

## Dr.Mohammed Al-Araji 2015/2016

### *Haemophilus influenzae*

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*Haemophilus influenzae* (formerly called **Pfeiffer's bacillus** or *Bacillus influenzae*) is a Gram-negative, coccobacillary, facultatively anaerobic bacterium belonging to the *Pasteurellaceae* family. *H. influenzae* was first described in 1892 by Richard Pfeiffer during an influenza pandemic. The bacterium was mistakenly considered to be the cause of influenza until 1933 when the viral etiology of influenza became apparent, and is still colloquially known as 'bacterial influenza'. *H. influenzae* is responsible for a wide range of localized and invasive infections. This species was the first free-living organism to have its entire genome sequenced. The sequencing project was completed and published in 1995.

### **Microbiology**

*H. influenzae* are small, pleomorphic gram-negative rods that are oxidase-positive, facultatively anaerobic, and nonmotile. In clinical specimens obtained from patients who have received beta-lactam antibiotics, *H. influenzae* can appear as filamentous rods. In vitro growth requires a CO<sub>2</sub>-enriched atmosphere, hemin (factor X), and nicotinamide adenine dinucleotide (NAD, factor V); therefore, isolation from clinical specimens on solid medium requires the use of chocolate agar or other X and V factor supplemented media. *H. influenzae* appear as transparent or slightly opaque colonies on solid media.

**Capsule** — The presence or absence of a polysaccharide capsule is an important distinguishing characteristic of *H. influenzae* species. The polysaccharide capsule can be serologically classified into six serotypes (a through f), while *H. influenzae* lacking a polysaccharide capsule are considered to be nontypeable. The type b capsule consists of a ribosyl and ribitol phosphate polymer and is the primary antigenic constituent of polysaccharide and polysaccharide conjugate Hib vaccines.

### **Serotypes**

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In 1930, two major categories of *H. influenzae* were defined: the unencapsulated strains and the encapsulated strains. Encapsulated strains were classified on the basis of their distinct capsular antigens. There are six generally recognized types of encapsulated *H. influenzae*: a, b, c, d, e, and f.<sup>[2]</sup> Genetic diversity among unencapsulated strains is greater than within the encapsulated group. Unencapsulated strains are termed

nontypable (NTHi) because they lack capsular serotypes; however, they can be classified by multilocus sequence typing. The pathogenesis of *H. influenzae* infections is not completely understood, although the presence of the capsule in encapsulated type b (Hib), a serotype causing conditions such as epiglottitis, is known to be a major factor in virulence. Their capsule allows them to resist phagocytosis and complement-mediated lysis in the nonimmune host. The unencapsulated strains are almost always less invasive; they can, however, produce an inflammatory response in humans, which can lead to many symptoms. Vaccination with Hib conjugate vaccine is effective in preventing Hib infection, but does not prevent infection with NTHi strains.<sup>[3]</sup>

## Diseases

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Most strains of *H. influenzae* are opportunistic pathogens; that is, they usually live in their host without causing disease, but cause problems only when other factors (such as a viral infection, reduced immune function or chronically inflamed tissues, e.g. from allergies) create an opportunity. They infect the host by sticking to the host cell using Trimeric Autotransporter Adhesins (TAA).

Naturally acquired disease caused by *H. influenzae* seems to occur in humans only. In infants and young children, *H. influenzae* type b (Hib) causes bacteremia, pneumonia, epiglottitis and acute bacterial meningitis. On occasion, it causes cellulitis, osteomyelitis, and infectious arthritis.

Due to routine use of the Hib conjugate vaccine in the U.S. since 1990, the incidence of invasive Hib disease has decreased to 1.3/100,000 in children. However, Hib remains a major cause of lower respiratory tract infections in infants and children in developing countries where the vaccine is not widely used. Unencapsulated *H. influenzae* strains are unaffected by the Hib vaccine and cause ear infections (otitis media), eye infections (conjunctivitis), and sinusitis in children, and are associated with pneumonia.

## Diagnosis

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*Haemophilus influenzae* requires X and V factors for growth. In this culture haemophilus has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor.

Clinical features may include initial symptoms of an upper respiratory tract infection (URTI) mimicking a viral infection, usually associated with fevers, often low grade. The URTI then may progress to a LRTI in

a few days with features often resembling a wheezy bronchitis. Sputum may be difficult to expectorate and is often grey to creamy in colour. The cough may persist for weeks without appropriate treatment. Be suspicious of patients with symptoms of a chest infection with wheezing who do not respond to penicillins or 1st generation cephalosporins.

Clinical diagnosis of *H. influenzae* is typically performed by bacterial culture or latex particle agglutinations. Diagnosis is considered confirmed when the organism is isolated from a sterile body site. In this respect, *H. influenzae* cultured from the nasopharyngeal cavity or sputum would not indicate *H. influenzae* disease, because these sites are colonized in disease-free individuals.<sup>[5]</sup> However, *H. influenzae* isolated from cerebrospinal fluid or blood would indicate *H. influenzae* infection.

### **Culture**

Bacterial culture of *H. influenzae* is performed on agar plates, the preferable one being chocolate agar, with added X (hemin) and V (nicotinamide adenine dinucleotide) factors at 37°C in a CO<sub>2</sub>-enriched incubator. Blood agar growth is only achieved as a satellite phenomenon around other bacteria. Colonies of *H. influenzae* appear as convex, smooth, pale, grey or transparent colonies.

Gram-stained and microscopic observation of a specimen of *H. influenzae* will show Gram-negative, rod shaped, with no specific arrangement. The cultured organism can be further characterized using catalase and oxidase tests, both of which should be positive. Further serological testing is necessary to distinguish the capsular polysaccharide and differentiate between *H. influenzae* b and nonencapsulated species.

Although highly specific, bacterial culture of *H. influenzae* lacks in sensitivity. Use of antibiotics prior to sample collection greatly reduces the isolation rate by killing the bacteria before identification is possible. Beyond this, *H. influenzae* is a finicky bacterium to culture, and any modification of culture procedures can greatly reduce isolation rates. Poor quality of laboratories in developing countries has resulted in poor isolation rates of *H. influenzae*.

*H. influenzae* will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates; the hemolysis of cells by *S. aureus* releases factor V which is needed for its growth. *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients such as factor V in these areas. Fildes agar is best for isolation. In Levinthal medium capsulated strains show distinctive iridescence.

### **Latex particle agglutination**

The latex particle agglutination test (LAT) is a more sensitive method to detect *H. influenzae* than culture.<sup>[8]</sup> Because the method relies on antigen rather than viable bacteria, the results are not disrupted by prior antibiotic use. It also has the added benefit of being much quicker than culture methods. However, antibiotic sensitivity testing is not possible with LAT alone, so a parallel culture is necessary.

### **Molecular methods**

Polymerase chain reaction (PCR) assays have been proven to be more sensitive than either LAT or culture tests, and highly specific. However, PCR assays have not yet become routine in clinical settings. Countercurrent immunoelectrophoresis has been shown to be an effective research diagnostic method, but has been largely supplanted by PCR.

### **Interaction with *Streptococcus pneumoniae***

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Both *H. influenzae* and *S. pneumoniae* can be found in the upper respiratory system of humans. In an *in vitro* study of competition, *S. pneumoniae* always overpowered *H. influenzae* by attacking it with hydrogen peroxide and stripping off the surface molecules *H. influenzae* needs for survival.

When both bacteria are placed together into a nasal cavity, within 2 weeks, only *H. influenzae* survives. When either is placed separately into a nasal cavity, each one survives. Upon examining the upper respiratory tissue from mice exposed to both bacteria species, an extraordinarily large number of neutrophils (immune cells) was found. In mice exposed to only one bacterium, the cells were not present.

Lab tests showed neutrophils exposed to dead *H. influenzae* were more aggressive in attacking *S. pneumoniae* than unexposed neutrophils. Exposure to dead *H. influenzae* had no effect on live *H. influenzae*.

Two scenarios may be responsible for this response:

1. When *H. influenzae* is attacked by *S. pneumoniae*, it signals the immune system to attack the *S. pneumoniae*
2. The combination of the two species triggers an immune system response that is not set off by either species individually.

It is unclear why *H. influenzae* is not affected by the immune response.

### **Treatment**

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*Haemophilus influenzae* produces beta-lactamases, and it is also able to modify its penicillin-binding proteins, so it has gained resistance to the penicillin family of antibiotics. In severe cases, cefotaxime and ceftriaxone delivered directly into the bloodstream are the elected antibiotics, and,

for the less severe cases, an association of ampicillin and sulbactam, cephalosporins of the second and third generation, or fluoroquinolones are preferred. (Fluoroquinolone-resistant *Haemophilus influenzae* has been observed.)

Macrolide antibiotics (e.g., clarithromycin) may be used in patients with a history of allergy to beta-lactam antibiotics. Macrolide resistance has also been observed.

## **Prevention**

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Effective vaccines for *Haemophilus influenzae* Type B have been available since the early 1990s, and is recommended for children under age 5 and asplenic patients. The World Health Organization recommends a pentavalent vaccine, combining vaccines against diphtheria, tetanus, pertussis, hepatitis B and Hib. There is not yet sufficient evidence on how effective this pentavalent vaccine is in relation to the individual vaccines.

Hib vaccines cost about seven times the total cost of vaccines against measles, polio, tuberculosis, diphtheria, tetanus, and pertussis. Consequently, whereas 92% of the populations of developed countries was vaccinated against Hib as of 2003, vaccination coverage was 42% for developing countries, and only 8% for least-developed countries.

## **History and vaccine composition**

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### **Polysaccharide vaccine**

The first Hib vaccine licensed was a pure polysaccharide vaccine, first marketed in the US in 1985.<sup>[5]</sup> Similar to other polysaccharide vaccines, immune response to the vaccine was highly age-dependent. Children under 18 months of age did not produce a positive response for this vaccine. As a result, the age group with the highest incidence of Hib disease was unprotected, limiting the usefulness of the vaccine. The vaccine was withdrawn from the market in 1988.

### **Conjugate vaccine**

The shortcomings of the polysaccharide vaccine led to the production of the Hib polysaccharide-protein conjugate vaccine.<sup>[5]</sup> Attaching Hib polysaccharide to a protein carrier greatly increased the ability of the immune system of young children to recognize the polysaccharide and develop immunity. There are currently three types of conjugate vaccine, utilizing different carrier proteins for the conjugation process, all of which are highly effective and safe: inactivated tetanospasmin (also

called tetanus toxoid), mutant diphtheria protein, and meningococcal group B outer membrane protein.

### **Combination vaccines**

Multiple combinations of Hib and other vaccines have been licensed in the United States, reducing the number of shots necessary to vaccinate a child. Hib vaccine combined with diphtheria-tetanus-pertussis-polio vaccines and Hepatitis B vaccines are available in the US. The World Health Organization (WHO) has certified several Hib vaccine combinations, including a pentavalent diphtheria-pertussis-tetanus-hepatitis B-Hib, for use in developing countries. There is not yet sufficient evidence on how effective this combined pentavalent vaccine is in relation to the individual vaccines

*Haemophilus influenzae* colonizes the human respiratory tract and is transmitted from person to person via airborne droplets and direct contact with respiratory secretions

*H. influenzae* has encapsulated (serotypes a through f) and non-encapsulated forms (nontypeable). The most important serotype is *H. influenzae* serotype b (Hib), which was a frequent cause of bacteremia, meningitis, and other invasive infections prior to the routine use of Hib conjugate vaccines in children. Other capsular serotypes and unencapsulated *H. influenzae* strains can also cause disease, mainly mucosal infections (sinusitis, otitis, bronchitis and pneumonia) but occasionally cause more invasive infections.