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MINIREVIEW

Haemophilus influenzae Infections in the *H. influenzae* Type b Conjugate Vaccine Era[▽]

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The widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccines has nearly eradicated invasive Hib disease where the vaccines are used. This success was accompanied by a shift in capsular serotypes of invasive *H. influenzae* disease, with nontypeable strains replacing type b strains as the most common bloodstream isolate, but there is no convincing evidence of a true increase in the incidence of non-serotype b invasive infections. *H. influenzae* causes predominantly mucosal infections. The introduction of vaccines for otitis media and global shifts in antimicrobial susceptibility emphasize the importance of continued surveillance of *H. influenzae* colonization and disease patterns.

The epidemiology and clinical manifestations of *Haemophilus influenzae* infections have undergone dramatic changes in the last 2 decades. The development and widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccines have nearly eradicated invasive Hib disease in children in countries where the vaccines are used widely. Hib conjugate vaccines induce protective humoral immune responses and also reduce circulating strains of Hib in the population by reducing nasopharyngeal carriage of Hib. Thus, these vaccines have had a profound impact on the incidence of disease and on the ecology of respiratory tract colonization by *H. influenzae*. More recent interventions are also impacting *H. influenzae*. These include pneumococcal conjugate vaccines, especially those with *H. influenzae* protein carriers and important global changes in antimicrobial susceptibility patterns of *H. influenzae*. Here we review the microbiology, epidemiology, and clinical manifestations of *H. influenzae* infection in the Hib conjugate vaccine era with particular attention to the evolving effects of vaccines and antimicrobial agents.

MICROBIOLOGY AND LABORATORY IDENTIFICATION

H. influenzae is isolated exclusively from humans, predominantly from the respiratory tract; no animal or environmental sources have been identified. A member of the *Pasteurellaceae* family, *H. influenzae* is a nonmotile, facultatively anaerobic, Gram-negative coccobacillus that requires special growth factors, hemin (factor X) and NAD (factor V). *H. influenzae* strains are divided into two groups depending upon the presence or absence of a polysaccharide capsule. Encapsulated strains are reactive with typing antisera, whereas unencapsulated strains are nonreactive and are thus referred to as nontypeable. Six serotypes, designated a through f, have been identified based on antigenically distinct polysaccharide capsules. Serotype b strains cause invasive infections in infants and children. Nontypeable strains commonly colonize the upper respiratory tract and cause mucosal infections in children and adults.

The recent observation that widely accepted methods do not reliably distinguish strains of *H. influenzae* from *H. haemolyticus* has important implications for clinical microbiology laboratories and also in the interpretation of published studies of colonization of the human nasopharynx (31). The ability to produce a clear hemolytic zone on blood agar has been the sole characteristic used to differentiate *H. haemolyticus* from *H. influenzae* (17, 21). Surprising recent observations revealed that a substantial proportion of *H. haemolyticus* strains are nonhemolytic (28, 31). Thus, laboratory tests used by clinical microbiology laboratories throughout the world do not reliably differentiate between these two species. Many nonhemolytic strains of *H. haemolyticus* are misidentified as *H. influenzae*. Misidentifying *H. haemolyticus* as *H. influenzae* has important implications in accurately elucidating colonization patterns and characterizing pathogenetic mechanisms of *H. influenzae*, because *H. haemolyticus* is a commensal and rarely causes disease. Analysis of 16S rRNA or the conserved P6 gene or IgA protease genes can be used for differentiation (31, 37). A simple method to reliably distinguish *H. influenzae* from *H. haemolyticus* is urgently needed. Finally, a note of caution, it is important to interpret the literature on respiratory tract colonization and infection by *H. influenzae* with this important limitation in mind, i.e., up to ~30 to 40% of strains recovered from respiratory tract cultures and identified as *H. influenzae* may in fact be commensal *H. haemolyticus*.

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IMPACT OF Hib CONJUGATE VACCINES ON Hib DISEASE

Prior to the development and use of Hib conjugate vaccines, Hib was the most common cause of invasive bacterial infection and bacterial meningitis in children in the United States; 3 to 6% died, and permanent sequelae, ranging from mild hearing loss to mental retardation, were seen in 20 to 30% of survivors. The conjugate vaccines induce bactericidal antibodies to capsular polysaccharide (polyribitol ribose phosphate [PRP]), a critical virulence factor that facilitates hematogenous dissemination. A key step in the development of the vaccines was the conjugation of PRP to a protein carrier, enabling the induction of antibody responses in infants at the peak age incidence of Hib infection. An unexpected finding was that vaccination reduced or eliminated nasopharyngeal carriage of Hib. In the pre-Hib vaccine era, the colonization rate of Hib in infants and children was 3 to 5%. The rate is close to zero in countries that employ widespread Hib vaccination. The reduction of circulating strains in the population results in a herd effect, contributing importantly to the efficacy of the vaccine.

Despite the immunogenicity and high efficacy of Hib conjugate vaccines in infants, a small number of Hib infections occur in vaccinated children. Children who experience Hib disease despite vaccination appear to have a defect in immunological priming, resulting in lower-avidity antibodies to Hib capsular polysaccharide (5, 24).

Higher rates of invasive Hib disease were observed in the prevaccine era among some indigenous populations, including Native Americans in the southwest United States, Alaska Native children, and Australian aboriginal populations. Immunization programs have resulted in marked reduction in invasive Hib disease in these populations. However, despite high rates of immunization, the incidence of invasive Hib disease remains higher in native populations than in nonnative populations (29, 41). The ability to respond to an Hib conjugate vaccine with protective immune responses appears to have both genetic and acquired components (46). The presence of low-response groups in a population will influence the efficacy of vaccine programs, emphasizing the importance of continued surveillance of invasive *H. influenzae* infection with careful attention to the distribution of capsular serotypes of disease-causing strains. The importance of continued surveillance is further emphasized by the potential for reservoirs for continued circulation of Hib under selected circumstances in selected populations (33).

While Hib conjugate vaccines have nearly eradicated Hib disease in developed countries that have adopted widespread use, a substantial global burden of Hib infection persists. Watt et al. (48) estimated that Hib caused ~8 million serious illnesses and ~371,000 deaths worldwide in 2000. These infections are almost entirely vaccine preventable.

IMPACT OF Hib CONJUGATE VACCINES ON INVASIVE NON-Hib DISEASE

The success of Hib conjugate vaccines raises the important question of how the alterations in invasive disease patterns and nasopharyngeal colonization by these vaccines may affect the epidemiology of *H. influenzae* infection more widely. In other words, will other serotypes or nontypeable strains of *H. influenzae* emerge as causes of invasive disease?

Several recent surveys have utilized various study designs to monitor invasive *H. influenzae* disease in several countries throughout the world in the Hib conjugate vaccine era (1, 3, 10, 19, 20, 22, 23, 27, 35, 46). The following statements summarize the main conclusions from these studies.

- A shift in the distribution of capsular serotypes of invasive *H. influenzae* disease has occurred, with nontypeable strains replacing type b strains as the most common bloodstream isolates. Strains of *H. influenzae* that cause invasive infection in the vaccine era are predominantly nontypeable.
- Accompanying the shift in serotype distribution resulting from prevention of serotype b disease in infants and children is a shift in the peak age incidence. The most common disease manifestation of invasive *H. influenzae* infection is bacteremia caused by nontypeable strains in adults.
- Infections caused by encapsulated non-type b serotypes, especially serotypes a and f, have been observed in selected geographic regions (1, 3, 8, 30, 45). However, evidence for strain replacement of type b strains with other capsular serotypes is lacking.
- Selected studies suggest an increasing incidence of invasive *H. influenzae* infection, particularly by nontypeable strains. However, there is no convincing evidence that a substantial or sustained increase in the incidence of nonserotype b infections has occurred as a result of widespread immunization with Hib conjugate vaccines.

Continued surveillance to carefully track the incidence, strain distribution, and clinical manifestations of *H. influenzae* disease is critical.

PATHOGENESIS OF *H. INFLUENZAE* INFECTION

Infection caused by encapsulated strains (including type b and other serotypes) involves invasion of the bloodstream and hematogenous dissemination. Capsular polysaccharide is the critical virulence factor that mediates invasion.

In contrast, the mechanism of pathogenesis of infection by nontypeable strains is predominantly by contiguous spread, with migration of bacteria from the nasopharynx to adjacent structures, including sinuses, the middle ear, the trachea, and lower airways. Thus, the first step in the pathogenesis of nontypeable *H. influenzae* infection is colonization of the upper respiratory tract. Approximately 20% of infants are colonized with nontypeable *H. influenzae* in the first year of life, and more than one-half of children are colonized by age five. Transmission of strains occurs frequently within households and among children in day care centers. Colonization and infection are mediated by multiple virulence factors, including adhesins, nutrient uptake systems, molecules that resist host factors, and others (for reviews, see references 2, 13, 32, 39, and 43). Biofilm formation by *H. influenzae* in the middle ear and the airways of cystic fibrosis patients is important for the pathogenesis of infection, particularly chronic and recurrent infections that characterize these clinical settings.

CLINICAL MANIFESTATIONS OF *H. INFLUENZAE* INFECTIONS

Upper respiratory tract infections. (i) Otitis media in children. Otitis media is the most frequently diagnosed bacterial illness in young children requiring office or clinic visits in the United States. Acute otitis media is an inflammation of the middle ear (the cavity between the eardrum and the inner ear), and episodes are characterized by fever, ear pain, and, in severe cases, discharge from the ear. The gold standard for an etiologic diagnosis, culture of middle ear fluid, requires tympanocentesis, which is an invasive procedure and is thus not routinely performed. However, based on results of middle ear fluid cultures obtained by tympanocentesis as part of clinical trials, *H. influenzae* is one of the most common causes of otitis media, accounting for 25 to 35% of episodes of acute otitis media (32).

Children who have 4 or more episodes of acute otitis media in a year or who experience at least 8 months of middle ear effusion in a year are defined as otitis prone. Up to 10% of children in the United States are otitis prone. Nasopharyngeal colonization by *H. influenzae* early in life is associated with the otitis-prone condition. The most common cause of recurrent otitis media is nontypeable *H. influenzae* (25). In developing countries, complications, including chronic suppurative otitis media, are frequent. Globally, up to 330 million people suffer from recurrent and chronic and otitis media (http://www.who.int/pbd/deafness/activities/hearing_care/otitis_media.pdf).

Since 2000, most infants in the United States have received the 7-valent pneumococcal conjugate vaccine. Widespread administration of this vaccine has resulted in a dramatic reduction in nasopharyngeal colonization by the 7 pneumococcal serotypes included in the vaccine. This has been accompanied by several important changes in the distribution of bacterial pathogens that cause otitis media: (i) a reduction in pneumococcal vaccine serotypes, (ii) a relative increase in pneumococcal nonvaccine serotypes, and (iii) a relative increase in *H. influenzae* and *Moraxella catarrhalis* causing nasopharyngeal colonization and otitis media (4, 9, 36). Two new pneumococcal conjugate vaccines have recently been approved for use, and these include a 13-valent vaccine in the United States and a 10-valent vaccine in Europe in which pneumococcal polysaccharides are conjugated to protein D, a surface protein of *H. influenzae* (34). These vaccines will have a significant impact on pathogens of otitis media, patterns of nasopharyngeal colonization, and the populations of bacterial strains that circulate in communities. Careful surveillance will be critical to track inevitable but not entirely predictable changes.

(ii) Sinusitis. Sinusitis is a complication of viral upper respiratory tract infection, estimated to occur following ~7% of episodes in children and less often in adults. Samples obtained by sinus puncture or endoscopy reveal that *H. influenzae* is an important pathogen in both acute and chronic sinusitis (6, 7, 11). Pneumococcal conjugate vaccines appear to have resulted in a relative increase in *H. influenzae* as a cause of sinusitis (7). Because treatment of most sinusitis is empirical, *H. influenzae* should be considered when making antibiotic choices in patients with the clinical picture of bacterial sinusitis.

(iii) Conjunctivitis. Nontypeable *H. influenzae* causes conjunctivitis in children, the elderly, health care workers, and immunocompromised hosts. Person-to-person transmission, particularly in day care centers and nursing homes, plays an important role in the spread of infection in these settings (14, 47).

Lower respiratory tract infections. (i) Exacerbations in adults with COPD. Chronic obstructive pulmonary disease (COPD) afflicts ~24 million Americans and is the fourth most common cause of death in the United States and in the world. The course of the disease is characterized by intermittent worsening or exacerbations that are associated with enormous morbidity, including missed work time, office and clinic visits, emergency room visits, hospital admissions, and, in the most severe cases, respiratory failure necessitating mechanical ventilation. The role of bacterial infection in COPD has long been a source of confusion and controversy, but more recently, investigation is beginning to elucidate the important role of bacteria in the course and pathogenesis of COPD (39). The best estimates are that approximately 30% of exacerbations are caused by viral infection and ~50% are caused by bacterial infection. The presence of a bacterial pathogen in the sputum of an adult with COPD does not establish the etiology of an exacerbation because the same pathogens colonize COPD patient airways during clinically stable periods. Several lines of evidence, as follows, support the conclusions that bacteria cause exacerbations of COPD and that nontypeable *H. influenzae* is the most common bacterial cause of exacerbations of COPD.

- Nontypeable *H. influenzae* is isolated from the lower airways of adults with exacerbations of COPD when the distal airways are sampled using bronchoscopy and the protected specimen brush.
- Acquisition of a new strain of *H. influenzae* is highly associated with the onset of exacerbation of COPD (31, 38).
- Adults with COPD who experience exacerbations associated with new strain acquisitions of *H. influenzae* develop strain-specific serum antibody responses (40).
- Acquisition of *H. influenzae* causes increased airway inflammation, a hallmark of exacerbations of COPD.

H. influenzae and other bacterial pathogens also play a more subtle role in the pathogenesis of COPD. In contrast to the sterile airways of a healthy lung, respiratory pathogens are present in the airways of 25 to 50% of clinically stable adults with COPD, with *H. influenzae* as the most common. These pathogens release highly active antigens that induce enhanced airway inflammation, which is a characteristic feature of COPD.

(ii) Pneumonia. Precise data on the role of *H. influenzae* as a cause of pneumonia in children and adults are lacking. In the pre-Hib conjugate vaccine era, Hib was thought to be a predominant cause in children. The role of nontypeable *H. influenzae* in childhood pneumonia remains unclear, but several indirect lines of evidence, including its high prevalence in nasopharyngeal colonization studies, its demonstrated pathogenic potential in otitis media and preliminary evidence of involvement in bronchitis, suggest that nontypeable strains play some role in lower respiratory tract infection in children (15).

Nontypeable *H. influenzae* causes community-acquired pneumonia in adults, particularly in the elderly and in patients

with COPD, HIV, and cystic fibrosis. Hence, antibiotics with activity against *H. influenzae* are recommended by Infectious Diseases Society of America guidelines when considering empirical treatment of community-acquired pneumonia in adults (26).

(iii) Infections in cystic fibrosis. Colonization and infection by *Pseudomonas aeruginosa* in patients with cystic fibrosis are often preceded by infection with nontypeable *H. influenzae* or *Staphylococcus aureus* early in the course of the disease. The presence of *H. influenzae* biofilms in the airways of children with cystic fibrosis suggests that biofilms may be important in early lung injury, facilitating later infection by *P. aeruginosa* (42).

Invasive infections. *H. influenzae* occasionally causes invasive infections in the post-Hib vaccine era; most such infections are caused by nontypeable strains. The highest incidence is in children less than 1 year old and in the elderly. The most common presentations are bacteremia and meningitis. Many but not all have some associated predisposing conditions, including immunosuppression, HIV infection, or a respiratory disorder. Nontypeable strains that cause invasive infection are genetically diverse.

Infections by other encapsulated strains have disease manifestations similar to those of type b strains, causing predominantly meningitis and invasive disease.

ANTIMICROBIAL SUSCEPTIBILITY

A comprehensive discussion of treatment is beyond the scope of this review. Antimicrobial susceptibility patterns of *H. influenzae* are undergoing changes, and as a result, patterns differ around the world. Depending on geographic location, approximately 20 to 35% of strains produce β-lactamase, which mediates resistance to amoxicillin (44). A decrease in the prevalence of β-lactamase-producing strains was observed recently in the United States, so this trend deserves surveillance (16). Alteration of penicillin-binding proteins, a second mechanism of β-lactam resistance, has been observed with increasing prevalence in isolates in Japan and Europe (12, 18). Most of these strains are genetically diverse, but clonal dissemination of antibiotic-resistant strains has also been observed. This mechanism of resistance has not yet been observed to any significant extent in the United States, but careful vigilance will be critical.

SUMMARY AND CONCLUSIONS

The spectacular progress in preventing Hib infections over the past 2 decades has resulted in changes in colonization and disease patterns of *H. influenzae*. In particular, the distribution of capsular serotypes in invasive disease has shifted from predominantly serotype b strains to predominantly nontypeable strains. There is no convincing evidence of an increased incidence of invasive disease by non-serotype b strains of *H. influenzae*, i.e., no strain replacement. However, this issue requires continued surveillance. The most common *H. influenzae* infections are mucosal, including otitis media in children and exacerbations in adults with COPD. Recent observations emphasize the importance of carefully distinguishing between *H. influenzae* and *H. haemolyticus* in the laboratory in order to have an accurate indication of *H. influenzae* colonization and disease patterns. The introduction of vaccines for otitis media that are having major effects on nasopharyngeal colonization patterns and global shifts in antimicrobial susceptibility create a "moving target" for monitoring *H. influenzae* disease and emphasize the importance continued vigilance.

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