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Research article

Haemophilus influenzae type b invasive infections in children hospitalized between 2000 and 2017 in a Pediatric Reference Hospital (PRH)

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ABSTRACT

Background: Uruguay incorporated the conjugate vaccine against *Haemophilus influenzae* b (Hib) in 1994. In 2008, the vaccine was changed from one with natural conjugated capsular polysaccharide to one with a synthetic polysaccharide component. We describe the frequency and characteristics of invasive Hib infections in children hospitalized in a Pediatric Reference Hospital (PRH) between January 1st, 2000 and December 31st, 2017.

Methods: Sterile site Hib isolations from hospitalized children were included. Clinical and microbiological characteristics were analyzed. Favorable conditions for the infection were considered: incomplete immunization, immunodeficiencies and associated pathologies. Two periods are described: 1, prior to vaccine change (1/1 st/ 2000- 12/31/08) and 2, post-change (1/1 st/09- 12/31st/17).

Results: 45 children were hospitalized: 5 in the first period and 40 in the second. The hospitalization rate per 10,000 discharges was 0.41 (95% CI 0.05–0.77) and 4.2/10,000 (95% CI 2.89–5.48), respectively ($p < 0.01$). The diagnoses at discharge were: meningitis/ventriculitis (20), pneumonia (16), bacteremia (3), epiglottitis (1), arthritis (1), cellulitis (3) and obstruction of the upper airway (1). Four children presented comorbidities. Twenty seven received less than 3 doses of anti-Hib vaccination and 18 were properly vaccinated (2 were immunodeficient). The median hospitalization was 14 days, 18 children required intensive therapy.

Conclusions: Observed change may be due to: incomplete primary series, inhomogeneous vaccine coverage and immunogenicity of the synthetic polysaccharide. To reduce this public health problem, epidemiological surveillance.

1. Introduction

The first polysaccharide vaccines against *Haemophilus influenzae* type b (Hib) were administered worldwide in the 1970s, with Finland being one of the first countries to demonstrate its high effectiveness in children over 18 months. In the late 1980s, the first conjugate vaccines against this germ emerged, with the advantage of being immunogenic in children under two years old. Hib was, until then, one of the agents that most frequently caused meningitis, epiglottitis, pneumonia, osteoarticular infections and occult bacteremia [1]. Uruguay was the first country in Latin America to include this vaccine in its Vaccination Scheme Certificate (VSC, shown in Figure 1) [2]. In 1994, using the French monovalent vaccine from the Pasteur Merieux Laboratory, universal vaccination against this agent started. That vaccine used purified natural polyribitol phosphate conjugated to tetanus toxoid (PRP-T) and was administered intramuscularly in the gluteal region [3].

Since 1999, in many countries of the region and the world, vaccination against Hib is administered as a pentavalent combined vaccine (Hib, DTPw, HB), currently applied at 2, 4 and 6 months, with a reinforcement at 15 months [4, 5, 6, 7]. Today, vaccine coverage is high in the country but inhomogeneous with regions where it falls significantly [8].

A rapid decrease in the incidence of invasive Hib disease was associated with this vaccination strategy: the results were positive and immediate [3]. Before the vaccination, in the Pediatric Hospital - Pereira Rossell Hospital Center (PH- PRHC), which is the pediatric hospital of national reference in the public subsector, there were approximately 60 cases of Hib meningitis per year, with a lethality of 10%. The year vaccination was introduced there were 27 cases of Hib disease in the

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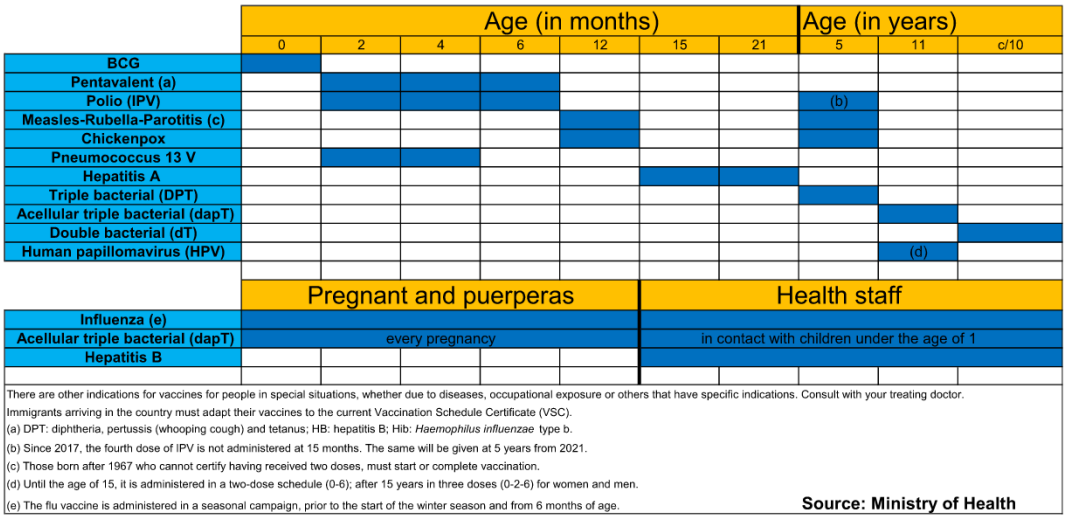

Figure 1. Current vaccination scheme certificate in Uruguay.

PH-PRHC; 23 of them corresponded to meningitis. In the year following vaccination (1995), this figure fell to 4 per year and in the subsequent years (1996-99) there were four cases of invasive disease (1 per year). This reality allowed adapting the empirical treatment guidelines of frequent paediatric pathologies, such as pneumonia and osteoarthritis [3]. In 2007, Uruguay implemented a reorganisation of its health system, which included changes in the management, financial and care model. This caused a migration of users from the public subsector to private suppliers [9]. Currently, the public subsector is mainly responsible for assisting people with high socioeconomic vulnerability [9]. In 2008, the Ministry of Health (MH) changed the pentavalent vaccine from one containing a naturally purified component against Hib PRP-T, to another one in which this component is synthetic polysaccharide.

In order to increase the knowledge about the epidemiological situation, we need to analyse the characteristics of those hospitalized children who carry this disease, their immune status and the presence of risk factors. The objective of this work is to describe the frequency and characteristics of invasive Hib infections in children hospitalized in a paediatric reference hospital in Uruguay, the PH-PRHC, between January 1st, 2000 and December 31st, 2017.

2. Materials and methods

A descriptive, retrospective study was carried out in which all children hospitalized in the PH-PRHC due to invasive Hib disease were included in the period from January 1st, 2000 to December 31st, 2017. Invasive disease was defined as the Hib isolation from a normally sterile body fluid (blood, pleural fluid, cerebrospinal fluid, joint fluid) [6]. The cases were identified from the database of the PH-PRHC Microbiology Laboratory. Those isolates obtained from normally non-sterile body fluids (secretions, exudates, etc.) and any other type of *Haemophilus* was excluded (no b, no typifiable). The following variables were described: age, sex, diagnosis at discharge, isolation site, production of beta-lactamases (Nitrocefin® test) [10, 11] and evolution during hospitalization (intensive therapy requirement, length of stay, death). We looked for predisposing factors to acquire Hib infection: doses of vaccine received for the age, primary or secondary immunodeficiencies and presence of comorbidity; for example: peritoneal ventricular shunt (DVP), genetic syndromes, etc [12, 13]. Children who received a complete primary series (3 doses) and developed an invasive Hib infection were considered [14]. In these children, basic studies were carried out to assess their immunological status: total serum immunoglobulins (IgG, IgA, IgM, IgE); plasma concentration of C3, C4 and CH50; serology for human immunodeficiency virus; abdominal ultrasound to rule out anatomic asplenia; hemogram with lamina with search of bodies of Howell and Jollie, serological response to anti-pneumococcal and tetanus vaccines. The frequency and hospitalization rate for 10,000 discharges were analyzed in two periods: period 1, pre-change (of immunogen type used in thevaccine, 1/1/2000-12/31/08) and period 2, post change (1/1/09- 12/31/17). Continuous variables were expressed in units and discrete variables in months or years.

In the comparison of hospitalization rates and the calculation of their confidence intervals, the Excell program was used. A value of $p < 0.05$ was considered statistically significant difference.

In the processing of the data, EpiInfo 7 was used. The research protocol was approved by the Ethics Committee of PH-PRHC.

3. Results

In the analysed period, 54 isolates of Hib were registered, corresponding to 45 children with invasive disease. 42.2% (19 of 45) were from Montevideo, the capital city, while the rest came from different locations in the interior of the country. Five children were hospitalized (0–2 cases per year) in period 1 and 40 in period 2 (4–6 cases per year). Distribution of cases and annual hospitalization rates per 10,000 discharges are shown in Figure 2. Rate of hospitalization for invasive disease to Hib per 10,000 discharges in period 1 was 0.41 (95% CI 0.05-0, 77) and 4.2 (95% CI 2.89–5.48) in period 2 ($p < 0.01$). Sex distribution was 31 males and 14 females. Average age is 17 months (2 months -14 years). 25 children (55.6%) were younger than one year old and 35 (77.8%) children under 2 years old.

The diagnoses at discharge were: infections of the central nervous system (CNS) 44.4% (acute suppurative meningoencephalitis [ASME] 18, ventriculitis 2), community acquired acute pneumonia (with or without empyema) 16 (35.6%), cellulitis 3 (6.7%), bacteremia 3 (6.7%), epiglottitis 1 (2.2%), knee arthritis 1 (2.2 %) and 1 upper airway obstruction (UAO) without epiglottitis with bacteremia (2.2%).

Five of 16 children with pneumonia presented empyema; one of them with co-infection with *S. pneumoniae* serotype 22F.

Table 1 shows distribution of children in both periods according to diagnosis at discharge.

Table 2 shows the number of isolations according to the type of sample. They were producers of beta-lactamases (Nitrocefin® test) 14 of the 54 isolates (25.9%) [10, 11].

Four children hospitalized in period 2 presented comorbidities: infection by Human Immunodeficiency Virus (HIV) in the stage of

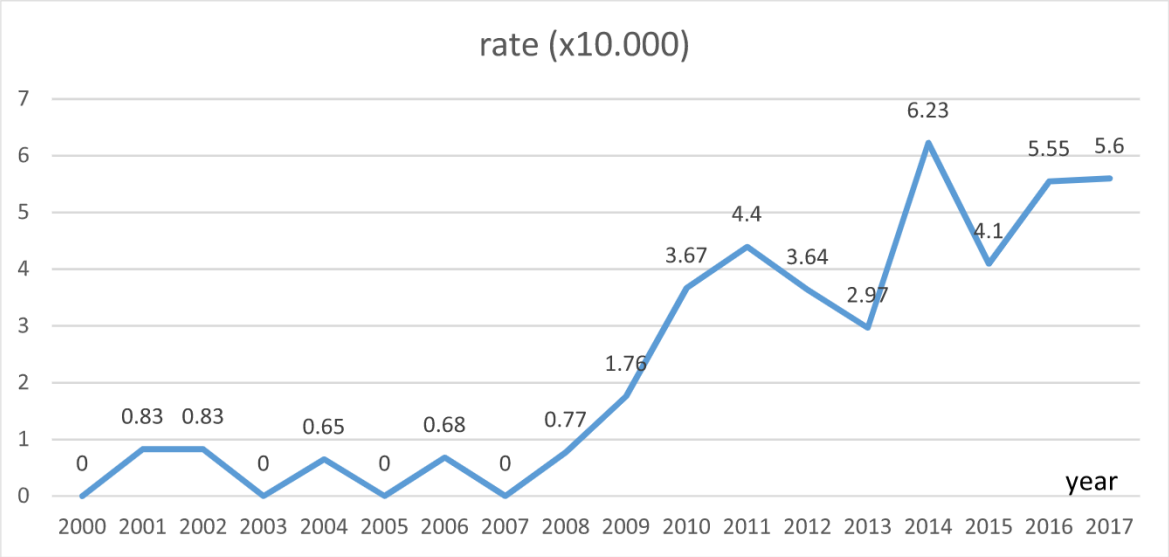

Figure 2. Evolution of discharge rates for invasive Hib infections in children from 0 to 14 years old in the HP-CHPR. Period 1/1st/2000 to 12/31st/2017.

Acquired Immunodeficiency Syndrome (AIDS) 1, VPS due to hydrocephalus 1, VPS due to skull tumor 1, Down syndrome 1.

Twenty-seven children received incomplete primary series of anti-Hib vaccination (less than 3 doses) and 18 were properly vaccinated for their age. Of the 27 children with incomplete primary series, 20 did not have the vaccines corresponding to their age. One child with complete primary series presented invasive disease in period 1 (year 2008) and 17 were hospitalized during period 2. When comparing the group of children with complete primary series and without it (as shown in Table 3), there were no statistically significant differences in the global number or in each of the diagnoses at discharge. The median number of days of hospitalization was 14 (6–27), 18 children required admission to the intensive care unit.

Lethality during the second period was 4.4 % (2 of the 45 children died during this period). One case was a girl of 6 months, previously healthy, with an incomplete vaccination schedule (2 doses of pentavalent with synthetic PRP), who presented ASME in 2009; and a 7-year-old male with Down syndrome, with a complete vaccination certificate (4 doses), who presented pneumonia with multiorgan failure in 2016.

This child had also received a synthetic PRP vaccine.

4. Discussion

In this series, and as happened in the world [15, 16], there was a decrease in invasive Hib infections following the first 15 years to the introduction of mandatory vaccination against this microorganism. Between 1999 and 2009, in the PH-HCPR there were between 0 and 2 cases per year of invasive disease due to Hib [17]. However, as of 2010, as observed in other countries [18, 19, 20], an increase in the frequency of infections by this agent was observed. As of 2010, there is a statistically significant increase in these infections in the population studied. The increase in the number of cases in recent years is also described in countries in the region such as Argentina [19, 20] and Chile [20] and in Europe, such as the United Kingdom [18, 21] and the Netherlands [22], although there could be different explanations for the same phenomenon. Possible causes include: reduction of indirect herd protection, drop of antibodies in children who did not receive booster doses, emergence of more virulent and/or contagious strains, differences in the immunogenicity of the different existing vaccines [20]. In Uruguay, the reorganisation of its health system in 2007 determined a decrease in around 25 % the population served in the PH-HCPR, bringing together the children with the most vulnerable conditions in the country. Undoubtedly, this determines a change in the characteristics of the denominator involved in the calculation of the rates of cases of invasive Hib disease. But on the other hand, it is described in this same centre and in this epidemiological context, a decrease in invasive disease and non-invasive by *S. pneumoniae*, after the universal implementation of the conjugate vaccine for this microorganism [23, 24].

CNS *infections* (meningitis/ventriculitis) were the most frequent, followed by pneumonia, as described in the prevacunal series [3, 14, 25]. Most of them occurred in children under 2 years, especially in children under 1 year, a widely documented finding [1, 3, 10, 18, 26]. There is a clear predominance of male over female (2–1), which is described as a

Table 1. Hospitalizations for *H. influenzae* type b in children from 0 to 14 years in the PH-HCPR in the two periods according to the type of capsular polysaccharide of the conjugate vaccine used and diagnoses at discharge.

Period	ASME	ACP	Bacteremia	Epiglottitis	Arthritis	Cellulitis
1- conjugated natural prp 2000–2008	-	4	-	-	1	-
2- Synthetic conjugated PRP 2009–2017	20*	12**	4***	1	-	3
Total	20	16	4	1	1	3

PH-HCPR = Pediatric Hospital - Hospital Center Pereira Rossell; PRP = poly-ribitol phosphate.
ASME: acute suppurative meningo-encephalitis, ACP: Acute community pneumonia.
* 1 death, 1 case corresponded to ventriculitis, 1 case presented co-infection with *S.pneumoniae*.
** 1 death.
*** 1 upper airway obstruction (UAO) without epiglottitis with bacteremia.

Table 2. Hospitalizations for *H. influenzae* type b in children from 0 to 14 years in the PH-HCPR. Number of Hib isolates according to sample type.

Sample	Number of isolations
Cerebrospinal fluid	14
Blood	19*
Pleural fluid	4
Joint fluid	1
Blood and pleural fluidl	3
Blood and cerebrospinal fluid	5**

* 2 correspond to the same patient.
** 2 blood cultures and a cerebrospinal fluid correspond to the same patient.

probable risk factor [16]. The production of beta-lactamases is also similar to that described in other series [10, 11].

In the United Kingdom, the increase in infections was linked to a decrease in the vaccination rates of the population, in addition to the application of a reduced scheme without a booster dose. In cases of well-immunized children, it is linked to the administration of the combined vaccine with acellular *B. pertussis*, diphtheria and tetanus. This vaccine produces lower levels of anti-Hib antibodies than in other combinations with complete cellular *B. pertussis*, so the vaccine efficacy would be lower and also the protection would last less time. These hypotheses could explain the increase in cases of vaccine failure in that country [15, 27, 28].

In Uruguay, the vaccination coverage for *Hemophilus influenzae* type b has been greater than 94% since its inclusion and to date [8]. However, the existence of an inhomogeneous coverage generates groups of the population with low coverage or inadequately vaccinated, producing a decrease in the herd effect and increasing the susceptibility to infections by this agent. In relation to this, various strategies have been generated, such as updating the vaccination scheme in children admitted to the PH-HCPR prior to discharge, which has been mandatory since 2016.

Although there are several risk factors for invasive disease due to this microorganism (such as overcrowding, daycare attendance, socioeconomic deficit, low educational level of parents, exposure to tobacco smoke) [5, 7, 15, 16, 29], in this series incomplete immunization for age was the most involved; while only 2 children presented an alteration of their immune system.

An important percentage (40%, N = 18) was presented as a vaccine failure, which makes it necessary to rule out immunodeficiencies. In this series, 1 primary immunodeficiency (linked to Down Syndrome) [30, 31, 32] and 1 secondary immunodeficiency (HIV in AIDS stage) were found. This assessment could not be completed in 8 children. Therefore, it is not possible to attribute solely responsibility to the type of immunogen used.

Table 3. Distribution of total diagnoses and relative to discharge according to complete or incomplete primary series of conjugate vaccine of *H. influenzae* type b.

Diagnosis	Vaccination failure (complete series) (n/%)	No vaccine failure (incomplete series) (n/%)	*P**
Community-acquired pneumonia	6/33,3	5/18,5	0,3
Empyema	2/11,1	3/11,1	1
Central Nervous System	6/33,3	14/51,9	0,36
Bacteremia	2/11,1	1/3,7	0,55
Epiglotitis	1/5,6	0	0,4
Arthritis	0	1/3,7	1
Upper airway obstruction and bacteremia	1/5,6	0	0,4
Celullitis	0	3/11,1	0,26
Total	**18/100**	**27/100**	

* *chi* square.

In 2008, Health Ministry (HM) changed the supplier of the pentavalent vaccine whose component against Hib was prepared based on purified natural PRP to another, in which it was synthetic. Literature endorses, and even considers somehow advantageous those vaccines that have synthetic polysaccharides as components [33, 34, 35, 36, 37]. There is no empirical immunological evidence to support the notion that there are differences in immune responses to the two Hib vaccine products. Serum dosing of specific antibodies against Hib was not performed in any of these children or in the population before, during or after this change.

5. Conclusions

The rate of hospitalizations per 10,000 discharges due to invasive Hib disease in the PH-HCPR increased more than 10 times from period 1 to period 2; this change was statistically significant. The factor most frequently involved in this series was having the primary series incomplete; although others are also probably involved, such as inhomogeneous vaccine coverage in the country. It is mandatory to continue monitoring the epidemiological situation and implement strategies to improve vaccination coverage.

Declarations

Author contribution statement

M. Delfino, C. Zabala and Lorena Pardo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

L. Fernández and C. Nieves: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

M. Más: Analyzed and interpreted the data.

P. Barrios, M. Mota, A. Varela and C. Gutiérrez: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

G. Algorta, S. Gutiérrez, G. Giachetto and M. Pírez: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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