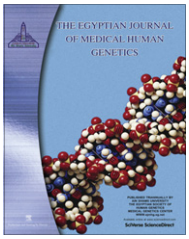
The Egyptian Journal of Medical Human Genetics (2012) **13**, 213–218

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Haemophilus influenzae type b pneumonia in Egyptian children under five years: A step toward the identification of the real burden in our community by the use of real-time polymerase chain reaction

Tharwat Deraz [a], Mervat Gamal Eldin Mansour [a,*], Sherin Albendary [b], Fatma Allam [a]

[a] *Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[b] *Department of Microbiology, Ain Shams University, Cairo, Egypt*

Received 10 January 2012; accepted 30 January 2012
Available online 28 February 2012

KEYWORDS

Haemophilus influenzae type b;
Egyptian children;
PCR;
Culture;
Antibiotic sensitivity

Abstract *Haemophilus influenzae* (Hi) causes more than 3 million cases of serious disease, mainly meningitis and pneumonia in children less than 5 years old with approximately 386,000 deaths each year worldwide. 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 to f). Invasive Hi diseases in children were almost exclusively caused by serotype b (Hib). To the best of our knowledge, the real burden of Hib pneumonia in Egypt is not clarified. Yet, few studies are published and concerned with Hib sepsis among neonates or Hib meningitis in children. We aimed in this study to identify the frequency of Hib pneumonia among Egyptian children below five years by the use of real-time polymerase chain reaction (PCR) technique, with insight on antimicrobial resistance of Hib strains in the Egyptian community. One hundred patients

* Corresponding author. Address: Abbass Elakad, Nasr City, Cairo, Egypt. Tel.: +20 01000837437.
E-mail address: mervatgmansour@gmail.com (M.G.E. Mansour).

Peer review under responsibility of Ain Shams University.
doi:10.1016/j.ejmhg.2012.01.005

with community-acquired pneumonia were investigated for Hib by both real-time PCR and bacterial culture. *Haemophilus influenzae* type b was diagnosed in 31% of the examined population by PCR, with sensitivity of 100% (95% CI: 0.86–1), specificity 100% (65% CI: 0.93–1), positive predictive value (PPV) 100% (95% CI: 0.83–1), and negative predictive value (NPV) 100% (95% CI: 0.93–1). Blood culture was positive in 12 patients only, with a sensitivity of 38% (95% CI: 0.22–0.57), a specificity of 100% (65% CI: 0.93–1), PPV 100% (95% CI: 0.69–1), and NPV 77% (95% CI: 0.68–0.86). Isolated Hib strains were sensitive to ceftriaxon in 91% of cases, followed by ampicillins in 31% and cotrimoxazole in 17%. Three patients had multidrug resistant strains of Hib.

Conclusion: *Haemophilus influenzae* type b infection is still an important and frequent pathogen causing community-acquired pneumonia in Egypt with changeable antibiotic sensitivity pattern. PCR represented a sensitive and rapid tool for the diagnosis of Hib pneumonia. Governmental plans to eradicate Hib in our community with the introduction of Hib conjugate vaccine in the national immunization program became indispensable.

1. Introduction

Haemophilus influenzae, a gram negative coccobacillus whose environmental niche is primarily restricted to the human respiratory tract, is classified on the basis of the production of a polysaccharide capsule: strain types a to f produce antigenically distinct capsules, and nontypeable strains produce no capsule [1]. Measurements of the gene contents of *H. influenzae* strains have revealed that the clinical type b Eagan strain possesses 270 kb of additional genomic material relative to strain Rd, a type d strain that has lost its capsule and is highly laboratory adapted. More-detailed analysis has shown that strain Eagan possesses gene regions not found in Rd, including the *cap* region genes, encoding the type b capsular polysaccharide; the *hif* gene cluster, encoding the pilus adhesion; the *hmw1* and *hmw2* gene clusters, encoding the high-molecular-weight adhesions; and the tryptophanase gene cluster; all related to the virulence factors of Hib strains [2]. It is estimated that the bacillus causes more than 3 million cases of serious disease, mainly meningitis and pneumonia in children less than 5 years old with approximately 386,000 deaths each year worldwide [3]. Invasive Hi diseases in children were almost exclusively caused by serotype b prior to the introduction of *H. influenzae* serotype b conjugate vaccine which had been replaced by nontypeable strains in several countries [4]. Community-acquired pneumonia (CAP) is a common and potentially serious infection in developing countries especially in children less than five years, causing up to 5 million deaths per year [5]. Since conventional diagnostic tools, such as radiology, microscopy, culture, and serology, fail to identify responsible pathogens in many occasions, the development of a PCR assay for target gene amplification has enabled the detection of low numbers of pathogens in clinical samples [6]. The World had disposed of *H. influenzae* Type b infection since the production of Hib conjugate vaccine, but what is the situation in our country? To the best of our knowledge, Egyptian data about the epidemiology of Hib pneumonia is lacking. In this study, we aimed to answer this question through identifying the frequency of Hib among Egyptian children with CAP under five years by the use of real-time polymerase chain reaction technique, with insight on the antimicrobial resistance of Hib strains in Egyptian community.

2. Patients and methods

2.1. Study settings

This is a cross-sectional observational study involving 100 children with community-acquired pneumonia, admitted to the Ain Shams University Children's Hospital from February 2009 to December 2009.

2.2. Case definition and data collection

All children under five years, of both sexes, with clinical pneumonia and were admitted to the hospital were enrolled. We excluded patients with bronchial asthma, chronic lung diseases, pulmonary tuberculosis (TB) and patients on antibiotic therapy for the current respiratory condition. According to the Integrated Management of Childhood Illness (IMCI) program of WHO, we identified clinical pneumonia when there is fast breathing, lower chest indrawing, inability to drink or feed, or cyanosis [5]. Demographic and clinical data of all patients together with their chest roentgenogram findings were recorded immediate to the enrollment of the patients. Patients were considered eligible after signing an informed consent by the parents or the assigned care givers.

2.3. Microbiological culture of Hib

One milliliter of blood was withdrawn from every patient and inoculated directly into 20 ml of trypticase soy broth in a blood culture bottle for aerobic culture at 37 °C. Positive blood cultures were sub-cultured on supplemented chocolate agar plates and incubated anaerobically under 10% CO_2 atmosphere for 24–48 h. Suspected bacterial colonies were identified by direct microscopic examination of Gram stained film, biochemical reaction and serotyping using Hib-specific antiserum by slide agglutination test; supplied by Denka-Seiken Co. Ltd., Tokyo, Japan. Antimicrobial susceptibility by disc diffusion method was performed for isolated strains.

2.4. Polymerase chain reaction

Total DNA was extracted from patients' sera using a MagNA Pure Compact system with MagNA Pure Compact NA isolation kit 1 according to the instructions of the manufacturer

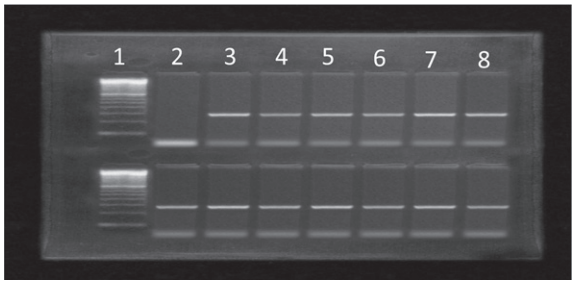


Figure 1 Detection of Hib by PCR. Lanes 3–8 show amplification of Hib. Lane 2: negative for Hib.

(Roche Applied Science, Mannheim, Germany; Cat. No. o3730964001). Amplification by PCR was done by Light Cycler-DNA Amplification Kit SYBR Green I (Cat. No. 2015137). The primer pairs used according to Hassan-King et al. [7] were for Hib 1 [GCG–AAA–GTG–ACC–TCT–TAT–CTC–TC] and Hib 2 [GCT–TAC–GCT–TCT–ATC–TCG–GTG–AA], and were similar and complementary to the nucleic acid sequence of conserved regions of the bexA gene specific for b serotype of *H. influenzae* (Fig. 1).

2.5. Statistical analysis

The data were coded, entered and processed on a computer using SPSS (version 15). The level $p < 0.05$ was considered the cut-off value for significance. Chi-Square test χ^2 was used to test the association variables for categorical data.

Fisher exact test was performed in a table containing values less than 5. Non-parametric data were tested by the Mann–Whitney U test. Sensitivity of the culture or m-PCR represents the proportion of patients with Hib pneumonia that tested positive. Specificity is the proportion of patients without Hib pneumonia that tested negative. Predictive value of a positive test (PPV) is the proportion of patients with positive tests that have disease. Predictive value of a negative test (NPV) is the proportion of patients with negative tests that do not have disease.

3. Results

3.1. Patients' cohort characteristics

The age of the studied patients ranged from 15 days of life to five years with a median of 8.5 months. In the selected age group, patients with Hib pneumonia showed significant lower age (median = 2 months, Interquartile range = 1.75–19 months) compared to non-Hib patients (median = 19 months, Interquartile range = 9–24 months; $p = 0.006$). Sixty percent of enrolled patients was male and 40% was female; however, no significant difference in gender was noticed between patients with Hib pneumonia and patients without ($p > 0.05$). None of the enrolled patients had received *H. influenzae* type b conjugate vaccine.

3.2. H. influenzae pneumonia and its presentation

The presenting symptoms of patients with Hib pneumonia were indistinguishable from those with non-Hib pneumonia. Fever, cough, expectoration, dyspnea and wheezes were common presenting symptoms in all studied patients, with significant predominance of cough ($p = 0.016$) and expectoration ($p = 0.01$) among patients with Hib pneumonia. Clinical signs of bronchial breathing, diminished air entry and fine crepitations were detected in all patients groups with significant presentation among non-Hib pneumonia patients. All patients had evidence of radiological findings on chest roentgenogram. Patients with Hib pneumonia had more localized areas of consolidation while patients with non-Hib pneumonia had more diffuse forms of consolidation ($p = 0.09$) (Table 1).

3.3. H. influenzae type b detection

Real-time PCR increased the diagnostic yield of Hib among the studied population. It was able to detect invasive Haemophilus disease in 31 patients who (31%) presented with CAP under the age of five while blood culture showed growth of Hib in 12 patients (12%) only. Real-time PCR had a sensitivity of 100% (95% CI: 0.86–1), a specificity of 100% (65% CI: 0.93–1), PPV 100% (95% CI: 0.83–1), NPV 100% (95% CI: 0.93–1) and diagnostic accuracy of 81%. While bacterial blood culture had a sensitivity of 38% (95% CI: 0.22–0.57), a specificity of 100% (65% CI: 0.93–1), PPV 100% (95% CI: 0.69–1), NPV 77% (95% CI: 0.68–0.86).

4. Discussion

Community-acquired pneumonia (CAP) is one of the most common serious infections in children. *H. influenzae* type b (Hib) remains one of the most common etiologic agents of CAP and invasive bacterial infection in children less than five years in countries where the Hib vaccine is not used [1,8,9]. We aimed in this work to have preliminary data about the frequency of Hib pneumonia, as the burden of the disease is still unclear in Egypt. Yet, no work to facilitate the affordability of safe and effective Hib immunization appears in recent future governmental health plans. Moreover, there are no training programs of health care workers regarding Hib disease surveillance, case definition, case management and laboratory diagnosis. We selected the age group younger than 5 years to be examined according to previous studies. It indicated that the highest incidence of hospital admissions due to severe lower respiratory infections is among children less than 5 years. Juvèn et al. [10] found 40% of admitted children due to CAP was less than 5 years. Klien [11] found that more than 52% of admissions due to community-acquired pneumonia were patients younger than 5 years. Though Hib conjugate vaccine is available in the Egyptian market early in this decade; yet it is not known by a large stratum of Egyptians that are adherent only to the national vaccination program. Thus unwillingly all enrolled patients were unvaccinated against Hib infection. We believe that the identification of Hib as a cause of respiratory disease in these patients is the first step in determining how frequently it may cause serious problems and, hence, how hard we should push governmental health organizations toward involvement of Hib conjugate vaccine in the national vaccination program in Egypt especially in the high risk group. Diagnosing Hib disease is difficult. To diagnose Hib meningitis, lumbar punctures must be performed and cerebrospinal fluid rapidly processed in a microbiological laboratory that has

Table 1 Clinical and radiological characteristics of patients with Hib pneumonia and patients without Hib pneumonia.

Characteristic	Patients with Hib pneumonia $n = 31$		Patients without Hib pneumonia $n = 69$		Chi-square test	
	n	%	*N*	%	χ^2	*p*-Value
Clinical symptoms						
Cough	31	100	55	79.7	5.73	0.016*
Expectoration	12	87	22	70.9	6.29	0.01*
Dyspnea	9	29	31	45	1.65	0.23
Wheeze	17	54.8	24	34.8	3.56	0.06
Clinical signs						
Fever	31	100	69	100	2.3	0.13
Sibilant rhonchi	10	32.3	17	24.6	3.56	0.06
Bronchial breathing	12	38.6	53	76.8	14.5	0.04*
Fine crepitations	20	64.5	64	92.8	12.7	0.04*
Decreased air entry	4	12.9	49	71	15.48	0.04*
Respiratory distress	23	74.2	52	75.4	1.66	0.2
Radiological pulmonary infiltrate						
Localized infiltrate	25	80.6	44	63.8	2.85	0.09
Diffuse infiltrates	6	19.3	25	36.2		

* *p* is significant if less than 0.05.

the technical capability to culture Hib. Hib pneumonia is even more difficult to diagnose. Although blood cultures are highly specific for Hib, they have a sensitivity of 20%, thus might lead to underestimated values of Hib pneumonia [4,12,13]. In our study, we used both bacterial culture and real-time PCR for optimizing the diagnosis of Hib pneumonia. In this study, PCR was able to diagnose Hib pneumonia in nearly one-third (31%) of the children admitted to the hospital due to community-acquired pneumonia with a sensitivity of 100% compared to confirmed positive culture in a 12% of cases with a sensitivity of 38%. Such spread of Hib pneumonia may be attributable to continued transmission and circulation of Hib in the adverse living conditions like household crowding, poverty, and poor indoor air quality in many of the Egyptian districts among unvaccinated children. To the best of our knowledge; the actual incidence of Hib pneumonia in Egyptian children is not determined yet. Feikin et al. [14] studied Hib meningitis and pneumonia in 11 developing countries retrospectively and recorded 4788 cases of Hib pneumonia out of 100,000 cases of children below 5 years in Egypt. A previous Egyptian study claimed that Hib infection rate is low in Egypt based on the presence of Hib-specific IgM and IgG in 64.7–75.5% of children below 5 years without mentioning the estimated rate [15]. The incidence of invasive Hib disease and pneumonia showed three phases of presentation worldwide. Early and before the introduction of Hib vaccine in 1985, Hib disease was considered the main cause of mortality-associated invasive bacterial diseases. In Canada, 10 centers reported 485 cases of invasive *H. influenzae* disease in 1985. In Latin America: Uruguay and Mexico represented the areas of highest disease burden and were among the earliest countries that involve Hib vaccine in their national immunization program [16]. In Africa and Asia, accurate comprehensive data on the burden of invasive Hib disease is insufficient. However, in Lombok-Indonesia, high incidences of Hib meningitis and pneumonia were reported in children younger than 2 years, from 1998 to 2002. In North America, American Indian and Alaska Native children have shown the highest risk of Hib disease in USA [17]. The epidemiology of invasive Hib infection has shifted dramatically in the post-Hib vaccination era. Vaccination has resulted in impressive reductions in Hib disease and reduced carriage of Hib among both vaccinated and non-vaccinated individuals through herd immunity [17]. In 2000, eight years after Canada had implemented their Hib immunization program, their Immunization Monitoring Program reported only four cases among children aged 5 years [18]. In a district in Malawi, Africa, the incidence of *H. influenzae* meningitis decreased from 20 to 40 per 100,000 to zero in 2005 after the vaccine was introduced in 2002 [19]. In The Netherlands, where vaccination was introduced in 1993, the incidence of invasive Hib disease among children younger than 5 years of age dropped from 28.7 per 100,000 in 1992 to 0.8 in 2002 [20]. Similar reductions of Hib disease have been observed in most other countries where vaccination has been implemented in the national vaccination programs and has almost led to the eradication of Hib infection [21]. Nevertheless, comparing the studies is difficult because pneumonia case definitions, study settings and methods of case ascertainment, as well as age group of the study populations differ. Nowadays, Hib disease is challenging physicians and researchers by a new rise in the rate of invasive disease. Some researchers described it as the deadly return of Hib [22]. However, it is difficult to assess whether the observed increases in rates of *H.* influenzae disease are true increases in disease or a result of improved surveillance over the past 20 years [23]. The observed abrupt increase in invasive Hib after the introduction of the vaccine has been caused by strains a and c to f and non-capsulate (nontypeable) strains which is attributed to be the main adverse event of the vaccine and shared in the current rise of invasive Hib disease. Wherefore; Hib remains an important disease pathogen that necessitates continuous surveillance to monitor for shifts in disease incidence, to understand the burden of invasive *H.* influenzae disease, and to develop public health prevention strategies. In this study, the majority of patients with Hib pneumonia were in the first 19 months of age with almost half of them (50%) in their first two months after birth; which indicate a high carriage rate among mothers. This finding is partly comparable to MacNeil and coworkers [18] who found that

the largest burden of Hib disease among children less than five years was in infants aged 1 year (63.5%), and (27.7%) in infants aged 1 month. With the emergence of nontypeable organisms, the age incidence of Hib disease significantly declined to less than one year [24]. There was no recorded sex difference between patients with Hib and patients with non-Hib pneumonia among our studied population. This was in agreement with the pathophysiology of Hib disease which had no difference in presentation in both sexes among children [25]. The WHO published data about the epidemiology of Hib disease with a slight predominance of Hib infection in certain races like American Indians, Alaskan Eskimos, Navajo, Apache, Yakima, Athabaskan, and Australian Aborigines but concluded no differences in sex distribution [1]. Like any other forms of bacterial pneumonia, fever, refusal to feed, cough and expectoration were chief presenting symptoms among patients with Hib pneumonia. Despite the fact that the physical examination of patients with Hib revealed predominance of bronchial breathing, diminished air entry and crepitations, yet physicians cannot differentiate etiological pathogens of pneumonia by their mere clinical presentation without bacteriological examination. In our study, PCR showed higher diagnostic yield than the bacterial culture which was negative in more than 50% of cases with PCR-proven diagnosis of Hib. Moreover it also provided rapid and unequivocal results of isolated Hib. We believe that PCR was particularly important not only in diagnosis of *H. influenzae* disease but also for typing of Haemophilus strains especially in the era of nontypeable strains. Many studies concluded that Polymerase chain reaction is a rapid and sensitive method for the detection of *H. influenzae* type b, which has gained greater importance in the last few years because it increased the detection rate by 94% [26–29]. Tajima and coworkers [30] recommended PCR as a robust method for Hib investigation because it can detect and quantify very small amounts of specific nucleic acid sequences. Though bacterial culture did not add to the diagnostic aspect of this study, yet it enabled us to identify antibiotic sensitivity and resistance of the isolated Hib colonies. Isolated Hib showed the highest sensitivity to cephalosporins (namely ceftriaxon) in 91% of occasions, followed by penicillin (namely ampicillins) in 33%, with the least sensitivity toward cotrimoxazole (17%). Multidrug resistant strains were found in 25% of isolates ($n = 3/12$). This is completely different from a previous study in Cairo, Egypt from 1991 to 1993 which addressed the highest sensitivity of isolated Hib from blood cultures of children with pneumonia to ampicillins and cotrimoxazole in 100% of cases with no recorded antibiotic resistance [31]. However and comparable to our results, Youssef et al. [32] studied Hib meningitis in Egyptian children and found that the majority of isolated strains to be resistant to ampicillins (79%) and chloramphenicol (87%) whereas the highest sensitivity was to ceftriaxon (100%). Fortunately, *H. influenzae* antibiotic resistance decreased in many countries from 1997 to 2007 where nationwide campaigns for the prudent use of antibiotics have been implemented by the health authorities and conjugate vaccines against *H. influenzae* type b was introduced globally [31]. However, antibiotic surveillance studies on this pathogen should be maintained since a rising resistance to amoxicillin and amoxicillin–clavulanic was observed recently in many localities [28,31]. Moreover antibiotic surveillance studies are necessary to determine trends in national, regional, and local susceptibility patterns and to effectively guide empirical antimicrobial therapy which varies between localities.

Our study has the limitation of the incapability to define the actual burden of Hib disease in our community as it cannot be calculated using passive, sentinel surveillance because of high probability of incomplete case ascertainment and the catchment population is usually not expressing the whole community. Nevertheless we tried to have preliminary data about disease frequency in the high risk group of children less than five years old which exceeded by far other communities including similar resource-limited countries.

5. Conclusion

H. influenzae type b infection is still an important and frequent pathogen causing community-acquired pneumonia in Egypt with changeable antibiotic sensitivity pattern. PCR represented a sensitive and a rapid tool for the diagnosis of Hib pneumonia. Governmental plans to eradicate Hib in our community with the introduction of Hib conjugate vaccine in the national immunization program became indispensable.

References

[1] Jin Z, Romero-Steiner S, Carlone GM, et al. *Haemophilus influenzae* type a infection and its prevention. Infect Immun 2007;75(6):2650–4.
[2] Meats E, Feil EJ, Stringer S, Cody AG, Goldstein R, Kroll JS, et al. Characterization of encapsulated and non-capsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. J Clin Microbiol 2003;41:1623–36.
[3] Garner D, Weston V. Effectiveness of vaccination for *Haemophilus influenzae* type b. Lancet 2003;361:395–6.
[4] Peltola H. 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. Clin Microbiol Rev 2000;13:302–17.
[5] Igor R, Lana T, Cynthia B, Harry Con Behalf of WHO Child Health Epidemiology Reference Group. Global estimate of the incidence of clinical pneumonia among children under five years of age. Bull WHO 2004;82:895–903.
[6] Klien J. Bacterial pneumonias. In: Foreign R, Cherry J, Demmler G, Kaplan S, editors. Textbook pediatric infectious diseases. Philadelphia: WB Saunders; 2004. p. 299–310.
[7] Hassan-King M, Adegbola R, Baldeh I, Mulholland K, Omosigho C, et al. A polymerase chain reaction for diagnosis of *Haemophilus influenza* type b in children and its evaluation during a vaccine trial. Pediatr Infect Dis J 1998;17:309–12.
[8] Ostapchuk M, Roberts DM, Haddy R. Community acquired pneumonia in infants and children. Am Fam Physician 2004;70(5):899–908.
[9] Gonzlez J, Orero A, Prieto J. Por el Grupo para el Estudio del Uso Racional de Antibióticos (URANO). Almacenamiento de Antibióticosen Los Hogares Españoles. Rev Esp Quimioter 2006;19:275–85.
[10] Juvèn T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000;19:293–8.
[11] Klien J. Bacterial pneumonias. In: Foreign R, Cherry J, Demmler G, Kaplan S, editors. Textbook of pediatric infectious diseases. Philadelphia: WB Saunders; 2004. p. 299–310.
[12] Isaacs D. Problems in determining the etiology of community-acquired childhood pneumonia. Pediatr Infect Dis J 1989;8:143–8.

[13] Shann F. Etiology of severe pneumonia in children in developing countries. Pediatr Infect Dis 1986;5:247–52.
[14] Feikin DR, Christopher BN, James PW, Ezzeddine M, Jay DW, Orin SL. Rapid assessment tool for *Haemophilus influenzae* type b disease in developing countries. Emerg Infect Dis 2004;10(7):1270–6.
[15] Redwan el-RM, Elsawy A. Surveillance of natural acquired antibodies to *Haemophilus influenzae* type b among children in Cairo-Egyptian. Hum Antibodies 2005;14(1–2):23–6.
[16] Rudan I, Campbell H. The deadly toll of *S. pneumoniae* and *H. influenzae* type b. Lancet 2009;374(9693):854–6.
[17] Stein RT, Marostca PJ. Community-acquired pneumonia: a review and recent advances. Pediatr Pulmonol 2007:15–23.
[18] MacNeil J, Amanda C, Monica F, Raydel M, Joan B, Nancy B, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease—United States, 1989–2008. CID 2011;53:1230–6.
[19] Daza P, Banda R, Misoya K, et al. The impact of routine infant immunization with *Haemophilus influenzae* type b conjugate vaccine in Malawi: a country with high human immunodeficiency virus prevalence. Vaccine 2006;24(37–39):6232–9.
[20] Leo MS, Arie VD, Ingrid VP, Corrie S, Lodewijk S, Paul V. Increase in genetic diversity of *Haemophilus influenzae* serotype b (Hib) strains after introduction of Hib vaccination in The Netherlands. J Clin Microbiol 2005;43(6):2741.
[21] Musser JM, Kroll JS, Moxon ER, Selander RK. Evolutionary genetics of the encapsulated strains of *Haemophilus influenzae*. Proc Natl Acad Sci 1988;85:7758–62.
[22] McVernon J, Trotter CL, Slack MP, Ramsay ME. Trends in *Haemophilus influenzae* type b infections in adults in England and Wales: surveillance study. Br Med J 2004;329:655–8.
[23] Watt JP, Wolfson LJ, O'Brien KL, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. Lancet 2009;374(9693):903–11.
[24] Bender JM, Cox CM, Mottice S, et al. Invasive *Haemophilus influenzae* disease in Utah children: an 11-year population-based study in the era of conjugate vaccine. Clin Infect Dis 2010;50:e41–6.
[25] Singleton R, Holve S, Groom A, et al. Impact of immunizations on the disease burden of American Indian and Alaska native children. Arch Pediatr Adolesc Med 2009;163:446–53.
[26] Sinaniotis CA, Sinaniotis AC. Community acquired pneumonia in children. Curr Opin Pulm Med 2005;11(3):218–25.
[27] Poppert S, Essig A, Stoehr B, Steingruber A, Wirths B, Juretschko S, et al. Diagnosis of *Haemophilus influenzae* by PCR and fluorescence in situ hybridization. J Clin Microbiol 2005;43:3390–7.
[28] LaClaire LL, Tondella ML, Beall DS, Noble CA, Raghunathan PL, Rosenstein NE, et al. Identification of *Haemophilus influenzae* serotypes by standard slide agglutination serotyping and PCR-based capsule typing. J Clin Microbiol 2003;41:393–6.
[29] Romeo S, Cesar M, Carlos J, Jaime I, Sonia V, María E, et al. Serological protection induced by *Haemophilus influenzae* type b conjugate vaccine in Mexican children: is a booster dose of the vaccine needed? Clin Vaccine Immunol 2010:1639–41.
[30] Tajima T, Nakayama E, Kondo Y, Hirai F, Ito H, Iitsuka T, et al. Etiology and clinical study of community-acquired pneumonia in 157 hospitalized children. J Infect Chemother 2006;12:372–9.
[31] Ostroff SM, Harrison LH, Khallaf N, Assaad MT, Guirguis NI, Harrington S, et al. Resistance patterns of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates recovered in Egypt from children with pneumonia. The Antimicrobial Resistance Surveillance Study Group. Clin Infect Dis 1996;23(5):1069–74.
[32] Youssef FG, El-Sakka H, Azab A, Eloun S, Chapman GD, Ismail T, et al. Etiology, antimicrobial susceptibility profiles, and mortality associated with bacterial meningitis among children in Egypt. Ann Epidemiol 2004;14(1):44–8.