64.8%]): 25 Hib; 7 Hia, 2 Hif and 1 Hie. Six cases of Hib infection were in infants too young to have received the Hib vaccine, 15 were aged 10 months to 5 years and four were older (6to 16 years). All seven cases of Hia infection occurred in children aged <2years and were isolated from 2016 onwards. Bajanca-Lavado et al (92) reported on 29 cases of Hib infection identified in Portugal between 2010 and 2018. Half of the cases occurred in 2017to2018 and 72% were in pre-school aged children. Eighteen were Hib vaccine failures: three were infants, seven were aged between 13 and 47 months and eight were ≥4 years of age. Only one child had an underlying comorbidity.

In France, a country with high Hib vaccine coverage (95% to 98% over the last decade) (93), the number of invasive Hib cases almost doubled in 2018 from <10 per year in previous years (94). The majority of cases were in children aged <5 years and there were at least 10 vaccine failures. After a change in the Hib immunization program from 3+1 to 2+1 doses in 2013, mean anti-PRP IgG titers were lower and peaked at 6 to11 months rather than at 2 years of age as seen with a 3+1 schedule. Antibody titers also declined to <1µg/mL at a much younger age (4 to 5 years versus 20 years).

In a Swedish Arctic Region, invasive *H. influenzae* infections declined by 89.1% ($p<0.01$), including *H. influenzae* meningitis by 95.3% ($p<0.01$) and 'all-cause' bacterial meningitis by 82.3% ($p<0.01$) in children aged 0 to4 years following general infant Hib vaccination (95).

Data on invasive Hib disease incidence in Eastern Europe and Central Asia are limited. The data that exist suggest the pre-vaccine incidence was low. For example, Hib meningitis incidence in children aged <5years in Bulgaria and Moscow was 6.7/100 000 (96) and 5.7/100 000 respectively (97). One factor contributing to low incidence figures is the high proportion of culture-negative cases of purulent meningitis, possibly reflecting prior

antimicrobial therapy or limited laboratory capacity. One approach, advocated by WHO, is to allocate culture-negative cases to bacterial pathogens in the same proportion as microbiologically-confirmed cases (31). Using this approach, Griffiths and colleagues (98) adjusted the incidence of Hib meningitis prior to introducing Hib conjugate vaccine in Belarus and Uzbekistan upwards to 10.8/100 000 and 18.7/100 000 respectively. In 2006, Ukraine was the first country in Eastern Europe to incorporate Hib protein-conjugate vaccine into its infant immunization program. Between 2007 and 2009, hospitalized children <2 years of age in Ukraine with radiographically-confirmed pneumonia were significantly less likely to have been immunized with Hib vaccine than age-matched controls (51% of cases versus 67% controls [p<0.001]) (99). A retrospective study in a tertiary hospital in Budapest, Hungary undertaken between 2004 and 2017 identified 34 adult invasive *H. influenzae* infections(100). Most cases presented as pneumonia (62%) due to NTHi (79%), followed by Hif (11%), Hia (5%) and Hib (5%). One-third of patients were 65 years or older with a clinically significant underlying comorbidity, including cardiovascular disease, diabetes mellitus, and malignancy.

EASTERN MEDITERRANEAN REGION

All countries in the WHO Eastern Mediterranean Region have introduced Hib vaccine. In 2019 the average coverage of three doses of Hib vaccine was 82%(101). Data on individual countries are shown in Table 3. Reports on invasive Hib disease before Hib vaccination was initiated are limited to a few countries (102). Pre-vaccine data from Egypt showed that Hib was the most common cause of meningitis (39%) in children <6 years of age with a case fatality rate of 27%(103). In Jordan, 32% of cases of bacterial meningitis in children aged 2 months to 12 years were due to *H. influenzae* with 95% of Hib meningitis occurring in

children <2 years of age (104). Pre- and post-vaccine introduction incidence data are available for Oman, which introduced Hib immunization in 2001. Before Hib vaccines were introduced, the highest incidence of Hib meningitis in Oman was in infants aged <1 year (23/100 000 in 1999). After Hib immunization was introduced in 2001, the incidence declined to 4/100 000 by 2003 (102). In Qatar, a study of bacterial meningitis conducted from 1998 to 2000 recorded the incidence of bacterial meningitis as 2.24/100 000 with the highest rate observed in infants aged <1 year, and where Hib accounted for 24% of cases (105). In Saudi Arabia, Hib meningitis incidence was 16.9/100 000 in those aged <5 years, but with marked regional variation (106). A prospective study of bacterial meningitis in children from both Karachi and Hyderabad in Pakistan was conducted in 2004, before Hib vaccines were introduced (107). The estimated incidence of Hib meningitis was 7.6/100 000 in children <5 years of age and 38.1/100 000 in children aged <1 year. All countries in this region have now introduced Hib vaccines, with Bahrain being the first country in 1998 and Egypt and Iran the last in 2014.

Currently, there are little data on the emergence of NTHi or non-b serotypes of *H. influenzae* causing invasive disease in this region.

AFRICAN REGION

Hib conjugate vaccines have been introduced into the NIPs of all 47 member states in the WHO African Region and the average coverage of three doses of Hib vaccine was 73% with wide variation between countries (108). Data on individual countries are shown in Table 4. The Gambia introduced the Hib protein-conjugate vaccine in 1997, being the first African country to do so. Several other African countries followed suit, although 15 years elapsed before all countries in the region had introduced the vaccine. An important study in The

Gambia established Hib as a significant cause of pediatric pneumonia, something that had not been appreciated previously(7). This trial showed vaccine efficacy of 21% against radiographic pneumonia, 36% efficacy against hypoxic pneumonia and 95% efficacy against invasive Hib disease(7). Before The Gambia introduced routine Hib vaccination, Hib meningitis incidence was 297/100 000 in infants <1 year of age and 60/100 000 in children aged <5 years(109). In neighboring Senegal, the incidence of Hib meningitis was 132/100 000 in children aged <1 year (110).

The GAVI-Alliance and the Hib Initiative have been major players in providing subsidized vaccines for low-income countries in Africa. All studies from African countries indicate that, with the sole exception of South Africa, Hib vaccination has led to a >90% decline in Hib meningitis or invasive disease. However, in South Africa, 5 years after vaccine introduction, Hib disease rates were only reduced approximately 65% in the <1 year age group (111) and a decade after Hib vaccination was implemented cases were reported to have increased from 0.7 to 1.3/100 000 in children aged <5years (112). To some extent the increase was due to low vaccine coverage and waning immunity of a 3-dose primary schedule without a booster, with Hib vaccine failure occurring in both HIV positive and negative children. South Africa introduced enhanced surveillance of invasive *H. influenzae* infections in 1999 following the introduction of Hib vaccination (112). During the first 10 years of Hib immunization there was no significant increase in other capsulated serotypes or NTHi (112). From 2003-2009, 135/263 (51%) cases of invasive Hib disease occurred in fully immunised children and 55% of the Hib vaccine failures were in children aged ≥18 months (112). South Africa introduced an 18 month booster dose of Hib vaccine in 2009 in a pentavalent vaccine (DTaP-Hib-IPV). Invasive Hib disease in infants aged <1year then declined significantly from

5.2 cases/100 000 in 2010 to 1.6 cases/100 000 in 2017 (p<0.001) and remained <0.2/100 000 in children aged 1 to 4 years from 2013 to 2018.

In 2018 the on-going surveillance program in South Africa (GERMS) identified 327 cases of invasive *H. influenzae* infection, of which 201 provided isolates for typing(113). Seventeen percent (34/201) were Hib and 64% (129/201) were NTHi. There were 25 isolates (12%) of other capsulated serotypes. Hib isolates were more likely than NTHi isolates to be from cases of meningitis (8/34, 24% versus 15/129, 12%, p=0.01). The highest incidence of invasive *H. influenzae* infection (all types) was in children aged <5 years, with a second peak in adults aged 25 to 44 years. NTHi incidence was highest in infants with a second peak in adults aged > 65 years (2.9/100 000 and 0.3/100 000 and 0.3/100 000 respectively). (Figure 4)

In contrast to South Africa, long-term surveillance in Kenya has demonstrated the effectiveness of a 3-dose primary schedule of Hib vaccine and no booster dose in the second year of life (114, 115). In Kenya there has not been an increase in invasive infections by other serotypes or NTHi (114). The reasons why a booster dose is necessary to achieve control in some settings, but not in others remain unclear (see discussion below).

The Gambia uses a 3-dose primary series of Hib immunization without a booster dose. This resulted in excellent control of invasive Hib disease for 14 years after the vaccine was introduced with consistently high coverage, low carriage rates and high levels of protective antibodies(116). In order to measure the impact of pneumococcal conjugate vaccine introduction, surveillance for meningitis, sepsis and pneumonia was established in Eastern Gambia (in Basse in 2008 and in Fuladu West in 2011). This surveillance identified increased Hib infections in Eastern Gambia between 2011 and2013, despite high vaccine coverage. In

2013, the incidence of invasive Hib disease in this region was 90/100 000/year in infants <1 year and 26/100 000/year in children <5 years of age (117). The primary reasons for Hib infection were vaccine failure (39%) and onset of infection in infants before receipt of two doses of Hib vaccine (39%). HIV infection did not appear to be a factor (117). The surveillance in Eastern Gambia identified 57 cases of invasive *H. influenzae* infection: 24 (42%) Hib, 17 (30%) NTHi and 10 (18%) Hia (110). Fourteen of the Hib infections (58%) were cases of meningitis, whereas NTHi presented principally as bacteremic pneumonia (12/17 [71%]). Case fatality was 17% (4/24) for Hib and 21% (5/17) for NTHi(117). It is also noteworthy that molecular studies of lung aspirate specimens collected in The Gambia more than 10 years after the introduction of Hib vaccines, indicate that *H. influenzae* (mostly NTHi) may still cause up to 23% of cases of radiographic pneumonia. In this study there was sufficient DNA for full multi-locus sequence typing (MLST) in 4/12 samples; with three samples yielding NTHi, whereas one sample in a child with HIV contained Hib(118).

Data on pediatric bacterial meningitis have been generated in 26 sub-Saharan African countries by the WHO's Invasive Bacterial Vaccine-preventable Diseases Surveillance Network (IB-VPD)(119-126). In South East Africa 1670/49 844 (3.3%) meningitis cases were laboratory-confirmed as *H. influenzae* (232/1670 [13.9%], which was the most uncommon organism identified (119). Whilst most were Hib, cases of Hia, Hic, Hie and NTHi were also reported. Interestingly, Boni-Cisse noted that NTHi had become a significant cause of meningitis in Cote d'Ivoire.(127)

Several West African countries, participating in IB-VPD reported bacterial meningitis cases in children aged <5 years in the years 2010-2016. In Lomé, Togo, there were 23 cases of *H. influenzae* meningitis, and 20 of the isolates were available for serotyping. There were 9

cases of Hib meningitis, with the number declining by year, with only 1 case in 2015; while 2/2 cases of Hic occurred in 2012 and 5/7 cases of Hie were in 2015 (124). In Nigeria, 19/153 (12%) pediatric bacterial meningitis cases were due to *H. influenzae* (123). Serotyping was performed on 16/19, with Hib being the predominant serotype. In contrast to the findings in Togo, the number of Hib isolates increased from 1 in 2012 to 8 in 2016 (total 11/16, 69%). Hia and Hic caused 3 and 2 infections respectively in 2015(123). In Senegal, *H. influenzae* was responsible for 10/115 (9%) of cases, with serotyping data available for 5 cases. Hib was the causative serotype in 4/5 (2 in 2014 and 2 in 2016). There was also 1 case of Hic (121). In the Gambia, *H. influenzae* was responsible for 11/69 cases (15.9%), with Hib causing 6/11 (54%) of cases (122). In Ghana, 5/73 (6.8%) microbiologically confirmed cases of bacterial meningitis were caused by *H. influenzae:* 1 Hib, 1 Hic, 2 Hie and 1 NTHi (125). It is of note that, although Hib vaccine was introduced in the Gambia, and Senegal in 1997 and 2005 respectively, Hib continues to cause pediatric bacterial meningitis, albeit at a low rate. Although the number of cases is small, the identification of Hia, Hic and Hie as causative agents of pediatric bacterial meningitis underlines the importance of continuing surveillance and serotyping of isolates.

In sub-Saharan Africa, in countries with a high prevalence of SCD and low rates of Hib vaccine coverage, invasive Hib infections remain one of the most frequent causes of bacteremia in children with SCD (128). Improving uptake of Hib vaccine should result in a significant decline in invasive Hib infections in these children. However, SCD children will remain at potential risk of bacteremia from non-type b strains.

Other than for South Africa, in countries of sub-Saharan Africa immunization programs employing three primary doses of Hib vaccine and no booster dose appears to be sufficient

to achieve good control. A lack of robust surveillance in many countries may mask potential limitations of the vaccine's impact in populations with high HIV prevalence or limited vaccine coverage. In order to appreciate the ongoing burden of Hib meningitis in Africa, surveillance using molecular methods is needed. The difficulty of making an etiologic diagnosis of pneumonia may also mask the role of NTHi and Hib as ongoing causes of pneumonia with consolidation. There are only limited data on other *H. influenzae* serotypes or NTHi. Concerning Hib disease, the primary challenges for NIPs in sub-Saharan Africa are optimizing the timeliness of the primary series of doses and increasing coverage in displaced or migrating sub-populations.

SOUTH EAST ASIA REGION

After 2009 Hib immunization was introduced throughout the WHO South East Asia Region. An exception was Thailand, which although adding Hib vaccine to the national infant immunization program in 2019 as a three-dose primary course without a booster dose, the vaccine had been available in the private market since the late 1990s. In 2019 the average coverage of three doses of Hib vaccine in the South East Asian Region was 89%, although rates between countries are highly variable (129).Data for individual countries in the region are shown in Table 5.Data on the incidence of invasive disease and pneumonia across the region are limited. In 1998 Miller (130) estimated 135 000 deaths due to Hib and 92% of these occurred in three countries in this WHO region: India, Bangladesh and Indonesia. In India, most deaths were from meningitis and pneumonia (131), although the incidence of severe Hib pneumonia is most likely to have been substantially underestimated (132). This is probably true for other countries in the region. The reported incidence rates for meningitis vary widely from an estimate of 9.5/100 000 in Thailand(133) to 122/100 000 in India, and

there are no reliable data for the incidence of pneumonia and bacteremia or sepsis. However, it is likely that the true incidence of meningitis reported for Thailand may also have been underestimated due to antimicrobial use prior to admission and taking of appropriate cultures (133, 134).

In India, with the largest population of any country in this region, the decision was made to introduce Hib immunization in 2011, but by 2015 only 8 of 29 Indian states had initiated Hib immunization in the public sector. Therefore, given the recent introduction of the vaccine, the earlier availability of the vaccine through the private sector, the widespread use of antibiotics and the lack of access to reliable diagnostic laboratories, it is very difficult to determine the impact of Hib immunization on the epidemiology of *H. influenzae* disease in this region. A study modelling the national, regional and state-level burden of Hib infections in children in India from 2000 to 2015(135) estimated Hib deaths in children aged <5 years fell from 82 600 (UR, 52 300–112 000) to 15 600 (UR, 9800–21 500) representing an 81% decline in death rates. The mortality rate varied between states with Uttar Pradesh (9300 [UR 5900–12 700]) and Odisha (1100 [UR, 700–1500]) reporting the highest number of deaths in 2015. It is of note that Hib mortality had declined before nationwide implementation of Hib conjugate vaccine, reflecting child survival trends in India(136) as a result of improved maternal and child health provision. Introduction of Hib vaccine in several states corresponded with a more rapid reduction in morbidity and mortality associated with Hib infection. The recent establishment of hospital-based sentinel surveillance for invasive bacterial diseases in India (HBSSPIBD) (137) will be of great benefit in determining the impact of Hib and pneumococcal conjugate vaccine immunization programs in India.

Generally, there are little data on the impact of introducing Hib vaccines on invasive *H. influenzae* disease in the WHO South East Asian Region. Two sentinel studies have demonstrated that the burden of invasive Hib disease should be reduced after Hib vaccine has been used NIPs for many years. The first, a large vaccine-probe trial conducted in Lombok, Indonesia reported a significant reduction in the Hib meningitis incidence, but it did not prevent pneumonia in children <5 years of age (6). This study also revealed that, contrary to the previously widely held belief, the incidence of Hib disease was high in the region and potentially vaccine preventable clinical meningitis was as much as 158/100 000, whilst vaccine preventable pneumonia was 1467/100 000 (6). The second study was from Bangladesh where significant reductions in meningitis and radiographically confirmed pneumonia incidence was reported in children aged <2 years (138). To date there are no published data on the impact of Hib immunization on bacteremic pneumonia in this region.

Given the relative lack of data, it is important that surveillance networks are established not only to monitor the impact of vaccination on Hib disease, but to also monitor the effect on *H. influenzae* epidemiology given the resurgence of disease by non-type b strains seen elsewhere in Europe, North America and Australia.

WESTERN PACIFIC REGION

According to the WHO, the Western Pacific Region has an annual birth cohort of almost 24 million with 121 million children aged <5 years. Both Australia and New Zealand incorporated Hib vaccine in their NIPs in 1993 and 1994 respectively. However, other countries within the region have only included Hib vaccines into their immunization programs within the last 10 to 15 years. The notable exception is China, which so far has not adopted Hib vaccines for its 17 million infants born annually. In China, Hib vaccines are

available in the private market, with only approximately 55% coverage for the full immunization schedule (139). Consequently, despite 19/26 countries reporting ≥90% completion rates of the third Hib vaccine dose during infancy, in 2019 just 24% of infants in this WHO region received 3 primary doses of Hib vaccine by 1 year of age(140).

The data for individual countries in the region are shown in Table 6.

In 2015, in the Western Pacific Region, there were an estimated 370 000 cases of invasive Hib disease and 3800 deaths(36). This compares with estimates of 1.5 million cases and 27 200 deaths in the year 2000 for this region (35). Meanwhile, the proportion of Hib-related deaths in the region attributed to China has almost doubled from 40% in 2000 to 74% in 2015 (36). Data on *H. influenzae* disease incidence in China are limited, although one study estimated that 29% of pediatric pneumonia was due to Hib (141)(132). In contrast, neighboring Mongolia, which introduced Hib vaccines in 2000, has had a 93% reduction in Hib meningitis cases and no Hib-related deaths since 2012 (36, 142).

Invasive Hib disease rates have fallen 95% following the introduction in 1993 of Hib vaccines into the Australian infant NIP. Recent surveys have not found a subsequent increase in replacement disease by encapsulated non-b strains (143, 144). A review of 238 invasive *H. influenzae* cases in South Australia and the Northern Territory between 2000 and 2011 reported an annual incidence rate in the Indigenous population of 9.0/100 000 compared to 0.7/100 000 in non-Indigenous people (143). Indigenous children aged <5 years had the highest rates of invasive *H. influenzae* disease. This included Hia whose annual incidence of 10.5/100 000 was stable and outranked that of Hib, NTHi and other non-b serotypes in this age group. Another recent review of 737 invasive cases from the state of Queensland found that the incidence of Hib and encapsulated non-b strains between 2000 and 2013 did not

change significantly (144). The highest observed rates for encapsulated types were for Hia and Hib in Indigenous populations. However, across age groups invasive NTHi infections predominated year-on-year and the incidence increased significantly during the study. The highest annual disease incidence rates for NTHi of 14.8 and 16.5/100 000 were in infants and in those aged >90years respectively.

In 2018 the age of administration of the Hib booster dose in Australia was moved from 12 months to 18 months (145). To assess the likely impact of this change, incidence rate ratios (IRR) and vaccine failure trends were calculated using Hib case surveillance scheme data from 2000 to 2017, where 153/345 cases reported between 2000 and 2017 were in children born after 2000, and 51 (33%) occurred in Indigenous Australian children. The IRR for Indigenous children was 8.34 (95%CI: 5.83–11.79) when compared with non-Indigenous children with no evidence of a decline over these years. There was also no evidence of an increase in vaccine failures during the study period. Between 2000 and 2017 invasive Hib disease declined by 55%, with a persistent marked disparity between Indigenous and non-Indigenous children (145). These results indicate the need to monitor any potential impact of moving the booster dose to 18 months of age, particularly in Indigenous children.

After Hib vaccine introduction in New Zealand hospital admissions for Hib meningitis and epiglottitis in children aged <15 years decreased sharply (146). From 1993 to 1995, meningitis rates declined by 82% from 8.63/100 000 in 1993 to 1.58/100 000 in 1995. Epiglottitis rates fell similarly by 87% from 3.67/100 000 in 1993 to 0.48/100 000 in 1995. In the period between 1991 and 2014, annual invasive Hib disease rates in this age group fell to be as low as 0.12/100 000. There was a similarly declining trend in Hib bacteremia

hospitalization rates (146). Moreover, hospitalization rates for Indigenous Māori and non-Māori children for Hib disease were similar.

In Japan, Hib vaccine was introduced on a voluntary basis in 2008 and was then incorporated into the national infant immunization program in 2013. Active population-based surveillance of culture-proven pediatric invasive *H. influenzae* infections was conducted from 2008 to 2017 in 10 prefectures in Japan (approximately 23% of the total Japanese population) (147). Over the 10 years of the study, 566 cases of invasive *H. influenzae* infection, including 336 meningitis cases, were identified. From 2013 to 2017 invasive *H. influenzae* infections among children <5 years of age declined by 93% (IRR: 0.07, 95%CI 0.05–0.10; p<0.001]) compared with cases in 2008 to 2012. Although there have been no identified cases of invasive Hib disease, since 2014 in the post-Hib vaccine era NTHi and Hif invasive infections have been identified in children <5 years of age. NTHi is now the major cause of invasive *H. influenzae* disease in Japan (148).

WHO IS NOW AT RISK OF INVASIVE Hib DISEASE?

In countries that have established infant immunization programs, including Hib protein-conjugate vaccine, invasive Hib infections are no longer a major cause of bacterial meningitis in young children, but cases of invasive Hib disease do still occur, especially in older adults with underlying comorbidities. Collins and colleagues investigated the epidemiology of all cases of invasive Hib disease in England and Wales from 2009 to 2012 (149). The incidence of invasive Hib disease in children aged <5 years was 0.06/100 000 (two cases) compared with an incidence of 35.5/100 000 prior to routine Hib immunization. All 106 cases of invasive Hib infection that occurred between 2009 and 2012 were followed-up. The median age at disease onset was 49.4years (interquartile range 16.9–67.5 years).

Seventy-three percent of the cases occurred in adults, many of whom had underlying comorbidities (77%) and presented with pneumonia (56%). Chronic heart disease (n=17, particularly in those >65 years of age) and chronic lung disease (n=17, mainly among those >45 years of age) were the most frequently reported comorbidities. The Hib-associated mortality was 9.4% (10/106). Data from the US show a similar pattern (51). Between 2009 and 2015, 77 cases of invasive Hib disease were identified by the ABC surveillance sites. The median age was 49 years (interquartile range 4 to 62 years). Twenty-three cases (29.9%) occurred in children aged <5 years, of whom 18 were either unvaccinated, incompletely vaccinated or too young to have been immunized. In adults, 37/54 (82.2%) cases presented with bacteremic pneumonia with underlying comorbidities in 62.9%, including chronic obstructive pulmonary disease (COPD), chronic heart disease, diabetes and obesity. The shift of invasive Hib disease from young children to adults could be due to the absence of natural boosting from exposure to Hib organisms before Hib vaccines were introduced(150).

There is an increased risk of invasive Hib disease in patients with primary immunodeficiency disorders (e.g. agammaglobulinemia, early complement component deficiencies), those with anatomical or functional asplenia, HIV infection, undergoing radiotherapy or chemotherapy for malignancy or post-haematopoietic stem cell transplantation,(151). In the US, a single booster dose of Hib vaccine is recommended for these high-risk groups whenever a primary course has not been completed(151).

IS A BOOSTER DOSE OF Hib VACCINE NEEDED?

Evidence of waning immunity following the introduction of a primary immunization series, without a booster dose, prompted 3 countries; the United Kingdom, South Africa and Mexico, to add a booster dose. Worldwide, 50/83 high-income countries, as defined by the

World Bank (152) recommend a booster dose at 11 to 18 months. By contrast, booster doses are only used by 19/56 upper middle-income countries and 5/50 lower middle-income countries. All 29 low-income countries employ a 3-dose primary series of Hib vaccine (at 6,10 and 14 weeks of age) without a booster dose, as recommended by the WHO Expanded Program on Immunization (EPI) schedule(153), and adopted by the vaccine alliance (GAVI)(154), when it began supporting Hib vaccine introduction in the poorest countries globally in 2000.

A meta-analysis of 20 randomized clinical trials, which had been conducted in 15 countries compared different Hib vaccination schedules (3+0, 3+1 and 2+1) and different intervals between the primary, and the primary and booster doses. (155). There was no difference between the schedules in terms of prevention of invasive Hib disease, clinical effectiveness or the immunologic response. A further meta-analysis reported 3 and 2-dose primary immunization courses of Hib vaccine in infancy showed similar vaccine effectiveness for (156). A 3-dose primary schedule had a vaccine efficacy of 82% compared to 79% for a 2-dose primary series. A modelling study estimated that a primary series plus a booster dose within one year would result in greater reduction in the incidence of Hib disease compared to a primary series without a booster dose(157). However, this assumed high vaccine coverage of 90-100%, which has not yet been achieved in many countries. The model also looked at delaying the booster dose for two years after the primary vaccination series, and found that this had little impact on direct protection(157).

It is unclear why a booster dose appears to be necessary in some settings, but not in others. Among the factors that may be of importance are the type of vaccine used. Almost all countries now use combination vaccines, which include Hib, with either a whole cell or

acellular pertussis component. Some DTaPHib vaccines were reported to be associated with reduced Hib immunogenicity, but the clinical significance of this finding is unclear. Almost all countries currently using a Hib-acellular pertussis combination vaccine do include a booster dose in their schedule, but this may reflect national income rather than issues with the vaccine. Use of DTaPHib or DTaPHibIPV does not significantly alter the immunogenicity and antibody functionality of the individual vaccine components, and several countries using DTaPHib combination vaccines have reported protective levels of anti-PRP antibodies and high vaccine effectiveness.(158). The protein carrier used for the Hib component does not appear to be a factor. Tetanus toxoid (TT) conjugated Hib vaccine has been adopted widely by low-income countries, both in those with sustained control, such as Kenya (115), and in others with evidence of waning immunity, such as The Gambia (117), so this seems to be an unlikely explanation.

It is difficult to determine the long-term impact of a primary immunization series without a booster dose in countries where high quality population-based surveillance is not in place and sustained high coverage over a prolonged period of time has not yet been achieved.

The impact of changes to the timing of a booster dose has been reported from France, where a 3+1 schedule of Hib vaccine was used from 1992 to 2012, with vaccine given at 2,3,4 months and a booster dose at 16 to 18 months. In 2013 the schedule was changed to a 2+1 schedule, with a 2-dose primary course at 2 and 4 months plus a booster dose at 11 months. In 2018, cases of invasive Hib disease increased with several examples of vaccine failure in children aged <5 years(94). This 2+1 schedule was associated with a decline in anti-PRP antibodies to <1 µg/mL by the age of 4 to 5 years. Although this is only preliminary data,

it does suggest that a booster dose administered in the second year of life rather than before the first birthday may achieve sustained high levels of anti-PRP antibodies.

INVASIVE NTHi DISEASE

Few reports exist describing the epidemiology of non-Hib disease prior to routine Hib immunization. A prospective, enhanced population-based surveillance of all invasive *H. influenzae* infections, conducted in six regions in England and Wales over the 2 years before routine Hib immunization was introduced, indicated that the majority (approximately 90%) were caused by Hib, while approximately 10% were due to NTHi. Other serotypes of *H. influenzae* accounted for <1% of invasive infections (14). Twenty-three percent of NTHi cases were in children <5 years of age and 62% occurred in adults; including 39% in adults aged >65 years. The annual attack rate of NTHi was 14.9/100 000 in neonates and 0.39/100 000 in those >65 years of age. Bacteremia without a detectable focus was the predominant presentation of invasive NTHi disease, being present in 37% of cases, while pneumonia accounted for 27% and meningitis for 12% of cases.

When Hib protein-conjugate vaccines were first introduced in the early 1990s, there was concern that as these vaccines reduced nasopharyngeal carriage of Hib, other *H. influenzae* species might replace Hib in the vacated ecological niche in the nasopharynx and cause more invasive disease. Indeed, as described above, NTHi is now responsible for the majority of invasive *H. influenzae* disease in every age group in countries where Hib vaccination programs are well established. This is most probably due to multiple factors such as: the increased number and survival of pre-term and extremely pre-term infants; the increasing number of patients with underlying chronic medical disorders and immunocompromized conditions and an aging population.

Between 1992 and 2006, the Health Protection Agency in the UK co-ordinated surveillance data from Europe and other regions as part of the EU-IBIS program(40, 77). There was a 3.5% (95%CI, 2.1%–5.2%) year-on-year annual increase from 1996 to 2006 in invasive non-type b *H. influenzae* disease in all age groups in 14 countries (40). Overall, 97% of invasive non-b *H. influenzae* infections were caused by NTHi (40). Since 2007, European data collation has been undertaken by the ECDC(159) In 2008 and 2009, the overall incidence of invasive NTHi disease from 29 reporting countries was 0.41/100 000 and 0.36/100 000 respectively, with higher incidence rates reported in Sweden (1.78/100 000 and 1.58/100 000 respectively) and Norway (1.58/100 000 and 1.48/100 000 respectively) compared to other European countries. The increase in NTHi infections was particularly notable among adults aged ≥60 years (78). In Italy during 2017 to 2018, the majority of invasive *H. influenzae* infections were caused by NTHi (76.1%) (89). Similar increases in NTHi infections have also been reported from other European countries, including Greece(160), Germany (161), England(86), Poland (162) and Finland (88).

The CDC ABC sites have also documented an increase in invasive NTHi disease. In 1989, 80% of invasive *H. influenzae* disease in the US was caused by Hib and 17% was from NTHi. By 2008, Hib was responsible for only 3% of cases with 68% due to NTHi (51). Continuing surveillance from 2009 to 2015 reported highest incidence (1.22/100 000) and highest case fatality rates (16%) for NTHi compared to Hib (0.03 and 4% respectively) and other capsulated serotypes (0.45 and 11% respectively)(51). The incidence of all invasive *H. influenzae* disease had increased by 16%, compared with 2002 to 2008, driven by increases in NTHi and Hia disease(51). The highest incidence rates of invasive NTHi disease were in children aged <1 year (3.18/100 000) and those aged >65 year (4.47/100 000). These

age groups also had the highest case fatality rates (51). NTHi are now the major cause of invasive *H. influenzae* infection in all age groups in the US, with a substantial disease burden, particularly in neonates, pregnant/post-partum women and older adults(163).

In Canada, most invasive *H. influenzae* disease is now caused by non-b *H. influenzae*. Between 2007 and 2015 the annual rate of invasive *H. influenzae* non-b disease in Canadian jurisdictions increased from 0.83/100 000 in 2007 to 1.57/100 000 in 2015, while invasive Hib disease incidence remained at 0.05–0.14/100 000 over the same period (164). In Ontario, the majority of invasive *H. influenzae* disease in all age groups between 2004 and 2015 was due to NTHi. Invasive non-b *H. influenzae* disease incidence increased from 0.67 to 1.60/100 000 over this time period, with the highest rates in infants, young children and older adults(164). NTHi is now the main cause of invasive *H. influenzae* disease in Canada.

Neonatal NTHi infections are well described, with an incidence of 1·6 to 4·9/100 live-births (165-167) accounting for approximately 5% of all neonatal sepsis episodes(166) . The European EU-IBIS study reported a 10-fold higher incidence of invasive NTHi disease compared with Hib in neonates, with 81% of cases occurring in the first 7-days of life (40). Neonatal NTHi sepsis develops rapidly (usually within the first 24-hours after birth) and has a fulminant course with substantial case fatality rates, especially in preterm infants. NTHi can colonize the female genital tract and an ascending infection can cause both maternal and fetal infection, with maternal sepsis, septic abortion, preterm delivery and increased complications during labor as possible consequences. In the EU-IBIS study, for example, NTHi caused more infections in women than men aged 25 to 44 years and there may be an increased risk of invasive NTHi infection in women of child-bearing age. Between 2008 and 2017, the ABC surveillance network identified NTHi invasive infection in 390 women of

child-bearing age. Of these, 86/390 (27.8%) were pregnant or post-partum. Pregnant/post-partum women were more likely to have NTHi bacteremia compared to non-pregnant women of child-bearing age (91% versus 56.1%; p<0.001) and less likely to have ≥1 underlying comorbidity (31.4% versus 67.7%; p<0.001]). Three mother/infant pairs with invasive NTHi at delivery were identified. The neonatal isolate shared the same sequence type as that of the corresponding maternal isolate(163).

There is increasing evidence that NTHi invasive infection in early pregnancy may be associated with fetal loss, but as this diagnosis relies on post-mortem placental and fetal samples, it is likely to be under-recognized. In a retrospective survey of all post-mortem samples at <24 weeks gestation, NTHi was identified in 20% of cases with histologically confirmed chorioamnionitis, and matched fetal and placental samples (168).

Following the neonatal period NTHi invasive disease in children has a low and relatively stable incidence. In countries with mature Hib vaccination programs, the median age at onset of invasive *H. influenzae* disease has now moved from early childhood to late adulthood since the majority of invasive infections are now due to NTHi, which has a median age at disease onset of approximately 60 years (40). The majority of invasive NTHi cases in children (40%–70%) and adults (60%–80%) are seen in those with underlying comorbidities, notably chronic respiratory disease and impaired immunity(166, 169-171). While only 38% of cases in a recent Swedish study had identified comorbidities(172), one-third of patients in that study were aged >80 years. Interestingly, the authors found B-cell immunity disorders, including chronic lymphatic leukaemia and multiple myeloma, to be common among patients with invasive NTHi, suggesting humoral immunity may have an important protective role to play (172). The authors also speculated that immunosenescence with age-related decline in B-cell function might explain the increasing incidence of invasive NTHi

infections(172). Nevertheless, NTHi invasive disease can also occur in previously healthy individuals. A study of such infections in children in Arkansas(173) found that 32% of the children had no predisposing factors for serious infection.

The clinical presentation of invasive NTHi disease varies with age. Almost all neonatal cases develop early-onset sepsis without a focus. Meningitis is more commonly seen in older infants and children and then declines with age. Overall, pneumonia is the most frequent presentation with invasive NTHi disease and NTHi pneumonia incidence increases with age, particularly in older adults with underlying respiratory tract comorbidities, such as COPD and emphysema (169, 174). Other clinical presentations, such as epiglottitis (once a characteristic of invasive Hib disease), skeletal and soft tissue infections, are uncommon, but cholecystitis appears to be a particular feature of invasive NTHi infection(175). The morbidity rate of invasive NTHi infections is high in older adults. In a Swedish study, 48% of 101 invasive NTHi cases developed severe sepsis or septic shock and 20% required intensive care(172).

In the US, 4683 cases of invasive NTHi disease were reported from 2008 to 2017 in the ABC surveillance system(163). The overall average annual incidence of invasive NTHi infection was 1.29/100 000 with an overall case fatality rate of 15.6% and considerable variation between different age groups. In children aged <1 year the average annual incidence was 5.94/100 000, with a case fatality rate of 8.3%. In contrast, in adults aged ≥80 years the average annual incidence was 10.08/100 000, with a case fatality rate of 25.2%. Of the 188 neonates with invasive NTHi infection; 152 (81%) were diagnosed in the first 24 hours of life and 134 (71%) were preterm. The average annual incidence amongst neonates and preterm infants was 43/100 000 and 320/100 000 respectively (154). Furthermore, 309 women of

childbearing age developed invasive NTHi disease, of whom 86 (27.8%) were pregnant or post-partum.

Several studies have reported that invasive NTHi infection causes significant mortality with case fatality rates of 12%–22% (163, 169, 176). Case fatality rates for invasive NTHi disease have also been reported to be higher in studies with an extended follow-up after the initial NTHi infection, suggesting NTHi may preferentially target those in poor health who, even if they survive the initial infection, may subsequently succumb to their underlying illness. The 28-day case fatality rate for invasive NTHi disease in the Swedish study was 8%, but 1-year post infection the case fatality rate had risen to 29% (172).

In countries with limited surveillance, or where serotyping of clinical isolates is incomplete, detecting an increase in NTHi infections will be difficult. Several factors may be responsible for the increase in NTHi infections and it is possible that all have all contributed to some extent. One possible explanation is strain replacement disease following the successful implementation of Hib immunization programs, although the evidence for this is limited (177). In England and Wales, invasive NTHi infections in children aged <15 years increased by 3 to 4% per year from 1994 to 2008, but there was no detectable effect on NTHi epidemiology during the resurgence of invasive Hib infections from 2000 to 2003 (178). It is also well-described that vulnerable populations, such as preterm infants, older adults, individuals with underlying cardiac and respiratory comorbidities, malignancy and immunosuppression, are particularly susceptible to invasive NTHI infection(178). In recent years medical advances have increased the survival rates for these groups resulting in a larger number of individuals susceptible to invasive NTHi infections. Other possible reasons for the increase may be the result of increased awareness of NTHi infections, alterations in

clinical practice, improved diagnostic testing, including the use of automated blood culture systems and molecular typing of isolates, together with more comprehensive epidemiologic surveillance.

In marked contrast to capsulated *H. influenzae* strains, NTHi are highly heterogenous and there is no obvious vaccine candidate(179). One outer membrane protein, Protein D, is highly conserved and produced by almost all strains(180). It is the protein carrier for eight of the pneumococcal polysaccharide antigens in the 10-valent pneumococcal protein-conjugate vaccine (Synflorix^{TM} GSK). Use of an 11-valent prototype of this vaccine resulted in a 35.3% reduction in NTHi acute otitis media in children (181), but to date the licensed vaccine has failed to show any significant effectiveness against invasive NTHi infections and has no effect on carriage. A multicomponent NTHi vaccine, containing protein D, protein E and a fusion protein of another surface exposed protein, PiLA, is under investigation as a possible vaccine to prevent NTHi infections in adults with COPD(182). Other routes of vaccine administration have also been under consideration. Oral NTHi vaccination has been proposed for patients with recurrent exacerbations of COPD. A Cochrane review(183) concluded that oral administration of an NTHi vaccine did not yield consistent reductions in NTHi-mediated exacerbations but did demonstrate a significant reduction in the number of antibiotic courses prescribed. Subject age and gender were significant confounding factors in these studies(184).

Albritton et al described a “cryptic genospecies of Haemophilus biotype IV” isolated from genitourinary tract specimens(185). Quentin et al (186) further investigated these strains, which have been designated *H. quentini*. These strains can be differentiated from other NTHi strains by DNA hybridisation, 16S rRNA gene sequencing and multilocus enzyme

electrophoresis (187). There have been case reports of *H. quentini* as a cause of neonatal bacteremia (188) and bacteremia in an adult with multiple myeloma(189) . It has also been isolated from urine and urethral cultures in adult males(190). Between 2016 and 2018, the Public Health Ontario laboratory in Toronto identified 7 cases of invasive *H. quentini* infection(191). Two of the isolates were from neonates, 3 were adult females of child-bearing age (2 were known to be pregnant) and 1 was an elderly female. There was also an atypical *H. quentini* biotype III from an elderly male with varices. *H.quentini* strains were identified as non-typeable , biotype IV plus possessing *sodC*, lacking *fucK* together with detection of the *H. quentini*-specific 16S rRNA gene, and near-full-length 16S rRNA gene sequencing, tests which require referral to a reference laboratory. Given the need for testing not generally available in routine diagnostic laboratories *H. quentini* may well be an under-recognized pathogen in adults, especially in pregnant women(191).

Differentiating NTHi and *H. haemolyticus*

H. haemolyticus, commonly regarded as a harmless commensal, is closely related to NTHi. Both colonize the upper respiratory tract. The increasing recognition of NTHi as a significant cause of invasive disease in neonates, the immunocompromized and older adults means that it is important to differentiate between these two *Haemophilus* species, especially as *H. haemolyticus* has only been implicated rarely as a genuine human pathogen(192). In order to understand invasive NTHi disease epidemiology and to ultimately optimize any future vaccine policy, identifying NTHi accurately is important as it is for guiding patient management and effective antimicrobial stewardship.

However, in the clinical laboratory the 2 species are difficult to distinguish by classical phenotypic methods as they have similar colonial morphologies, are X and V factor

dependent, and *H. haemolyticus* isolates can be non-hemolytic. Automated biochemical differentiation systems do little better with misidentification rates as high as 10% with some commercial systems(193). In upper airway sites, from 4 to 61% of phenotypic NTHi isolates are *H. haemolyticus*, while in contrast, invasive blood isolates believed previously to be *H. haemolyticus* may have been NTHi strains instead(192, 193). In contrast, Anderson et al(194) reported 7 cases of invasive disease due to *H. haemolyticus* strains that had previously been mis-identified as NTHi.

In order to progress laboratory performance, comparative genomic analysis has been used to identify genes unique to either NTHi or *H. haemolyticus*. These genetic targets have then been incorporated into MLST and polymerase chain reaction (PCR) assays to allow both species to be detected and differentiated from one another(195). Unfortunately, as NTHi undergoes high levels of gene recombination within and between other *Haemophilus spp.*, none of the single gene-based assays will provide 100% diagnostic accuracy. Matrix-assisted laser desorption/ionisation time of flight analysis (MALDI-TOF), a technology now available in many clinical laboratories has also been used. However, the discriminatory power of MALDI-TOF depends upon the quality of the supporting reference library, which for *Haemophilus spp.* needs further improvement and global standardization before MALDI-TOF can be recommended as the diagnostic test of choice over multi-plex PCR assays targeting more than one gene (192). Recently, whole genome analysis has revealed that some isolates, previously classified as *H. intermedius* are in fact hemin (X-factor) independent strains of *H. haemolyticus* (196), and suggests that the use of whole genome analysis rather than phenotypic methods of identification may be required to accurately identify *Haemophilus* species.

IMPACT OF Hib CONJUGATE VACCINES ON ANTIMICROBIAL RESISTANCE IN *Haemophilus influenzae*

Ampicillin-resistance in *H. influenzae* was first observed in the early 1970s and increased steadily in the pre-Hib vaccine era (16). Resistance was usually mediated by a β-lactamase, usually *bla*TEM-1/2 (197), and occasionally *bla*ROB-1(198). In addition, there were occasional reports of BLNAR and BLPACR strains during this period originating from Japan and other countries (16, 199).

During the northern hemisphere respiratory season of 1999 to 2000, an international surveillance study found the overall prevalence of β-lactamase positive *H. influenzae* respiratory tract isolates was 16.6%, with wide variations between countries; 3.2% of isolates in Germany were β-lactamase positive, whereas prevalence of these resistant strains was 64.7% in South Korea(17). During the 1990s the prevalence of β-lactamase positive strains started to decline, contemporaneously with the introduction of Hib immunization. However, this was possibly coincidental since the majority of respiratory tract isolates were NTHi. In Canada β-lactamase resistant strains fell from 32% in 1993 to 19% in 2000; in Spain resistance decreased from 28% in 1998 to 16% in 2000, and in the USA from 36% in 1994 to 26% in 2002, while in Japan resistance rates fell from 25% in 1995 to 3% in 1999(16).

With the decline in invasive Hib infections and the increase in invasive NTHi infections, antimicrobial resistance remains a challenging issue and resistance in NTHI is found in all parts of the world (200). NTHi are often associated with considerable rates of resistance, with similar rates of β-lactamase positive (BLPAR) and BLNAR strains(201). Strains resistant to extended cephalosporins, including cefotaxime and cefixime, have been reported in

Japan(199), Norway(202) and Spain (203). Consequently, WHO has recognized the importance of increasing resistance among *H. influenzae* isolates, and *H.influenzae* has been included in the recent list of high-priority antimicrobial resistant pathogens (204). Meanwhile, resistance to carbapenems (205, 206), quinolones (207)and macrolides(208, 209) has also been reported and more worryingly, strains exhibiting multiple drug resistance are emerging (210). A detailed description of antimicrobial resistance and its mechanisms is outside the scope of this review, but interested readers are directed to other publications on the subject(16, 41, 200).

Outside of China, where the vaccine is not included in their NIP, Hib conjugate vaccination has led to the near global elimination of invasive Hib infections requiring antimicrobial chemotherapy and a corresponding reduction in resistant Hib strains. However, the increasing antimicrobial resistance in NTHi is of concern, and is also likely best addressed by developing an NTHi vaccine.

CONCLUSION

The sustained low incidence of invasive Hib infection in all age groups in all countries that have introduced Hib protein-conjugate vaccine illustrate the remarkable success of routine Hib vaccination programs that have provided both direct and indirect protection to vaccine recipients and their communities respectively(211). Vaccine failures in fully-immunized children are rare and Hib vaccines are safe and well-tolerated. The coverage of the third dose of Hib vaccine ranges from 76% in the WHO African and European Regions (75, 108), to 82 % in the Eastern Mediterranean Region (101) and to 87% in the Region of the Americas and South East Asian Region(43, 129). The overall coverage in the Western Pacific Region is only 23%(140), reflecting that China has yet to include Hib vaccines into their NIP. Most

invasive Hib cases now occur in older adults who have underlying comorbidities. Although invasive Hib infections are now rare, the continuing identification of cases of invasive Hib disease stresses the importance of maintaining Hib immunization with high rates of coverage in all countries to ensure this infection does not re-emerge as a major pediatric infection.

In the pre-vaccine era, NTHi was an infrequent cause of invasive infection, but it is now acknowledged as the major pathogen responsible for invasive *H. influenzae* disease in countries with good surveillance mechanisms and full typing capabilities. NTHi infections occur predominantly in infants, particularly neonates, older adults, those who are immunosuppressed or who have underlying comorbidities, and women of childbearing age. Several studies have shown an increased burden of NTHi in individuals who have an increased susceptibility to infection, resulting in frequent intensive care admission, high case fatality rates, and many sequelae in those who survive the infection. The reporting rate of NTHi infections in neonates is particularly striking, with most cases presenting as early-onset sepsis, especially in those born preterm where maternal infection has induced labor and preterm delivery. Furthermore, NTHi infections in neonates are likely to be underestimated, although the increasing notification rates in this age group suggest an increasing awareness and reporting of these infections. NTHi immunization of pregnant women could protect both the mother and her baby. However, the development of an NTHi vaccine is difficult because of the genetic diversity of unencapsulated NTHi strains and needing to identify multiple vaccine targets. Despite these challenges, investigations of potential NTHi vaccine candidates are ongoing.

Hia has emerged as a serious problem among the Indigenous populations of North America, to the extent that a Hia conjugate vaccine may be required to control these infections. In

contrast, Hif is now the most frequently isolated capsulated serotype of *H. influenzae* causing invasive disease in the European Region, but the number of cases remains relatively small. Hie infections have also been recognized increasingly, principally in the European Region. Like NTHI, both Hie and Hif often target the elderly, and a high proportion of cases occur in patients with underlying comorbidities. Hie appears to be more virulent than Hif.

The increasing appreciation of NTHi as a significant invasive pathogen and the emergence of non-b serotypes in some regions emphasizes the importance of future surveillance of invasive *H. influenzae* disease, including all serotypes and strains, age groups, underlying comorbidities, risk factors, clinical presentations and outcomes of the infections. National Reference Centers should establish comprehensive epidemiologic and microbiologic surveillance of invasive *H. influenzae* infections. Accurate identification and typing of invasive isolates using molecular methods together with antimicrobial susceptibility testing and determining novel antimicrobial resistance mechanisms should be undertaken. The low rates of invasive NTHi infections reported in some countries is more likely due to the low proportion of strains referred to reference laboratories and highlights the need to improve ascertainment of such cases.

Hib conjugate vaccines were the first glycoprotein vaccines developed. The use of these vaccines over the last 30-years has proven extremely successful, to the extent that young pediatricians may never see cases of acute epiglottitis or acute meningitis caused by Hib. Valuable lessons have been learned about the use of these glycoprotein vaccines, which have aided the development of this type of vaccine for other major bacterial pathogens, including *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Salmonella enterica serovar Typhi*. Sporadic cases of invasive Hib disease are still occurring. These, and the changing

epidemiology of invasive *H. influenzae* infections, exemplified by the increasing incidence of Hia in Indigenous populations in North America and NTHi globally, together with alterations in the age groups affected, emphasize the importance of epidemiologic and microbiologic population-based surveillance systems. Such systems should be implemented both before and for many years after a new vaccine has been introduced to establish its potential impact. Continuous surveillance is essential to inform future vaccination strategies, detect vaccine 'escape mutants' or replacement disease and vaccine-failures. Indeed, as Hib disease declined, surveillance systems have identified the increasing importance of NTHi, and in some regions of non-b serotypes, so that these species may themselves become future vaccine targets.

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Mary Slack is an independent Consultant Medical Microbiologist. She is currently a Professor in the School of Medicine at Griffith University, Gold Coast Campus Queensland, Australia. She was formerly Head of the Haemophilus and Pneumococcal Reference Laboratories at Public Health England, London, United Kingdom; Head of the WHO Collaborating Centre for *Haemophilus influenzae*and Head of the Global Reference Laboratory for *Haemophilus influenzae* in the WHO Global Vaccine-Preventable Invasive Bacterial Disease (VP-IBD) Surveillance Network. She did her clinical training at Cambridge University, Kings College Hospital, London and Oxford, United Kingdom. Previous appointments include University Lecturer in Bacteriology, University of Oxford; Consultant Medical Microbiologist, Public Health England and Research Scientist in the Institute of Hygiene and Microbiology, University of Würzburg, Germany. Her research interests include *Haemophilus influenzae* and *Streptococcus pneumoniae* infections; the impact of Hib and pneumococcal conjugate vaccines, the role of vaccines in combatting antimicrobial resistance and community acquired pneumonia.

Professor Allan W Cripps

Allan Cripps is a Research Professor at the School of Medicine, Griffith University and leads the Mucosal Immunology Research Group, Menzies Health Institute Queensland at Griffith University. He has a PhD in Immunology from the University of Sydney. He is a distinguished academic, clinical scientist and health services leader. In 2015 he was awarded the Officer of the Order of Australia (AO) for his distinguished service to tertiary education and to public health as a leading immunologist, academic and researcher in the field of immunisation. He has made a significant contribution to immunology through translational research and human clinical studies in the fields of: diagnostic technology; vaccine antigen discovery for respiratory infections particularly, non-typable *Haemophilus influenzae* and *Pseudomonas aeruginosa*; and mucosal immunisation for bacterial infections related to chronic obstructive pulmonary disease, otitis media and pneumonia. In 2012 he launched the first international peer-reviewed journal exclusively focused on pneumonia.

Professor Keith Grimwood

Keith Grimwood is Deputy Head of the School of Medicine (Research) and Professor of Infectious Diseases at Griffith University and a pediatric infectious diseases physician at Gold Coast University Hospital, Queensland Australia. He did his clinical training in pediatrics in New Zealand and Australia, undertook an MD through the University of Melbourne on the systemic and mucosal antibody responses to rotavirus infections, and completed specialist infectious diseases training at the University of Calgary, Canada. Previous academic appointments include Senior Lecturer in Paediatrics at the University of Melbourne, Australia; Professor of Paediatrics at the University of Otago-Wellington, New Zealand; Conjoint Professor of Paediatrics at the University of Queensland and Inaugural Director of the Queensland Children's Medical Research Institute, Brisbane, Australia. His clinical research interests include respiratory infections, especially cystic fibrosis and bronchiectasis, *Pseudomonas aeruginosa* and *Haemophilus influenzae* infections, and vaccine-preventable diseases.

Dr Grant Mackenzie

Grant Mackenzie is an epidemiologist/paediatrician. His medical training was at the University of Melbourne. He trained in paediatrics at the Royal Children's Hospital in Melbourne and in Darwin in tropical Australia. He completed an MPH and PhD at Flinders University and Menzies School of Health Research in Darwin. He has been a Clinical Epidemiologist with the MRC Unit, The Gambia at London School of Hygiene & Tropical Medicine since 2008. He also holds appointments as Associate Professor at the University of Melbourne, Murdoch Children's Research Institute in Melbourne and at London School of Hygiene & Tropical Medicine. He co-ordinates surveillance for pneumococcal disease and carriage in The Gambia evaluating the effectiveness of pneumococcal conjugate vaccine. He is currently conducting a cluster-randomised trial of different pneumococcal vaccine schedules. His research interests cover vulnerable children at increased risk of mortality with a motivation to reduce child mortality through vaccination interventions.

Professor Marina Ulanova

Marina Ulanova is Professor of Immunology at the Northern Ontario School of Medicine (NOSM) and Adjunct Professor at Departments of Biology, Chemistry, and Health Sciences of Lakehead University, Thunder Bay, Canada. Dr. Ulanova received her MD as well as MSc and PhD in Immunology in Moscow (Russia), and PhD in Clinical Immunology from University of Gothenburg (Sweden). She moved to Canada in 2000, completed postdoctoral training at the University of Alberta and became one of the funding faculty members at NOSM when the School was open in 2005. She runs a research program on immunoepidemiology of bacterial infections, vaccinology, and host-pathogen interactions. Her major research interests include immune response to *Streptococcus pneumoniae* and *Haemophilus influenzae* in immunocompromized adults. For the last 10 years, she has been involved in the development of a new vaccine to prevent invasive *H. influenzae* type a disease in North American Indigenous people.

Figure legends

Figure 1: Cartoon illustrating Hib conjugate vaccine. Polyribosyl ribitol phosphate (PRP) [composed of repeating units of 5-D-ribitol-(1→1)-β-D-ribose-3-phosphate] covalently linked to a carrier protein* (*chemical structure kindly provided by Dr Neni Nurainy, Biofarma, Indonesia).

Figure 2: Posteroanterior chest radiograph of an 11month old infant showing right-sided pneumonia caused by *H. influenzae* type b (reproduced with permission from Dr Grant Mackenzie**)**.

Figure 3: Number of cases of invasive *Haemophilus influenzae* infections in England, 1990-2018, by serotype (unpublished data from Public Health England, with permission from Dr Shamez Ladhani).

Figure 4: Number of cases of invasive *Haemophilus influenzae* infection, by serotype,, reported to NICD, South Africa, 2006-2018 (from GERMS-SA Annual Reports, 2006-2018, , with permission from Prof Anne von Gottberg, National Institute of Communicable Diseases, Johannesburg, South Africa)(113, 212-223)