Haemophilus influenzae Type b Vaccine Failure in Portugal

A Nationwide Multicenter Pediatric Survey

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on behalf of the Portuguese Study Group on Haemophilus influenzae Invasive Disease in Children

Background: Despite the high effectiveness of the *Haemophilus influenzae* type b (Hib) vaccine in preventing invasive disease (ID) in children, Hib vaccine failures (VFs) cases may still occur. This study aimed to characterize the Hib-VF cases in Portugal in a 12-year period and trying to identify the possible associated risk factors.

Methods: Prospective descriptive nationwide surveillance study. Bacteriologic and molecular studies were performed at the same Reference Laboratory. Clinical data were collected by the referring pediatrician.

Results: Hib was identified in 41 children with ID and 26 (63%) were considered VF. Nineteen (73%) cases occurred in children less than 5 years old; 12 (46%) occurred before the Hib vaccine booster dose at 18 months of age. Comparing the first and the last 6-year periods of the study, the incidence rate of Hib, VF and total *H. influenzae* (Hi) ID significantly raised ($P < 0.05$). VF cases corresponded, respectively, to 13.5% (7/52) and 22% (19/88) of total Hi-ID cases ($P = 0.232$). Two children died due to epiglottitis and 1 acquired sensorineural hearing loss. Only 1 child had an inborn error of immunity. The immunologic workup performed in 9 children revealed no significant abnormalities. All 25 Hib-VF strains analyzed belonged to the same clonal complex 6.

Conclusions: In Portugal, more than 95% of children are vaccinated against Hib, but severe Hib-ID cases still occur. No predisposing factors were clearly identified to justify the increased number of VF in recent years. Along with continued Hi-ID surveillance, Hib colonization and serologic studies should be implemented.

Key Words: *Haemophilus influenzae* vaccine failure

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Haemophilus influenzae (Hi) was one of the major causes of bacterial invasive disease (ID) in children and Hi type b (Hib) was estimated to be responsible for 60% of the Hi-ID cases in Portugal in the pre-vaccine period.[1] After the introduction of the Hib vaccine in the National Immunization Program (NIP) in 2000, a great decline in Hib ID was observed.[2]

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This study was registered and approved by the Ethics Research Committee of Centro Hospitalar Universitário Lisboa Norte.

Written signed informed consent was obtained from parents or guardians of participating children. All data were processed anonymously.

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In 2010, the Portuguese Society for Pediatric Infectious Diseases and the National Reference Laboratory for *Haemophilus influenzae*, based at the National Institute of Health in Lisbon, established a surveillance program on pediatric Hi-ID. Previously published data showed that Hi-ID, mainly due to noncapsular strains (nontypeable *Haemophilus influenzae* [NTHi]), was an important cause of morbidity in children living in Portugal but also some cases of Hib vaccine failures (VFs) were described.[3]

Several factors have already been discussed that may be involved in Hib-VF. These include those related to the vaccine (different conjugation proteins and use of combined vaccines), timing and number of doses administered, the concomitant administration of other vaccines that may lower the antibody titers, molecular changes of Hib strains (number of copies of *cap* b locus and/or the capability of losing the capsule), the host (mainly prematurity and immunosuppression) and loss of protective immunity.[4–12]

The aim of this study is to characterize the Hib-VF cases in Portugal in a 12-year period and trying to identify the possible associated risk factors.

METHODS

Bacterial Isolates

A total of 140 *H. influenzae* invasive isolates were recovered from children (0–17 years), between January 1, 2010, and December 31, 2021, during a prospective nationwide surveillance study, which included 40 hospitals. Bacteriologic and molecular studies were performed at the National Reference Laboratory for *Haemophilus influenzae*. Clinical data were collected by the referring pediatrician at the local hospital.

All strains were cultured on chocolate agar supplemented with polyvitex (BioMérieux, Linda-a-Velha, Portugal) and incubated at 35°C for 18–24 hours in 5% CO_2 atmosphere. Pure cultures were stored in Tryptic Soy Broth with 20% glycerol, at –80°C, for further study.

An ID case was considered when Hi was isolated from blood, cerebrospinal fluid or other normally sterile fluid.

The Hib vaccination schedule has always been at 2, 4, 6 and 18 months. Between 2010 and 2016, a Hib pentavalent vaccine was given at 2, 4 and 6 months and a Hib tetravalent vaccine was given at 18 months. Since 2017, the pentavalent vaccine is given only at 4 and 18 months and a hexavalent vaccine is given at the 2- and 6-month visits. The Hib cases were considered VF if the ID occurred ≥2 weeks after 1 Hib vaccine dose given after the first birthday, or ≥1 week after ≥2 doses, given at <1 year of age.[4,5]

Serotyping

DNA was extracted by a boiled procedure, previously described.[2]

The capsular status was determined by polymerase chain reaction amplification of the *bexA* gene and serotype was confirmed by amplification of capsule-specific genes, using previously described primers and conditions.[13]

Multilocus Sequence Typing

Multilocus sequence typing was performed as previously described.[14] Sequence type (ST) was assigned using the *H. influenzae* multilocus sequence typing website.[15]

Data were subjected to statistical analysis using nonparametric tests for comparing Hib, VF and total Hi ID and MedCalc Software Ltd., Ostend, Belgium Comparison of 2 rates (Available at: https://www.medcalc.org/calc/rate_comparison.php, Version 22.002. Accessed May 21, 2023) for comparison of incidence rates, with 95% confidence interval (CI) and the level of significance set at 0.05 ($P < 0.05$).

Population estimates were obtained at Pordata, statistics about Portugal and Europe (Available at: https://www.pordata.pt/. Accessed May 21, 2023).

RESULTS

Serotyping of Hi-ID isolates revealed a high percentage of encapsulated isolates 49.3% (69/140), with the remaining 50.7% (71/140) being characterized as NTHi. Among encapsulated isolates, most were characterized as serotype b (59.4%; 41/69), corresponding to 41 children in a total of 140 (29.3%). Non-b serotypes were characterized in 28 children's isolates as follow: 14.3% (20/140) serotype a, 1.4% (2/140) serotype e and 4.3% (6/140) serotype f.

Vaccination status was recorded for all 41 children with Hib-ID. According to previous definition, 26 (63.4%) were considered VF; 3 under 6 months with 2 doses; 10 between 6 and 18 months with 3 doses and 13 older than 18 months with a 3+1 schedule. Of the 15 (36.6%) non-VF cases, 8 (19.5%) were vaccinated according to their age but had only 1 dose in infancy and 7 (17.1%) had not been vaccinated (4 of these were less than 2 months old).

The distribution of VF cases per ages and year of diagnosis are showed, respectively, in Figures 1 and 2.

Children less than 5 years of age were predominant, corresponding to 73.1% (19/26) of the cases; 50% (13/26) of these occurred before the age of the Hib vaccine booster dose at 18 months old; 6 (23.1%) occurred between 12 and 17 months old and 26.9% (7/26) occurred in infants (<12 months old).

More cases of Hi-ID were notified in the last years of the study except for 2020. Comparing the last and the first 6-year periods of the study, there was a statistically significant difference in the incidence rate of: Hi-ID 0.84 versus 0.46/100,000 (incidence rate ratio [iRR]: 1.84; 95% CI: 1.29–2.64; $P < 0.001$); Hib-ID 0.29 versus 0.10/100,000 (iRR: 2.96; 95% CI: 1.44–6.54; $P = 0.001$) and VF 0.18–0.06/100,000 (iRR: 2.94; 95% CI: 1.19–8.29; $P = 0.011$). In these 2 periods, Hib was, respectively, responsible for 34.1% (30/88) and 21.1% (11/52) of the total Hi-ID cases ($P = 0.104$) and VF cases corresponded, respectively, to 21.6% (19/88) and 13.5% (7/52) of total Hi-ID cases ($P = 0.232$).

The distribution of diagnosis, sex and age in both VF and non-VF groups is showed in Table 1.

In the VF group, 2 patients died with epiglottitis and 1 child acquired sensorineural hearing loss. No major sequelae were reported in 23 children. There were no fatalities in the 15 cases of non-VF Hib ID.

Nineteen of 26 children were Portuguese native and 1 each came from Africa, Europe and South America (data were not available for 4 children).

Twenty children were considered previously healthy, 1 had a previous diagnosis of autoimmune lymphoproliferative syndrome, 1 had recurrent otitis media and for 4 we had no information. An immunologic workup (IgG, IgA, IgM, CH50 and lymphocyte subpopulations) was performed in 9 children but no significant abnormalities were found (1 child had selective IgA deficiency). Anti-polyribosylribitol phosphate (PRP) antibodies were not measured. The African child was HIV-negative.

Multi locus sequence typing was performed for all, except one of the 26 Hib-VF isolates. Most of isolates were characterized in ST6 (23/25; 92.0%); the remaining 2 isolates were characterized in ST190 and ST1231, which are single locus variant of ST6. Therefore all 25 Hib-VF analyzed belong to the same clonal complex 6.

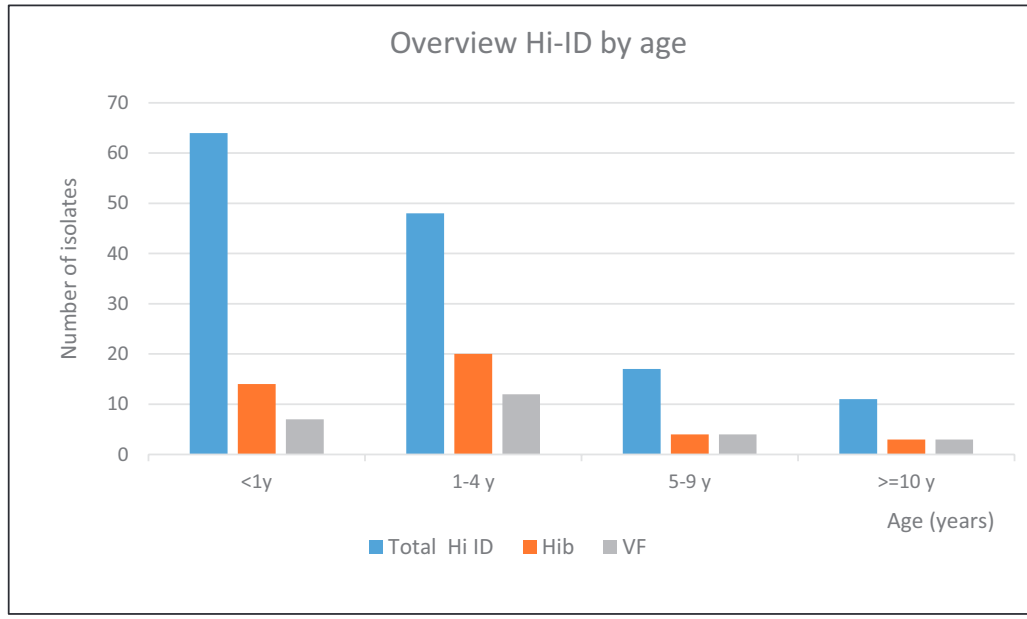

FIGURE 1. Distribution of Hi-ID isolates, collected during this study (2010–2021), by age groups, considering the total number of isolates (n = 140), Hib (n = 41) and VF (n = 26).

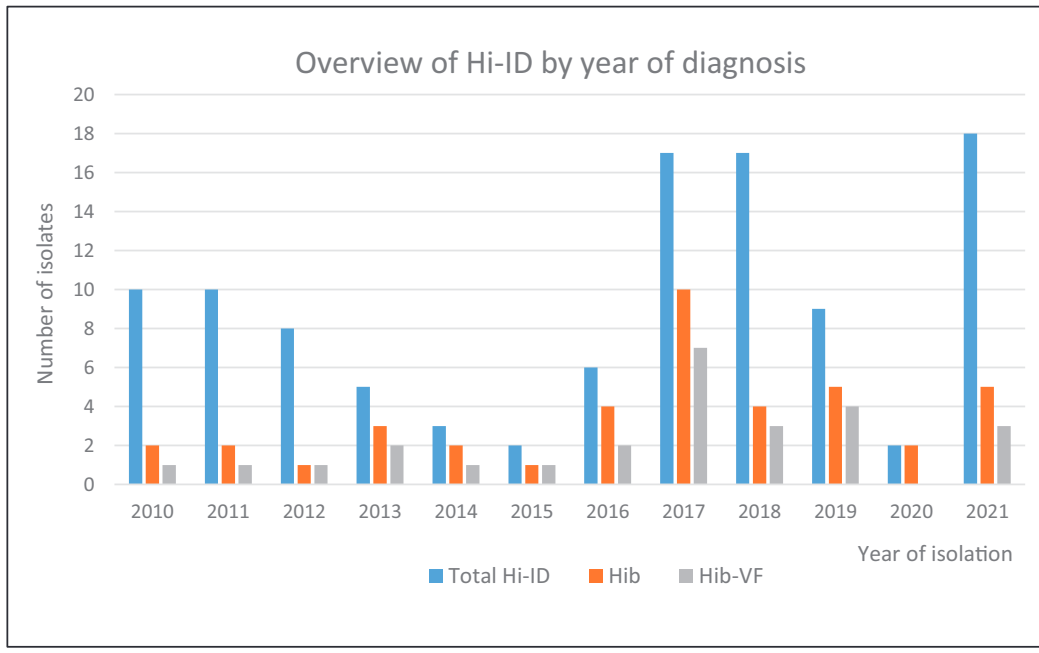

FIGURE 2. Distribution of isolates collected during this study (2010–2021), by year of isolation, considering the total number of Hi isolates (n = 140), Hib (n = 41) and VF (n = 26).

TABLE 1. Age, Sex and Diagnosis in VF and Non-VF Cases of Hib-ID

Characteristics	VF	Non-VF
Total	n	n
	26	15
Gender	n	n
Male	15	9
Female	11	6
Age	Median (minimum–maximum)	Median (minimum–maximum)
Months	19 (5–196)	3 (1–20)
Diagnosis	n (%)	n (%)
Meningitis	9 (34.6)	10 (66.7)
Bacteremia	2 (7.7)	3 (20)
Pneumonia	8 (30.8)	1 (6.7)
Epiglottitis	4 (15.4)	
Sepsis	1 (3.8)	1 (6.7)
Arthritis/osteomyelitis	1 (3.8)	
Facial cellulitis	1 (3.8)	

DISCUSSION

Portugal has a sustained vaccination coverage for Hib of more than 95% of the children, since 2000.[16] As the vaccines against Hib have an estimated effectivity of 95% and confer herd protection,[17] we could expect that Hib-ID virtually disappeared from our country.

However, there was a significant raise in the number of pediatric HI-ID, Hib-ID and VF cases between the first (2010–2015) and the second half (2016–2021) of the study. Although in 2020, there were fewer cases of Hi-ID, which was probably related to the strict population infection control measures implemented during the severe acute respiratory syndrome coronavirus 2 pandemic, and no cases of VF were found, numbers rebounded in 2021 as these restrictions were eased.

This study is based on voluntary notification of the Hi-ID cases that may have increased along the study years and the incidence estimates may not be accurate. However, this fact could not be the explanation for the increased percentage of VF cases in all notified Hi-ID cases, which also increased in the last years, albeit not reaching statistical significance.

Although most of the VF cases occurred in children less than 5 years old (73.1%; Fig. 1), all pediatric ages were affected and only 1 child had a previous disease, autoimmune lymphoproliferative syndrome that could compromise the vaccine efficacy. However, an immunologic screening was performed only in 9 children and anti-PRP antibodies were not measured. Clinical manifestations of Hib disease differed between the VF and non-VF groups (Table 1) with a predominance of respiratory disease in the VF group, as described by others.[18] However, severe disease occurred in fully vaccinated previously healthy children, leading to death in 2 of the cases, while no deaths were reported in the non-VF group.

Genetic characterization of the Hib isolates from VF, showed a great genetic clonality, with only 2 isolates being single locus variant of ST6. These results are in accordance with the results presented by other researchers,[19,20] who observed a slight increase in the genetic diversity of Hib, but not in the isolates responsible for VF.

The presence of multiple copies of the *cap* b locus has been associated with VF.[21] However, we have analyzed 5 of VF isolates by WGS and none revealed more than 1 copy of the *cap* locus.

It was not possible to analyze the VF rate by type of vaccine administered since different vaccines types were included in the NIP since 2000, and we did not have access to vaccine coverage by vaccine type and age cohort.

Recent data from different countries have, also, reported a resurgence of Hib-ID.

Data from Argentina 2011 to 2019 reported a transient emergence of Hib cases in 2015. In this period, there were 577 Hib cases but only 8 (1.4%) were reported as VF. Emergence of Hib-ID was mainly attributed to inadequate compliance with Hib vaccination schedule.[22]

Data from France suggests that a simplified vaccination schedule with a hexavalent vaccine at age 2, 4 and 11 months is responsible for an emergence of Hib VF cases and correlates with

lower antibody titers.[28] The number of cases analyzed is small and a difference in anti PRP titers was significant only in those 2 years old, which may reflect the shorter interval from the booster at 18 months in those with the 3 + 1 scheme. Also, since the considered period was 2017–2019, the 2 + 1 scheme was introduced in 2013 and only children less than 5 years were included, it was expected that the Hib-VF cases should be in children with the simplified scheme. Deghmane and Taha[23] then reported a decreased in Hi-ID in 2020 followed by a resurgence in 2021, as we noted in Portugal. However, in France, capsulated strains became prominent (Hib and Hia) while in our country, the proportion of capsulated and NTHi remained the same as it was in the period previously to the pandemic.

Interestingly, in the first year of the pandemic in the Netherlands, there was a decline in invasive bacterial disease but Hib-ID increased by 51%.[24] Several changes in the Hib childhood immunization schedule occurred in the Netherlands, namely the use of 3-component acellular pertussis followed by 3-component acellular pertussis hexavalent vaccines, and the adoption of 2 + 1 schedule (3, 5 and 11 months of age) since 2020. Although the sequent changes may lead to lower titers of IgG anti-PRP, they did not find a loss of vaccine effectiveness nor a vaccine escape variant.[10]

In Portugal, the pneumococcal conjugated 13-valent vaccine was introduced in the NIP in 2015, and the meningococcal B vaccine was introduced in 2020, both with 3 doses administered at 2, 4 and 12 months old. The first 2 doses are administered simultaneously with the hexavalent vaccine, at 2 months old, and pentavalent vaccine, at 4 months old. We are not aware of any study that documents an immunologic interference that lowers the expected Hib antibodies titers in infants exposed to this vaccination schedule. If a significant interference exists, we could expect some loss of individual protection, but mainly of herd protection because of lower antibodies titers may lead to a weaker impact in colonization, albeit maintaining protection against ID.[10,11] If that becomes true, then more cases of Hib-ID will emerge in the following years.

Also, a diphtheria, tetanus and acellular pertussis vaccine in pregnancy was recommended since 2016 and included in the NIP in 2017 with more than 85% of pregnant women vaccinated since 2018.[25] A blunting phenomenon has been described, which is responsible for lower antibody titers after vaccination in infants born to vaccinate mothers, although that appears not to decrease the immunogenicity of the Hib vaccine.[26]

Portugal is an important touristic destination and has a great income of people from Portuguese speaking African countries and Brazil, where the vaccination schedules and vaccine coverage are not as good as in our country. So, a continuous income of potential Hib colonized people may contribute for a recurrent introduction of Hib in our population, thereby reducing the herd protection effect.

A longitudinal study performed between 2015 and 2019 in Portuguese children up to 6 years old living in Lisbon showed an expected high rate of Hi nasopharyngeal colonization (84.3%), but Hib was detected only in 1 of 1524 samples (0.06%).[27] In the pre-vaccine period, serotype b carriage among Portuguese day-care center attendees was 3.8% suggesting that Hib vaccination nearly eliminated carriage of this serotype.[28]

The serologic inquiry performed in Portugal in 2015–2016 showed a prevalence of protective antibodies titers (IgG > 1.0 μg/mL) in 88.5% of the 2–4 years old, 86% of the 5–9 years old, 75.9% of the 10–14 years old and 79.4% of the 15 19 years old. Only 0.6% of the pediatric population had a titer below 0.2 μg/mL.[29] A new serologic survey would help to establish if the rate of children that achieved seroprotective titers against Hib-ID has changed from before to after the recent alterations in the Portuguese NIP, namely the concomitant use of hexavalent, conjugated pneumococcal and meningococcal B vaccines.

The Hib vaccination schedule in other European countries are quite variable, but most of them consider the booster dose at age 11–12 months old.[30] Almost a quarter of the Hib-VF cases occurred in children between 12 and 17 months old. An anticipation of the booster dose in the 2nd year of life, to be given at the age of 12 month, may be considered to achieve a higher protection in the beginning of the second year of life.

This study has a prospective design and nationwide sampling frame. All samples were analyzed in the same reference laboratory and there was no change on the surveillance system along the study. The weaknesses are (1) the passive surveillance that may have led to incomplete case capture; (2) the incomplete clinical and national vaccine type coverage data; (3) limited number of children undergoing an immune function work-up, which of itself was incomplete and namely not including the determination of PRP antibodies and (4) small numbers of cases, not allowing a significant statistical analyzes.

In conclusion, a high vaccination coverage, with more than 95% of children adequately vaccinated against Hib, does not eliminate the risk for ID and a few cases of VF may still occur every year. As for now, the significant increased number of Hib-ID and VF cases in recent years has no predisposing factors clearly identified that allow a positive intervention. The question whether any situation that may lead to lower anti-PRP antibodies in children (poor compliance, less immunogenic vaccines, less vaccine doses, interference by concomitant use of other vaccines or blunting) will promote an emergence of Hib-ID should be seriously addressed in the near future. Along with surveillance of Hi-ID, both Hi colonization and serologic surveillance studies should be implemented.

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APPENDIX

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