Archives of Razi Institute, Vol. 78, No. 1 (2023) 315-322


DOI: 10.22092/ARI.2022.358161.2165

***Original Article***

# Detection of Main Causative Agents among Young Children Suffering from Epiglottitis in Hilla City, Iraq

**Ahmed Ali Ahmed, J[1 *], Khalifa, B. S[2], Al-Hasnawy, H. H[1]**

*1. Department of Microbiology, College of Medicine, University of Babylon, Babylon, Iraq*
*2. Department of Pediatrics, College of Medicine, University of Babylon, Babylon, Iraq*

Received 21 March 2022; Accepted 24 November 2022
Corresponding Author: jwanalhamawandi@gmail.com

**Abstract**

Epiglottitis is a rapidly progressive epiglottis infection leading to upper airway edema. This study aimed to detect the main causative agent, viral infection, by immunofluorescence antibody technique and PCR technique and bacterial infection detection by specific gene among young children suffering from epiglottitis. This study included 85 young children aged 10-15 years. The virus was identified on 85 blood samples using the CER test Human simplex virus Card test; the results revealed that 12 (14.1%) specimens were related to virus infection, and the sera of patients showed anti-IgM to HSV-1 antibodies. HSV-1 was detected in blood samples by qPCR technique. Eighty-five saliva samples were collected from young children suffering from epiglottitis. The samples were cultured for 18-24 hours at 37°C. They were then cultivated for 18-24 hours on various selective media at 37°C. The colony morphology, microscopically, and biochemical testing were used to identify *Haemophilus influenzae* as a first Identification. Out of 85 clinical specimens, 63 (74.1%) were positive culture, while 22 (25.9%) had no growth on culture media; out of 63 specimens, only 22 (34.9%) isolates belonged to *Haemophilus influenzae* by biochemical tests, while 41 (65.1%) related to other types of microorganisms. VITEK 2 was used to validate bacteria isolates from young children suffering from epiglottitis. The findings indicate that 22 (34.9%) isolates related to *Haemophilus influenzae* have been confirmed with an excellent ID message confidence level (94 to 99.8% likelihood percentage). This method is characterized by quick bacterial detection. DNA was taken from all suspected isolates previously identified as *Haemophilus influenzae* using the vitek2 technology, and traditional PCR was used to amplify specific hel gene for *Haemophilus influenzae* primers utilizing these DNA samples. After that, when compared to an allelic ladder, gel electrophoresis revealed that all 22 (100%) samples of *Haemophilus influenzae* produced 101 bp DNA fragments. For isolates previously identified as *Haemophilus influenzae*, molecular identification of the ompP gene was performed. The results showed that 12 (or 54.5 percent) of the 22 isolates tested positive for this virulence gene. When compared to an allelic ladder, the presence of (459 bp) bands indicated positive results. In addition, the bexA gene was molecularly detected in 22 *Haemophilus influenzae* isolates, showing that only 8 (36.3 percent) of the isolates had this gene. When compared to an allelic ladder, the presence of a (343 bp) band indicated positive results for *bexA* gene pathogenicity; in conclusion, HSV (1) and Hib were considered almost causative agents of epiglottitis in young children.

**Keywords:** *Haemophilus influenzae*, Viral infection, Bacterial infection, HSV-1, Epiglottitis

## 1. Introduction

Epiglottitis is inflammation and swelling of the cartilaginous structure (1). Infection typically causes it. However, it can even happen due to a throat injury (2). The epiglottis could be a flap of tissue that sits below the tongue at the rear of the throat. Its primary operation is to shut over the cartilaginous tube (trachea), whereas the uptake stops food from getting into the airway (1).

The symptoms of epiglottitis typically develop quickly and find speedily worse, though they will develop over a couple of days in older youngsters and adults (3). The most common symptoms of epiglottitis in young youngsters are respiration difficulties, a symptom associated with a husky voice (4). Infection typically causes epiglottitis with *Haemophilus influenza* type B (Hib) microorganisms. Still, as epiglottitis, Hib will cause a variety of significant infections, resembling respiratory disorder and infectious disease (5). It spreads within the same approach because of the cold or respiratory illness virus. The bacteria are in the little droplets of secretion and secretion propelled into the air once an infected person coughs or sneezes (6). Within the central nervous system (CNS), the herpes simplex virus (HSV) generates a wide range of clinical symptoms. Infection of a baby with HSV after exposure to the virus after delivery will result in severe disseminated infection and death (7). Therapeutic therapy is achievable; however, antiviral medication should be started as soon as possible. As a result, rapid laboratory detection is critical for reducing morbidity, even though HSV infection has long-term consequences (8). H. influenzae, a gram-negative eubacterium with a predominantly human metabolic process tract environmental niche, is evaluated based on the formation of a carbohydrate capsule: strain variants a through f generate antigenically unique capsules, while non-typeable strains produce none (9). *H. influenzae* colonizes the nasopharynges of healthy humans and causes metabolic process infections, including acute redness media, sinusitis, bronchitis, and pneumonia, as well as invasive blood-borne infections like meningitis, septic arthritis, and redness media (8). This study aims to detect main causative agents (viral infection by immunofluorescence antibody technique and PCR technique and bacterial infection detection by specific gene among young children suffering from epiglottitis, detection of some virulence factor genes of bacterial isolates.

## 2. Materials and Methods

The research included 85 young children aged 10-15 years. Children with epiglottitis visited clinical private in Hilla city for the period between March 2021 to June 2021.

### 2.1. Blood Samples

A total of 5 milliliters of blood were drawn from 85 children. 2 ml of the sample was placed in an EDTA tube to acquire whole blood; all samples were maintained in a freezer at -20 °C; until utilized, while 3ml were taken while fasting and gradually placed into dispensable tubes containing isolating gel. The serum was centrifuged at 2000 g for about 10 minutes after 30 minutes of clotting at room temperature, then partitioned into small aliquots and kept at (-20 °C).

### 2.2. Saliva Sampling

Saliva samples were correctly collected in a contamination-free procedure for each child according to the method described by Navazesh (10). In addition, all patients' mouths were rinsed with purified water (10 mL) for 30– 60 seconds to verify that any material was removed. Non-stimulated clean saliva was collected in sterile laboratory cups and stored in a cool box with ice bags to preserve its viability until it was transferred to the laboratory for analysis. Each sample was then centrifuged at (3000 rpm) for 10 minutes to separate the unwanted free salivary particles. The clear salivary solution was then aspirated with (1l) micropipette tips and saved in a sterile Eppendorf tube for freezing at (-20 °C).

### 2.3. Identification of Virus

Viruses were identified using cer test human simplex virusCard test, approximately 100 mg of saliva specimen was transferred by a stick into a sterile Eppendorf tube, and then the tube was shacked to assure good sample dispersion. After that, 4 drops of the solution provided with the kit were added into the circular window marked with an arrow; finally, the results were read at 10 minutes by observing the coloring bands:

### 2.3.1. Identification of Human Simplex Virus Type 1 by Indirect Immunofluorescence Antibody Technique

Indirect (IFA) Kit for the simultaneous detection of IgM antibodies to the main etiological agents of respiratory tract infections. The IFA method is based on the reactivity of antibodies with antigen on the slide surface, and the immunoglobulin that is not linked to the antigen is eliminated during the washing process. The antigen-antibody complexes then react with fluorescein-labeled anti-human globulin in the next stage. An immunofluorescence microscope can be used to investigate it.

### 2.3.2. Extraction of Genomic Information

Genomic DNA was extracted from blood samples using the gSYAN DNA kit extraction kit Geneaid. The USA, as directed by the manufacturer.

### 2.3.3. PCR (Polymerase Chain Reaction) Technique

On positive HSV-1 samples, the PCR technique was used to perform DNA sequence analysis. This procedure was carried out according to Markoulatos, Georgopoulou (11), with the HSV-1 specific primer gene and the primer sequence in this study for the HSV gene (F: TGCTCCTACAACAAGTC, R: CGGTGCTCCAGGATAAA **)** and PCR condition of HSV shown in table 1.

**Table 1.** The PCR condition of HSV-1 in this study

| Gene name | Size of Bp | Conditions | References |
|---|---|---|---|
| HSV gene | 200 | 1: 94ºC, 2 min.<br>2: 94ºC, 30 sec.<br>3: 51.9ºC, 30 sec.<br>4: 70ºC, 20.0 sec.<br>6: 70ºC, 5 min.<br>7: 4ºC, | Markoulatos, Georgopoulou (11) |

## 2.4. Identification of *Haemophilus influenza*

### 2.4.1. Culture Characteristics and Microscopic Examination

Depending on its morphological characteristic (Form, size, color, limits, and texture of the colony), Biochemical tests were performed on each isolate following the inspection to complete the final identification, according to Maaroufi, De Bruyne (12), and the vitek2 method was employed for bacterial isolate identification.

### 2.4.2. Identification of Bacteria with the Vitek2 System

The pure colonies from growth media are a sample used to identify and transfer to Vitek 2 clinical microbiology as an automatic identification (ID) instrument device. All of the reagents and instruments required for the method of the Manufacturer Company. Before processing, inoculated cards and isolates were introduced to the system and processed within 30 minutes of inoculation. A vacuum chamber in the system is used to fill (inoculate) bacterial suspensions with GN cards. The identity card was then put near the transfer tube, which was inserted into the sample-containing check tubes into the matching suspension tube. The cassette may fit in up to ten tubes. Inside the Vitek 2 instrument machine, the filled cassette was placed in a vacuum chamber. The bacterial suspension was pushed into microchannels via the transfer tube, which filled all of the test wells when the vacuum was applied, and the air in the station was refilled. Before inserting inoculated cards into the circular incubator, a device stops the transfer tube and closes the card. The incubator may hold up to 30 cards. All card variants were incubated at 35.5 °C. Every 15 minutes, each card was withdrawn from the incubator and transferred to the reaction readings optical instrument, which was returned to the incubator before the next read time. At 15-minute intervals, data was collected during the incubation phase.

## 2.5. DNA Extraction of *Haemophilus influenzae* Isolated by Specific Gene and Detection of Some Virulence Factor Genes

A Genomic DNA purification kit was used to isolate DNA Templates from *Haemophilus influenzae* isolates, augmented by (Geneaid, USA). A UV-trans illuminator has been used to observe the image. The primer sequences used in this study include (*hel gene* F:GATCCGAATTCCTTAAAAGGAAT,

R:TTAAATATTGGATCCAGTAAAAACTGAAC/ *ompP gene* F:ATGAACAAATTTGTTAAATCA, R:TGCGATGTTGTATTCAGGTGTA/*bexA gene* F: CGTTTGTATGATGTTGATCCA,R: TGTCCATGTCTTCAAAATG) (Table 1). PCR conditions for each gene and virulence factor genes used in this work are in table 2.

**Table 2.** PCR conditions for genes used in this study

| Gene name | Size of Bp | PCR Conditions | References |
|---|---|---|---|
| *hel* | 101 | 1: 94C, 2 min.<br>2: 94ºC, 30 sec.<br>3: 56.3ºC, 30 sec.<br>4: 70ºC, 20.0 sec.<br>5: 70ºC, 5 min. | van Ketel, De Wever (13) |
| *ompP* | 459 | 1: 94ºC, 2 min.<br>2: 94C, 30 sec.<br>3: 52.0ºC, 30 sec.<br>4: 70ºC, 50.0 sec.<br>5: 94C, 30 sec.<br>6: 45.0ºC, 30 sec.<br>7: 70ºC, 50.0 se<br>8: 70ºC, 5 min. | Hendrixson, De La Morena (14) |
| ***bexA*** | 343 | 1: 94ºC, 2 min.<br>2: 94ºC, 30 sec.<br>3: 56.0ºC, 30 sec.<br>4: 70ºC, 40.0 sec.<br>5: 94C, 30 sec.<br>6: 49.0ºC, 30 sec.<br>7: 70ºC, 40.0 sec.<br>10: 70ºC, 5 min. | Maaroufi, De Bruyne (12) |

## 3. Results

In this study, in table 3, the virus was identified on 85 blood samples by using the cer test Human simplex virus Card test; the results revealed that 12 (14.1%) specimens were related to virus infection, and the sera of patients were shown anti-IgM to HSV-1 antibodies as shown in figure 1.

**Table 3.** Human simplex virus type 1isolates from blood samples

| No. of blood isolates | HSV isolates | Others MO |
|---|---|---|
| 85 | 12(14.1%) | 73 (85.9%) |
| Total | 85(100%) | |

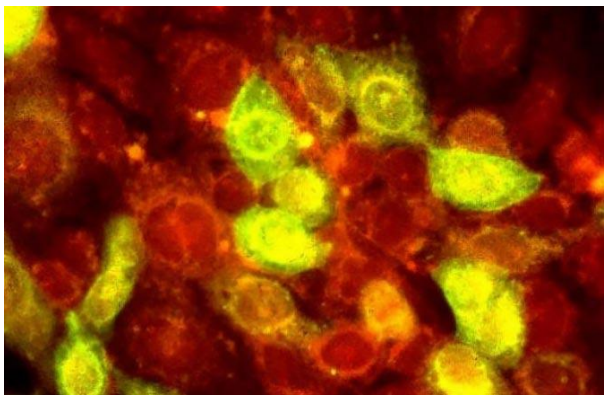

**Figure 1.** Detection of HSV-1 by indirect immunofluorescent assay (magnification power 400×)

HSV- 1from 12 samples that gave positive results by IFA, it was found that all 12 isolated were related to HSV-1 and produced the specific (200 bp) fragment compared with the ladder in figure 2.

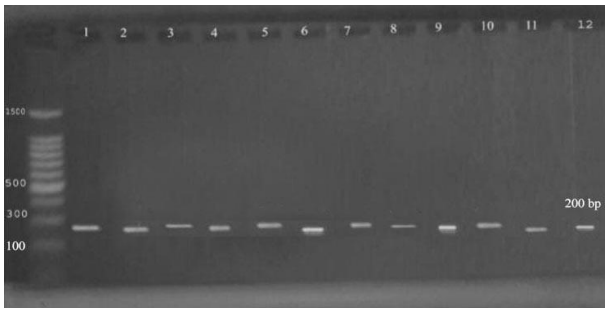


**Figure 2.** HSV-1 gene PCR products were seen under UV light at 301 nm after staining with ethidium bromide following electrophoresis at 70 volts for 50 minutes. The HSV-1 gene was found to be positive in Lanes 1-12, and the product size is (200 bp)

In these results, 85saliva specimens were collected from young children carrying epiglottitis. The samples were cultured for 18-24 hours at 37 °C. They were then cultivated for 18-24 hours on various selective media at 37 °C. Initial identification of *Haemophilus influenzae* was based on colonial morphology, microscopy, and biochemical tests. Out of 85 clinical specimens, 63 (74.1%) were positive cultures, while 22 (24.9%) had no growth on culture media; biochemical tests

identified only 22 (34.9%) isolates as belonging to *Haemophilus influenzae*, while 41 (65.1%) were appear other types of microorganisms, in table 4. Researchers used the automated VITEK 2 instrument with GN-ID cards carrying 64 biochemical tests to authenticate the bacteria isolates from young children suffering from epiglottitis. According to the findings, 22 isolates (34.9%) were found isolates related to *Haemophilus influenzae* have been confirmed with an excellent ID message confidence level (94 to 99.8% likelihood percentage). This method is characterized by quick bacterial detection.

The polymerase chain reaction technology relies on DNA polymerase's ability to create new strands of DNA that are complementary to the template strand, and the exact sequence will have been amassed in billions of copies by the end of the PCR reaction (Amplicon). Using the vitek2 technology, DNA was taken from all suspected isolates previously identified as *Haemophilus influenzae*, and traditional PCR was used to amplify specific hel gene for *Haemophilus influenzae* primers using these DNA samples, as shown in table 2. Then, compared to an allelic ladder, gel electrophoresis indicated that all 22 (100 %) samples of *Haemophilus influenzae* formed a 101 bp DNA fragment, as shown in figure 3.

For isolates previously identified as *Haemophilus influenzae*, molecular identification of the ompP gene was performed. The results showed that 12 (or 54.5 percent) of the 22 isolates tested positive for this virulence gene. When compared to an allelic ladder, positive findings were discovered by the presence of (459 bp) bands, as illustrated in figure 4. In addition, the bexA gene was molecularly detected in 22 *Haemophilus influenzae* isolates, showing that only 8 (36.3 percent) of the isolates had this gene. A (343 bp) band compared to an allelic ladder revealed positive results for bexA gene pathogenicity, as illustrated in figure 5.

**Table 4.** The presence of *Haemophilus influenzae*, as well as other etiological agents, was linked to the sample that was isolated

| N. of isolates | Culture (+) | Culture (-) | *H. influenzae* Culture (+) | Others |
|---|---|---|---|---|
| 85 | 63 (74.1%) | 22 (25.9%) | 22 (34.9%) | 41 (65.1%) |
| Total | 85 (100%) | | 63 (100%) | |

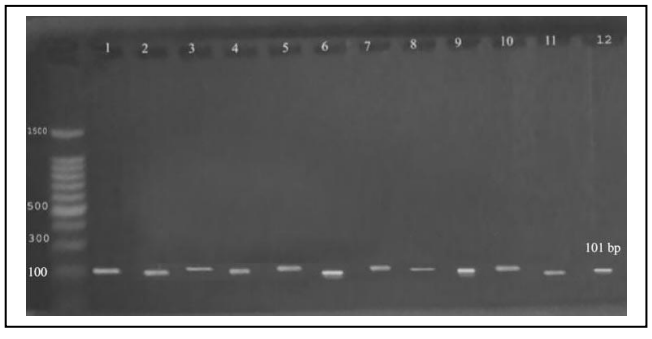


**Figure 3.** Hel gene PCR products were observed under UV light at 301 nm after staining with ethidium bromide following agarose gel electrophoresis at 70 volts for 50 minutes. The HSV-1 gene was found to be positive in lanes 1-12, and the product size is (101 bp)

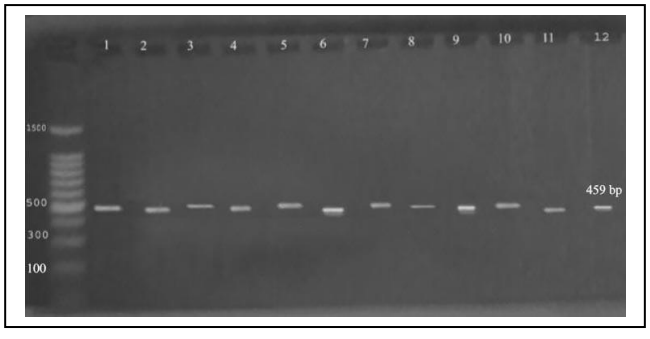


**Figure 4.** OmpP gene PCR products were detected under UV light at 301 nm after being stained with ethidium bromide following electrophoresis at 70 volts for 50 minutes. The HSV-1 gene was positive in Lanes 1-12, and the product size is (459 bp)

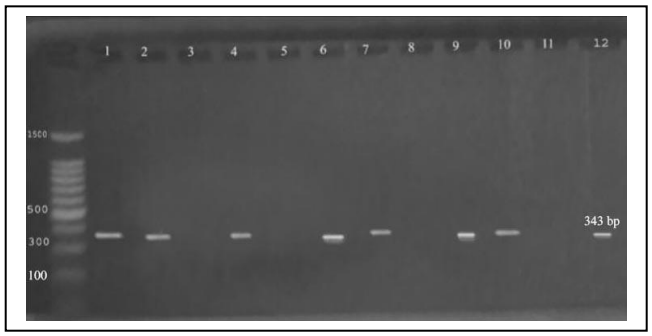


**Figure 5.** After staining with ethidium bromide, agarose gel electrophoresis at 70 volts for 50 minutes for bexA gene PCR products was observed under UV illumination at 301 nm. Lanes 1, 2, 4, 6, 7, 9, 10, and 12 were all positive for HSV-1, and the product size was (343 bp)

## 4. Discussion

Epiglottitis is almost always caused by an infectious agent, bacterial, viral, or flora (11). The most prevalent cause of infection in children is *Haemophilus influenzae* type B (HIB) (15). However, since the widespread availability of vaccines, this has decreased considerably—different bacteria (16). The fungus Pseudomonas aeruginosa and the species Pseudomonas aeruginosa have been identified in agitated patients. Thermal, caustic, or foreign body processes are common non-transmissible causes (17). Previous virus infection may facilitate the development of a microorganism superinfection (18). Viruses that can induce a beneficial infection include VZV, HSV, and EBV (12). The airway in the medical population is significantly different from that of an adult (17). The subglottis is the narrowest part of a child's or a child's airway, whereas the glottis is the narrowest part of an adult's airway (19).

Furthermore, a child's cartilaginous structure comprises much more supple animal tissue than an adult's (16). The suppleness of the cartilage provides a ball-valve effect, in which every inspiration drags a dropsical epiglottis over the laryngeal airway, causing symptoms in children (11). Infections of the cartilaginous structure caused by *H. influenzae* and other viruses induce significant swelling of the epiglottis and supraglottis in people of all ages (4). This edema can swiftly spread to neighboring structures, resulting in symptoms of airway obstruction (6). Other pathogens such as S. Pneumoniae, S. aureus, and beta-hemolytic true bacteria spp. are also major pathogens in adults and children (20). Epiglottitis has become uncommon with the advent of standard childhood immunization against *Haemophilus influenzae* serotype B, which was historically the most prevalent cause of epiglottitis (18). Microorganism epiglottitis is a common secondary bacterial infection that develops due to acute tracheal virus infection or chronic endotracheal introduction (12). The gene ompP, which encodes the precursor style of an outer membrane supermolecule, has a 70 percent sequence identity with OmpT (19). OmpP has chemical change activity similar to OmpT. Even though this gene was found in confined subgroups of *Haemophilus influenzae* strains, its exact origin remained unknown (11). Within the cap locus (21). Genes in region two appear to be involved in the synthesis of serotype-specific polysaccharides, whereas genes in region three appear to be engaged in some capsule postpolymerization process; similar to what was previously observed in Neisseria meningitidis and haemolytica (22). Serotype b strains were responsible for most invasive *H. influenzae* infections before the release of the *H. influenzae* serotype b (Hib) conjugate vaccine (18). As a result of the widespread use of the Hib conjugate immunizing agent, Hib infections have declined dramatically, and non-Hib strains now cause the majority of invasive infections (12). Macromolecular amplification techniques were used to support the bexA gene sequence of the Hib Eagan strain for nonculture detection of *H. influenzae* in clinical specimens (19) as a marker for *H. influenzae* (1).

Epiglottitis is an inflammation of structures above the insertion of the glottis and is most often caused by viral and bacterial infection. Human simplex virus and *Haemophilus influenzae* type b (Hib) were considered almost causative agents of epiglottitis in young children.

## Authors' Contribution

Study concept and design: J. A. A. A.
Acquisition of data: J. A. A. A.
Analysis and interpretation of data: B. S. K.
Drafting of the manuscript: B. S. K.
Critical revision of the manuscript for important intellectual content: H. H. A.
Statistical analysis: J. A. A. A. and B. S. K.
Administrative, technical, and material support: J. A. A. A. and B. S. K.

## Ethics

Valid consent from the children's parents was obtained prior to their inclusion in the report.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. Woods CR. Epiglottitis (Supraglottitis). Clinical features and diagnosis. UpToDate; Waltham, MA: UpToDate. 2020.
2. Swain SK, Nahak B, Debta P. Clinical characteristics and treatment of acute epiglottitis: A retrospective study of 28 cases. J Acute Dis. 2020;9(3):109.
3. Parthasarathy V, Snyder B, Saddawi-Konefka R. A lot to choke on: case of adult epiglottitis with concurrent peritonsillar abscess in a patient with a sore throat. J Emerg Med. 2018;55(6):841-4.
4. Tristram D. Laryngitis, Tracheitis, Epiglottitis, and Bronchiolitis. Introduction to Clinical Infectious Diseases: Springer; 2019. p. 75-85.
5. Beltrami D, Guilcher P, Longchamp D, Crisinel PA. Meningococcal serogroup W135 epiglottitis in an adolescent patient. Case Rep. 2018;2018:bcr-2017.
6. Slack M, Cripps A, Grimwood K, Mackenzie G, Ulanova M. Invasive Haemophilus influenzae infections after 3 decades of Hib protein conjugate vaccine use. Clin Microbiol Rev. 2021;34(3):e00028-21.
7. Pittet LF, Curtis N. Postnatal exposure to herpes simplex virus: to treat or not to treat? Pediatr Infect Dis J. 2021;40(5):16-21.
8. Kang HM, Kang JH. Effects of nasopharyngeal microbiota in respiratory infections and allergies. Clin Exp Pediatr. 2021;64(11):543.
9. Reidenberg JS. Where does the air go? Anatomy and functions of the respiratory tract in the humpback whale (Megaptera novaeangliae). Madag Conserv Dev. 2018;13(1):91-100.
10. Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993;694(1):72-7.
11. Markoulatos P, Georgopoulou A, Siafakas N, Plakokefalos E, Tzanakaki G, Kourea-Kremastinou J. Laboratory diagnosis of common herpesvirus infections of the central nervous system by a multiplex PCR assay. J Clin Microbiol. 2001;39(12):4426-32.
12. Maaroufi Y, De Bruyne J-M, Heymans C, Crokaert F. Real-time PCR for determining capsular serotypes of Haemophilus influenzae. J Clin Microbiol. 2007;45(7):2305-8.
13. van Ketel RD, De Wever B, Van Alphen L. Detection of Haemophilus influenzae in cerebrospinal fluids by polymerase chain reaction DNA amplification. J Med Microbiol. 1990;33(4):271-6.
14. Hendrixson DR, De La Morena ML, Stathopoulos C, St Geme III JW. Structural determinants of processing and secretion of the Haemophilus influenzae Hap protein. Mol Microbiol. 1997;26(3):505-18.
15. Desloges I, Taylor JA, Leclerc JM, Brannon JR, Portt A, Spencer JD, et al. Identification and characterization of OmpT-like proteases in uropathogenic Escherichia coli clinical isolates. Microbiologyopen. 2019;8(11):915.
16. Cerqueira A, Byce S, Tsang RS, Jamieson FB, Kus JV, Ulanova M. Continuing surveillance of invasive Haemophilus influenzae disease in northwestern Ontario emphasizes the importance of serotype a and non-typeable strains as causes of serious disease: a Canadian Immunization Research Network (CIRN) Study. Can J Microbiol. 2019;65(11):805-13.
17. Lu'ukia Ruidas M. Chapter IX. 7. Gastrointestinal Foreign Bodies.
18. Kivekäs I, Rautiainen M. Epiglottitis, acute laryngitis, and croup. Infections of the ears, nose, throat, and sinuses. 2018:247-55.
19. Dekker JP, Frank KM. Salmonella, Shigella, and yersinia. Clin Lab Med. 2015;35(2):225-46.
20. Iskander M. Development and evaluation of a core genome MLST schema for Haemophilus influenza. 2017.

21. Carloni I, Ricci S, Rubino C, Cobellis G, Rinaldelli G, Azzari C, et al. Necrotizing pneumonia among Italian children in the pneumococcal conjugate vaccine era. Pediatr Pulmonol. 2021;56(5):1127-35.

22. Nanduri SA, Sutherland AR, Gordon LK, Santosham M. 23—Haemophilus influenzae Type Vaccines. Plotkin's Vaccines, 7th ed; Plotkin, SA, Orenstein, WA, Offit, PA, Edwards, KM, Eds. 2017:301-18.