AUCTORES
Globalize your Research

Journal of Clinical Case Reports and Studies
Fattma A. Ali *

Open Access

Review Article

The Role of Virulence Factors of Haemophilus Influenza

Muna Muhammad Najeeb, Fattma A. Ali *, Ahmed Akil Khudhair Al-Daoody, Yasmeen Moustafa Khalil

[1]Medical microbiology department /College of Health Sciences/ Hawler Medical University, Erbil/Iraq

[2]Microbiology, Education College, Salahaddin University, Erbil, Kurdistan, Iraq.

***Corresponding Author:** Fattma A. Ali. College of Health Sciences/ Hawler Medical University, Erbil/Iraq.

Received date: December 11, 2024; **Accepted date:** December 30, 2024; **Published date:** January 03, 2025

Citation: Fattma A. Ali, Ahmed Akil Al-Daoody, Cheman H. Hamid, Sawsan M. Sorche, (2024), The Role of Virulence Factors of Haemophilus Influenza**,** *J, Clinical Case Reports and Studies*, 6(1); **DOI**:**10.31579/2690-8808/237**

Abstract

Background: Haemophilus influenzae is a pathogenic bacterium that frequently leads to serious infections, especially in newborns. Haemophilus influenzae is a Gram-ve coccobacillus belonging to the Pasteurellaceae family. It is microscopic in size, ranging from 0.3 to 1 micrometers. Its facultative anaerobic can survive with or without oxygen and has the ability to change its shape. It grows in an environment with increased levels of carbon dioxide. The development of this organism on chocolate agar is facilitated by a medium that contains two erythrocyte factors: factor X (hematin) and factor V (phosphopyridine nucleotide).

Objectives: Our study aimed to carry out the role of the virulence factors of haemophilus influenza that facilitate the pathogenesis of infections caused by this bacteria.

Main body: The pathogenicity determinants of H..influenzae contribute to its capacity to colonies and induce illness in the host. The following are important virulence.factors of H.. influenzae: Capsule used for inhibition of phagocytosis to avoid detection by the host's immune system. Pili : are adhesion molecules that enable the binding of cells or microorganisms to surfaces . IgA Protease : H..influenzae secretes IgA1 protease, an enzyme that specifically splits human IgA1 antibodies. Endotoxin (Lipopolysaccharide - LPS) This endotoxin has the ability to initiate an inflammatory reaction in the host. Beta- lactamase : Beta-lactamase is a bacterial enzyme that provides resistance to beta-lactam antibiotics .

Conclusion: H. influenzae can be categorised into six separate categories, known as serotypes a, b, c, d, e, and f, based on the presence of a polysaccharide capsule. Types A, E, and F are also isolated, although less frequently than type B. C and D are hardly identified. All serotypes, especially type b, are frequently responsible for lower respiratory tract infections, such as pneumonia .In addition, they have the potential to induce various additional severe illnesses including meninigitis , epiglottitis, cellulitis , septic arthritis and bacteremia.

Key Words: haemophilus influenza; virulence factors; pathogenesis; unencapsulated; encapsulated

1.Introduction

Haemophilus influenzae is a pathogenic bacterium that frequently leads to serious infections, especially in newborns. Richard.. Pfeiffer originally documented it in 1892. Throughout an influenza outbreak, he discovered the presence of *H. influenzae* in the sputum of patients and put upa hypothesis suggesting a direct link between this bacteria and the clinical illness often referred to as influenza. The organism was given the name Haemophilus by Charles-Edward Winslow, et al. in 1920. The discovery that influenza is caused by a virus and that *H. influenzae* can induce secondary infection was made in 1933 [1]. *Haemophilus influenzae* is a Gram-ve coccobacillus belonging to the Pasteurellaceae family. It is microscopic in size,ranging from 0.3 to 1 micrometers. Its facultative anaerobic can survive with or without oxygen and has the ability to change its shape. It grows in an environment with increased levels of carbon dioxide. The development of this organism on chocolate agar is facilitated by a medium that contains two erythrocyte factors: factor X(hematin) and factor V (phosphopyridine nucleotide). These factors are released when red blood cells are lysed [2] . *H.influenzae* can be categorised into six separate categories, known as serotypes a, b, c, d, e, and f, based on the presence of a polysaccharide capsule. These serotypes are identified by their particular reactivity to serum agglutination. *H..influenzae* type b is notable dueto its polyribosyl. ribitol phosphate capsule, whichis responsible for 95% of invasive disease in children and over 50% of invasive disease in adults .Less frequent pathogens, the other capsular kinds, are responsible for a smaller proportion of infections. The majority of isolates are non-typeable , indicating the absence of a polysaccharide. capsule and the resulting lack of agglutination with antiserum [3] .The mostcommonly encountered and prevailing variant

is*H.. influenzae* type b which primarily affects youngsters and individuals with weakened immune systems. Types A, E, and F are also isolated, although less frequently than type B. C and D are hardly identified. All serotypes,especially type b, are frequently responsible for lower respiratory tract infections, such as pneumonia. In addition, they have the potential to induce various additional severe illnesses, including meningitis, epiglottitis, cellulitis, septic.arthritis, as well as empyema andbacteremia as diagram (1) [4] . Diagnostic cultures of blood, cerebrospinal fluid (CSF), and other typically sterile fluids are obligatory under the suitable conditions. Whenever possible, it is advisable to gram-stain specimens obtained for culture. The presence of capsular antigen in blood, CSF, or concentrated urine can be detected using immunoelectrophoresis, latex agglutination, or enzyme-linked immunosorbent assay. This diagnostic method can identify the antigen in up to 90 percent of cases of meningitis that have been confirmed through culture. Untreated Haemophilus influenzae infection can lead to immediate fatality, especially through meningitisand epiglottitis. Currently, there is a tendency to utilize certain injectable third generation cephalosporins as the first treatment option forpotentially life-threatening Haemophilus influenzae infections in children older than the newborn stage. The typically used drugs for this purpose are cefotaxime or ceftriaxone. Supportive care is crucial in managing Haemophilus influenzaeinfection in children, in addition to antibiotictherapy [5].

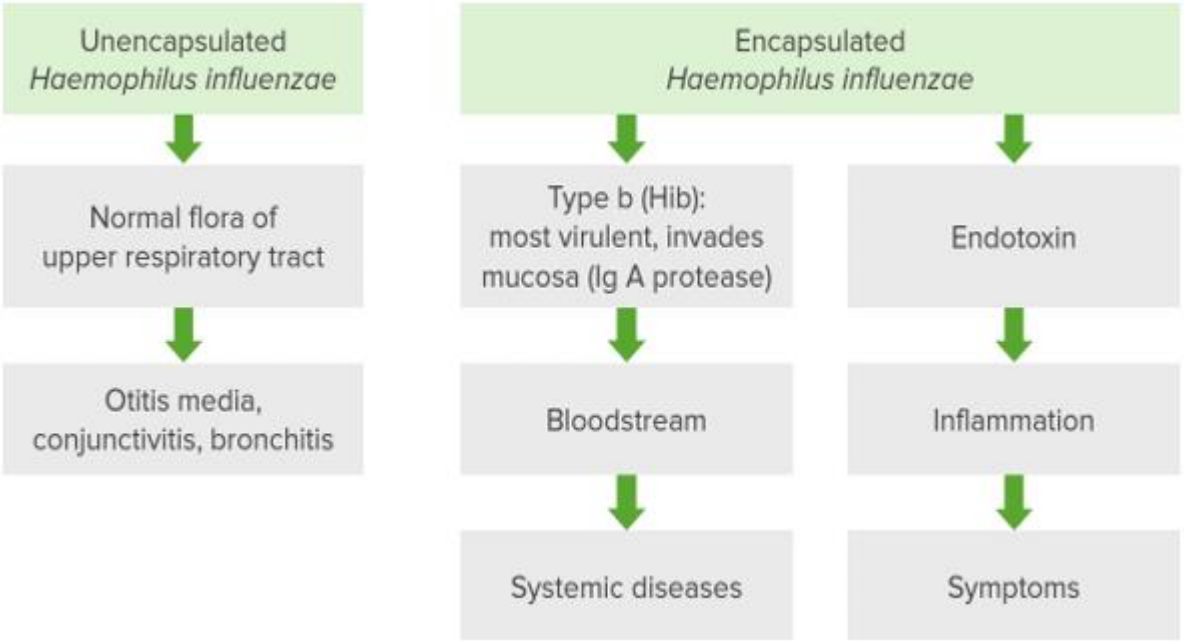

Diagram (1) [6]

This diagram shows the how the types of the bacteria can cause infections, in which bacteria divided to encapsulated and encapsulated. 1- Unencapsulated bacteria are part of normal flora of upper respiratory tract may cause mild infection in childlike otitis media, conjunctivitis and bronchitis. 2- Encapsulated bacteria mainly type b is the most virulent strain that invades mucosa by IgA protease in the bloodstream that cause systemic diseases may complicated to bacteremia or encapsulated bacteria produce endotoxin that induce inflammation lead to appearance of symptoms of bacterial infection.

1. Main Body

The pathogenicity determinants of *H. influenzae* contribute to its capacity to colonies and induce illness in the host. The following are important virulence factors of *H. influenzae*:

1.1. Capsule

The polysaccharide capsule of *H.influenzae* is a significant determinant of its ability to cause disease. The bacterium uses inhibition ofphagocytosis to avoid detection by the host's immune system [7]. *H.influenzae* serotypes are categorized according to their capsular antigens, with the type b (Hib) capsule being specifically linked to invasive illnesses .The capsule of *Haemophilus influenzae* is a significant virulence factor due to its antiphagocytic properties, which provide protection against phagocytosis bymacrophages or neutrophils. *H.influenzae* is able to evade destruction and adhere to epithelial cells in the airways [8]. The native type B capsule consistsof linear teichoic acid, which contains ribose, ribitol,and phosphate, and is referred to as such.Polyribosyl-ribitol-phosphate is a compound. The process of capsule synthesis is determined by the capB genes, which exist in two identical copies (17-18 kb) within the chromosome and are connected by a small area (1-1.3kb). The genetic sequence in this area includes a gene known as bexA, which is responsible for producing a protein necessary for transporting the capsular material to the surface of the cell .The remaining 5 serotypes possess the cap gene alone in a single copy. Approximately 2% of *H.influenzae* type b individualspossess a single copy of the gene. The absence of the other copy results in the inability to produce capsules, and these strains are referred to as capsule-deficient mutants [9]. Contrary to encapsulated type b bacteria, which are linked to decreased ability to stick to and invade host cells, mutant strains have a significantly. Enhanced capacity to adhere and infiltrate the macro organism, occurring at a frequency of 50 times [10].

1.2. Pili

Pili are adhesion molecules that enable the binding of cells or microorganisms to surfaces. Pili and non- pilus adhesions aid in the adherence of *H.influenzae*to host cells, facilitating colonization and the initiation of infection [11] . The pili are an additionalelement that contributes to the pathogenicity of *H.influenzae*. They are present in the encapsulated strains of serotype b and in over 50% of the cases. Encapsulated strains. The composition of these structures includes a single substantial protein(HifA) together with two minor proteins (HifD and HifE).Pili facilitate the adherence of the bacterial cell to the eukaryotic cell by attaching to glycoproteins and glycolipids on the eukaryotic cell's surface. *H.influenzae* possesses a single copyof each gene that encodes fimbria proteins, namely hifA, hifD and hifE. Within this gene cluster, there are two additional genes (hifV and hifS) that encode proteins responsible for synthesizing and protectingfimbria proteins during their export from the cell. Non typable *H.influenzae* possesses an additional fimbria protein known as P5-fibrin, which bears resemblance to one of the outer membrane proteins (P5) [12]. Exterior membrane The number of *H.influenzae* proteins ranges from six to

eight. Some of these antigens, such as P2 and P6, are currently undergoing intensive research. May be used into vaccine formulations targeting none capsulated pathogens. Antibodies targeting P2 havebactericidal properties and provide protection. P2 proteins are the predominant outer membrane proteins. These are porins characterized by a very changeable outer portion that exhibits differences among different strains. Their intracellular region, situated within the outer membrane, possesses a conserved amino acid sequence. The extrinsic region of these proteins may undergo temporal variations due to individual modifications in the structural genes associated with P2. This results in a persistent manifestation of certain *H.influenzae* infections.P6 is a protein located on the outer membrane that is produced on the surface of both encapsulated and non typable strains. The gene responsible for encoding it exhibits a significant level of conservation, resulting in a notable similarity in terms of its nucleotide sequence across various strains [13].

1.3. IgA Protease

H.influenzae secretes IgA1 protease, an enzyme that specifically splits human IgA1 antibodies. This enzyme facilitates the evasion of the host's mucosalimmune responses by the bacteria [14]. TheIgA1 protease is a crucial element in determining the pathogenicity of *H.influenzae*. It works bydeactivating human immunoglobulin A1 and aiding in the colonization of mucosae. Approximately 95% of strains that cannot be typed possess the gene responsible for encoding this enzyme is known asn iga. The protease activity is maintained to a higher degree in invasive isolates blood and cerebrospinal fluid and isolates obtained from sputum. This particular virulence factor is more commonly seenin strains originating from the upper respiratory tract, particularly in non typable strains. A second IgA protease has been identified, which is more prevalent in isolates from individuals with COPD [15].

1.4. Endotoxin (Lipopolysaccharide - LPS):

H.influenzae, similar to other Gram-ve bacteria,contains endotoxin on its outer membrane. This endotoxin has the ability to initiate an inflammatory reaction in the host [16]. *H. influenzae*, like other Gram-ve microbes, possesses a lipopolysaccharide. However, its lipopolysaccharide has a shorter polysaccharide chain and is referred to as lipooligosaccharide. Aside from having the distinct lipooligosaccharide, which is a characteristic feature of Gram-ve bacteria's endotoxins, aids in evading the process of opsonization and phagocytosis by mimicking molecular structures found in the hostorganism. This is because the lipooligosaccharide has ends that are sialated, meaning they have sialic acid attached to them. These sialated ends have a comparable structure and antigenic properties to the sialated oligosaccharides found in the sphingolipids of the human body [17].

1.5. Beta- lactamase

Beta-lactamase is a bacterial enzyme that provides resistance to beta-lactam antibiotics, such as penicillins and cephalosporins. *H.influenzae* has the ability to generate beta-lactamase, which is amethod it employs to counteract the impact of beta-lactam antibiotics. The enzyme hydrolyzes the beta-lactam ring compound found in these antibiotics, causing them to lose their efficacy against the bacterium [18] . The phenomenon of ampicillin resistance in *H.influenzae* was first documented in the early 1970s [19]. β-Lactams have historically been employed for the treatment of *H.influenzae* infections, but, resistance has arisenand spread. Ampicillin resistance in bacteria*H.influenzae* has become prevalent worldwide, withvaried incidence rates ranging from 8 to 30% in diverse European countries and North America, to over 50% in certain Eastern Asian countries [20].There have been two described mechanisms of β- lactam resistance. One method involves thebreakdown of β-lactam by enzymes called (?TEM-1 or ROB-1 β-lactamases. Bacteria with these enzymes are referred to be β-lactamase-positive ampicillin-resistant. The other involves decreased β-lactam affinity for penicillin binding protein 3 owing to change in the ftsI gene [21].

The classification of *H.influenzae* that is resistant to antimicrobial agents is intricate. The term often used, β-lactamase-negative ampicillin-resistant is applied to isolates that exhibit resistance to ampicillin, but do not show any indication of β- lactamase [22] . The isolates exhibited resistance to Ampicillin as a result of significant changes in theftsI gene, which encodes the penicillin-binding protein 3. These mutations caused a decrease inthe affinity for β-lactam [22]. Nevertheless, certain isolates exhibited such changes, yet were not the strain has demonstrated resistance to ampicillin as determined by phenotypic testing. Isolates that have critical mutations in penicillin-binding protein 3 , regardless of their resistance profile, are classified as genomic BLNAR [23] . The term often used is "β-lactamase positive" in reference toamoxicillin. The term "clavulanic acid-resistant" (BLPACR) refers to isolates that have resistance to amoxicillin/clavulanic acid and show indications of β-lactamase synthesis . The identified isolates were found to have both the β-lactamase gene and significant mutations in the ftsI gene [1]. Certain isolates had this profile, however, they did not demonstrate resistance to amoxicillin clavulanic acid based on phenotypic testing. The genomic BLPACR isolates are denoted as genomic BLNAR [22]. Resistance caused by modified penicillin- binding proteins has become a significant mechanism of β-lactam resistance in various bacterial pathogens, including non typeable *H.influenzae* . In recent years, numerous nations have shown a significant rise in the occurrence of BLNAR non typeable *H.influenzae* isolates, especially among respiratory tract isolates [23] . Geno typically characterized BLNAR isolates make up 15-30% of all non typeable *H.influenzae* isolates in Australia the USA and Europe, and a concerning 50% in Japan [24].

1.6. Pathogenesis

The exact mechanism by which *H.influenzae* infections develop is not fully comprehended, however, the presence of the type b polysaccharide capsule significantly contributes to its ability to cause disease. Enclosed microorganisms have the ability to enter the epithelium of the nasopharynx and directly infiltrate blood vessels. Non typable strains have reduced invasiveness, although they, along with typable strains, elicit an inflammatoryresponse that leads to disease; the significance of exotoxin generation in pathogenicity is not considered significant. The nasopharynx of themajority of healthy persons is colonized by non- typeable *H.influenzae* strains, while type b *H.influenzae* strains are present in just a small percentage (1 to 2 percent) of normal youngsters. Occurrences of type b infection are prevalent, particularly in nurseries and child care centers. In such cases, the preventive use of antibiotics may be employed. Immunization with type b polysaccharidehas shown efficacy in avoiding infection, and vaccines are currently accessible for regular [25].

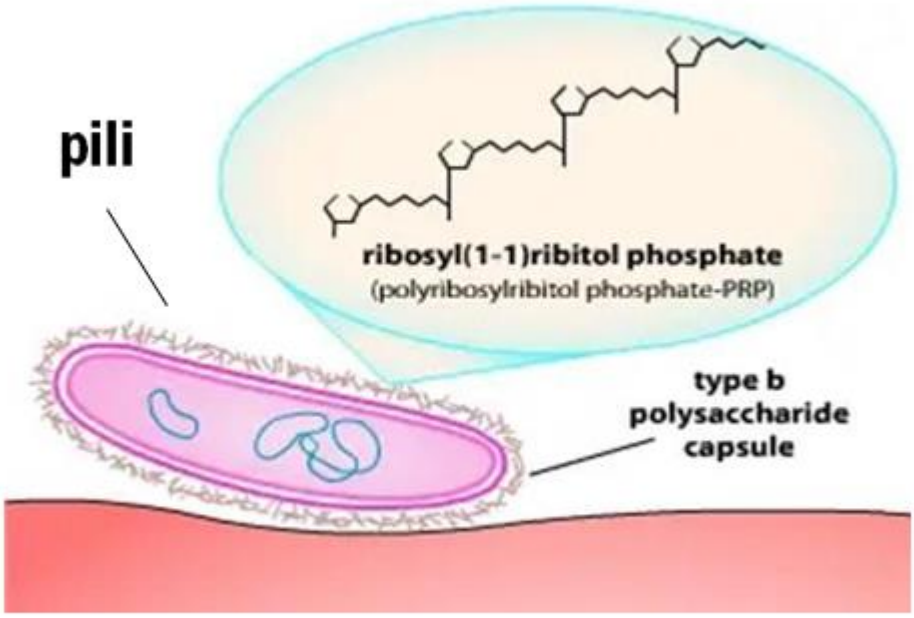

Figure (1) [26] .

This figure shows some of important virulence factors that are required for the pathogenesis of*H.infleunzae* including 1- Polysaccharide capsule which is important for inhibition of phagocytosis to avoid detection by the host's immune system. 2- Are adhesion molecules that enable the binding of cells or microorganisms to surfaces. Pili adhesions aid in the adherence of *H.influenzae* to host cells, facilitating colonization and the initiation of infection .

2. Conclusion

Haemophilus influenzae is a pathogenic bacterium that frequently leads to serious infections, especially in newborns. *Haemophilus influenzae* is a Gram-ve coccobacillus belonging to the Pasteurellaceae family. It is microscopic in size, ranging from 0.3 to 1 micrometers. Its facultative anaerobic can survive with or without oxygen and has the ability to change its shape. It grows in an environment with increased levels of carbon dioxide. The development of this organism on chocolate agar is facilitated by a medium that contains two erythrocyte factors: factor X (hematin)and factor V (phosphopyridine nucleotide). Thesefactors are released when red blood cells are lysed. H.influenzae can be categorised into six separatecategories, known as serotypes a, b, c, d, e, and f, based on the presence of a polysaccharide capsule. Types A, E, and F are also isolated, although less frequently than type B. C and D are hardly identified. All serotypes, especially type b, are frequently responsible for lower respiratory tract infections, such as pneumonia .In addition, they have the potential to induce various additional severe illnesses, including meningitis, epiglottitis, cellulitis, septic.arthritis, as well as empyema and bacteremia. The pathogenicity determinants of *H.influenzae* contribute to its capacity to colonies and induce illness in the host. The following areimportant virulence factors of *H.influenzae* Capsule The polysaccharide capsule of *H.influenzae* is a significant determinant of its ability to cause disease. The bacterium uses inhibition ofphagocytosis to avoid detection by the host's immune system. Pili : are adhesion molecules that enable the binding of cells or microorganisms tosurfaces. Pili and non-pilus adhesions aid in the adherence of *H.influenzae* to host cells, facilitating colonization and the initiation of infection. IgA Protease *H.influenzae* secretes IgA1 protease, an enzyme that specifically splits human IgA1 antibodies. Endotoxin (Lipopolysaccharide - LPS) *H.influenzae* similar to other Gram- ve bacteria, contains endotoxin on its outer membrane. This endotoxin has the ability to initiate an inflammatoryreaction in the host. Beta- lactamase Beta- lactamase is a bacterial enzyme that providesresistance to beta-lactam antibiotics, such as penicillins and cephalosporins. *H.influenzae* has theability to generate beta-lactamase, which is amethod it employs to counteract the impact of beta-lactam antibiotics. The enzyme hydrolyzes the beta-lactam ring compound found in these antibiotics, causing them to lose their efficacy against the bacterium.

References

1. Hall, E., A. Patricia Wodi, Hamborsky, J., Morelli, V. and Schillie, S. (2021). Epidemiology and Prevention of Vaccine-Preventable Diseases.
2. Bamberger EE, Ben-Shimol S, Abu Raya B, Katz A, Givon-Lavi N, (2014), Israeli Pediatric Bacteremia and Meningitis Group. Pediatric invasive Haemophilus influenzae infections in Israel in the era of Haemophilus influenzae type b vaccine: a nationwide prospective study. *Pediatr Infect Dis J*.;33(5):477-481.
3. Gozum GG, Tatarina-Nulman O, John M. (2020), Case Report: Invasive Non Type b Haemophilus influenzae in Immunocompromised Children. *Am J Case Rep*. 11; 21: e920853.
4. Romaneli MTDN, Tresoldi AT, Pereira RM, Garcia MT, Hofling CC, (2019), INVASIVE NON- TYPE B HAEMOPHILUS INFLUENZAE DISEASE: REPORT OF EIGHT CASES. *Rev Paul Pediatr*.;37(2):156-160.
5. Bachiller Luque, P., Eiros Bouza, J.M. and Blanco Quirós, A. (2016), Clinical manifestations, diagnosis and treatment of Haemophilus influenzae infection. Anales De Medicina Interna (Madrid, Spain: 1984).; 17(4), pp.204–212.
6. www.lecturio.com. Haemophilus | Concise Medical Knowledge. 2020
7. Peltola H. (2014), Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. *Clinical microbiology reviews*. 13(2), 302-317.

8. Osmosis. Haemophilus influenzae: Video, Anatomy & Definition. 2016.
9. Meats E, Feil EJ, Stringer S, et al. (2013), Characterization of encapsulated and noncapsulated Haemophilus influenzae and determination of phylogenetic relationships by multilocus sequence typing. *J Clin Microbiol*.; 41:1623-1636.
10. Koneman E, et al. (2006). Color Atlas and Textbook of Diagnostic Microbiology. Baltimore (MD): *Williams & Wilkins*.
11. Bakaletz L. O. (2015), Adhesins as effector molecules in virulence of Haemophilus influenzae. *Trends in microbiology*.; 3(7), 235-238.
12. Cripps AW, Otczyk DC, Ravuru M, Hausdorff WP. (2010), The role and pathogenicity of non-typeable Haemophilus infl uenzae in invasive and mucosal respiratory diseases. 20th ECCMID.10-13; Vienna, Austria. S178 (abstract book).
13. Chang A, Kaur R, Michel LV, Casey JR, Pichichero M. (2014), Haemophilus influenzae vaccine candidate outer membrane protein P6 is not conserved in allstrains. *Hum Vaccin*.; 7:102-105.
14. Kilian M., Mestecky J., Schrohenloher R. E. (2017), Pathogenic species of the genus Haemophilus and Streptococcus pneumoniae produceimmunoglobulin A1 protease. Infection and immunity; 26(1),143-149.
15. Barbosa AR, Giufre M, Cerquetti M, Bajanca- Lavado MP. (2011), Polymorphism in ftsI gene and {beta}- lactam susceptibility in Portuguese Haemophilus influenzae strains: clonal dissemination of beta- lactamase-positive isolates with decreased susceptibility to amoxicillin/clavulanic acid. *J Antimicrob Chemother*.; 66(4):788–796.
16. Murphy TF, Lesse AJ, Kirkham C, Zhong H, Sethi S, (2011), A Clonal group of nontypeable Haemophilus influenzae with two IgA proteases is adapted to infection in chronic obstructive pulmonary disease. *PLoS One*.;6:e25923.
17. Sethi S., Murphy T. F. (2011), Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. *Clinical microbiology reviews*.; 14(2), 336-363.
18. Garcia-Cobos S, Campos J, Cercenado E, et al. (2018), Antibiotic resistance in Haemophilus influenzae decreased, except for β-lactamase-negative amoxicillin-resistant isolates, in parallel with community antibiotic consumption in Spain from 1997 to 2007. *Antimicrob Agents Chemother*.; 52: 2760-2766.
19. Hoban DJ, Zhanel GG, Kim I, Karlowsky JA. (2014), Characterization of Beta-Lactamase-Producing Strains of Haemophilus influenzae from the Surveillance Years (2000–2002)." *AntimicrobialAgents and Chemotherapy*.; 48(7), 2457–2464.
20. Skaare D, Anthonisen IL, Caugant DA, Jenkins A, Steinbakk M, (2014), Multilocus sequence typing and ftsI sequencing: a powerful tool for surveillance ofpenicillin-binding protein 3-mediated beta-lactam resistance in nontypeable Haemophilus influenzae. *BMC Microbiol*.; 14:131.
21. Setchanova LP, Kostyanev T, Markovska R, Miloshev G, Mitov IG. (2013), Serotypes, antimicrobial susceptibility, and beta-lactam resistance mechanisms of clinical Haemophilus influenzae isolates from Bulgaria in a pre-vaccination period. *Scand J Infect Dis*.; 45(2):81-87.
22. Bajanca-Lavado MP, Simoes AS, Betencourt CR, Sa-Leao R . (2014), Characteristics of Haemophilus influenzae invasive isolates from Portugal following routine childhood vaccination against H. influenzae serotype b (2002–2010). *Eur J Clin Microbiol Infect Dis*.; 33(4):603–610.
23. Puig C, Calatayud L, Marti S, Tubau F, Garcia- Vidal C, (2013), Molecular epidemiology of nontypeableHaemophilus influenzae causing communityacquired pneumonia in adults. *PLoS One*.; 8(12), e82515.
24. Hoshino T, Sato Y, Toyonaga Y, Hanaki H, Sunakawa K. (2013), Nationwide survey of the development of drug resistance in the pediatric field in 2007 and 2010: drug sensitivity of Haemophilus influenzae in Japan (second report). J Infect Chemother : Off *J Jpn Soc Chemother*.; 19(3):495–503.
25. Musher, D.M. (2016) Haemophilus Species. 4th ed.
26. Microbeonline. (2019) Virulence Factors of Haemophilus Influenzae - Microbeonline.

DOI:10.31579/2690-8808/237