Epidemiology and molecular characterization of invasive disease in children twenty years after the implementation of *Haemophilus influenzae* serotype b vaccine in Portuguese Immunization Programme

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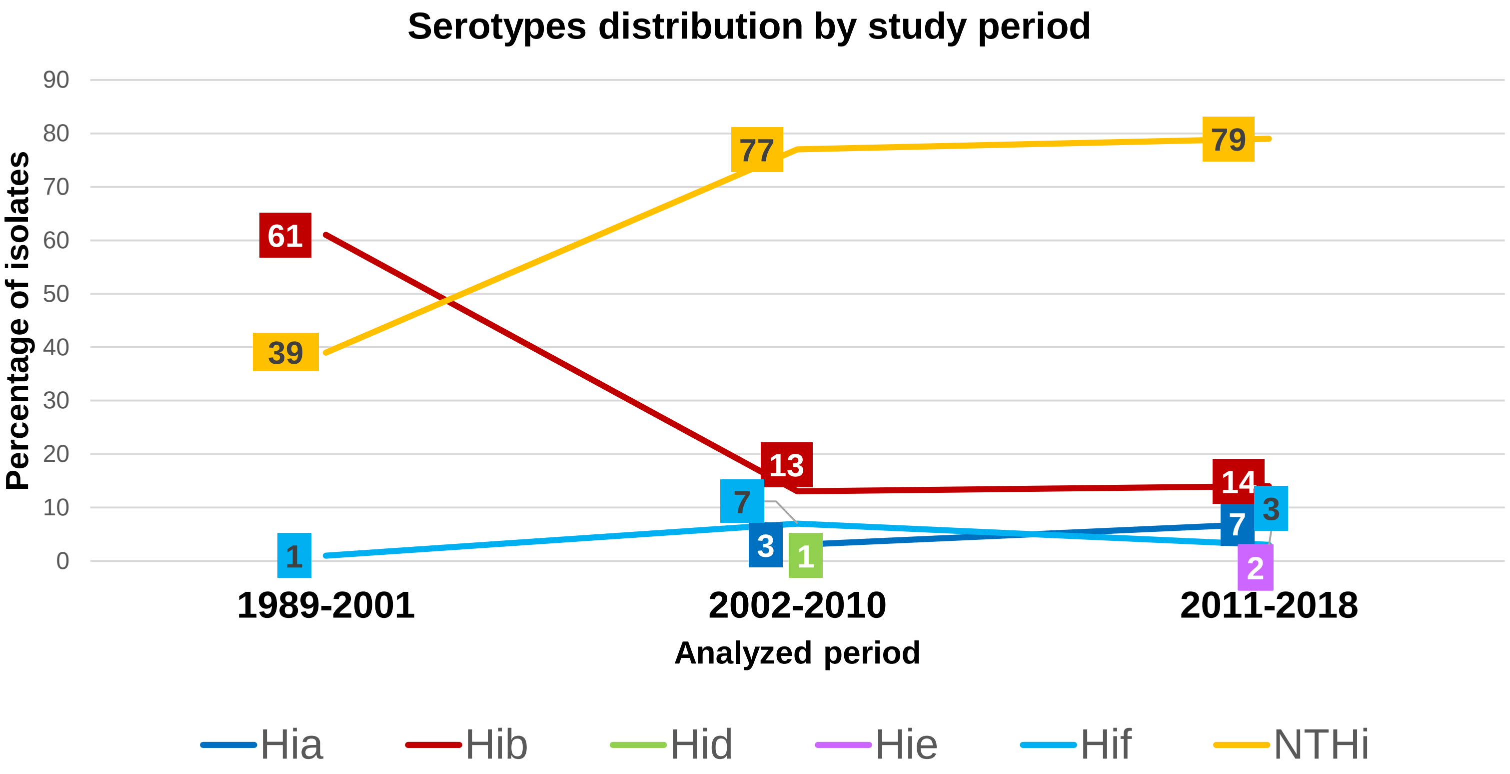
Introduction and aim of the study

- *Haemophilus influenzae* is a human-restricted pathogen responsible for severe childhood invasive disease, being classified as encapsulated (serotypes a-f) or non-encapsulated (NTHi), depending on the presence or absence of a polysaccharide capsule
- The implementation of the vaccine against *H. influenzae* serotype b (Hib), in our National Immunization Programme, in June 2000, lead to a reduction in invasive disease due to serotype b, together with the emergence of NTHi, and encapsulated non-b-type isolates
- The vaccine schedule is 3+1 doses (2, 4, 6 months of age) and a booster dose (18 months)

- **This study aims to characterize *H. influenzae* invasive disease in children, twenty years after the introduction of the Hib vaccine in National Immunization Programme**

Overview of *H. Influenzae* invasive disease in Portugal



- Bajanca P, Caniça M, & the Multicenter Study Group. Emergence of noncapsulated and encapsulated non-b-type invasive *Haemophilus influenzae* isolates in Portugal (1989-2001). *J Clin Microbiol*, 2004; 42:807-10.
- Bajanca-Lavado, M.P. Simões A.S. Betencourt C.R. Sá-Leão R. The Portuguese Group for the Study of *Haemophilus influenzae* infection. Characteristics of *Haemophilus influenzae* invasive isolates from Portugal following routine childhood vaccination against *H. influenzae* serotype b (2002-2010). *Eur J Clin Microbiol Infect Dis*, 2014; 33:603-10.
- Heliodoro CIM, Bettencourt CR, Bajanca-Lavado MP, on behalf of Portuguese Group for the Study of *Haemophilus influenzae* invasive infection 2020. Molecular epidemiology of invasive *Haemophilus influenzae* disease in Portugal: an update of the post-vaccine period, 2011–2018. *Eur J Clin Microbiol Infect Dis* 39:1471–1480.

Material and Methods

Samples

- 120 *H. influenzae* invasive isolates from January 2010 – December 2020

Capsular status

- Serotype characterization: PCR amplification of capsule-specific genes - a to f (Falla et al, 1994)

MLST

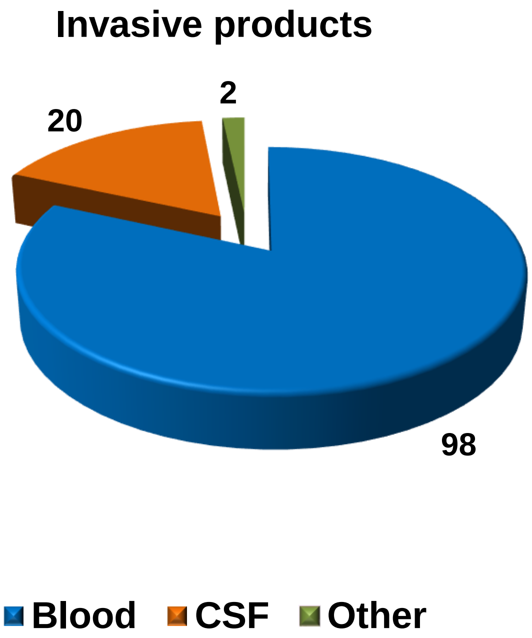
- Multilocus Sequencing Type (amplification and sequencing of internal fragments of 7 housekeeping genes: *adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi*, and *recA* (Meats et al, 2003).
- Sequence type (ST) was assigned through (https://pubmlst.org/hinfluenzae/)

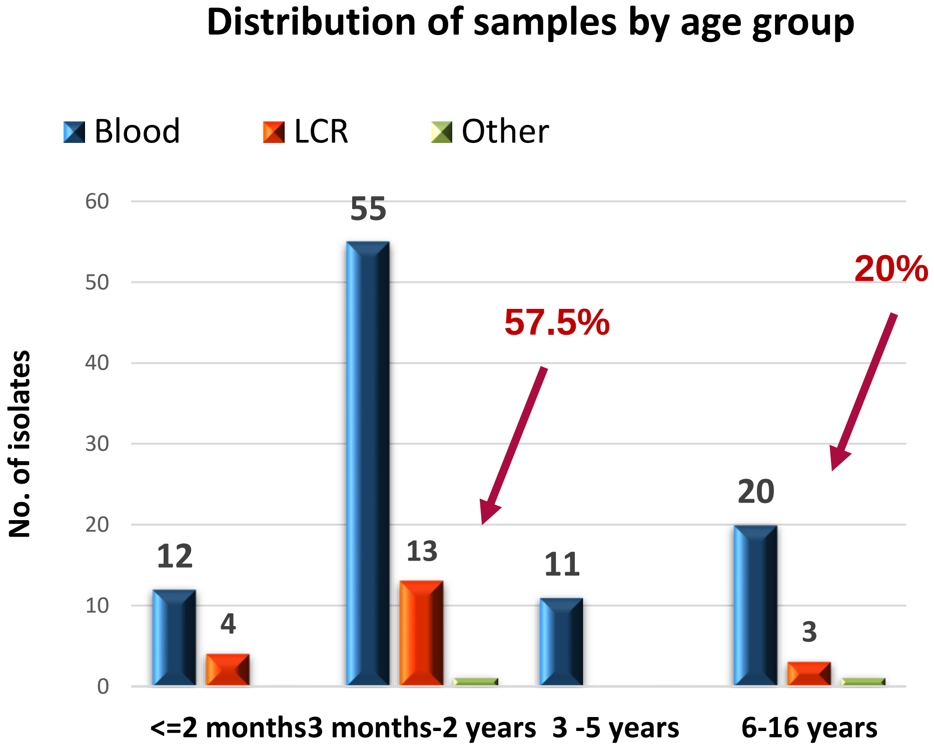
Project with pediatricians:

Invasive *H. influenzae* disease in children - an ongoing project evolving clinical data

- Falla, T.J., et al., PCR for capsular typing of *Haemophilus influenzae*. J Clin Microbiol, 1994. 32(10): p. 2382-6.
- Meats, E., et al., Characterization of encapsulated and noncapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. J Clin Microbiol, 2003. 41(4): p. 1623-36.

Isolation products



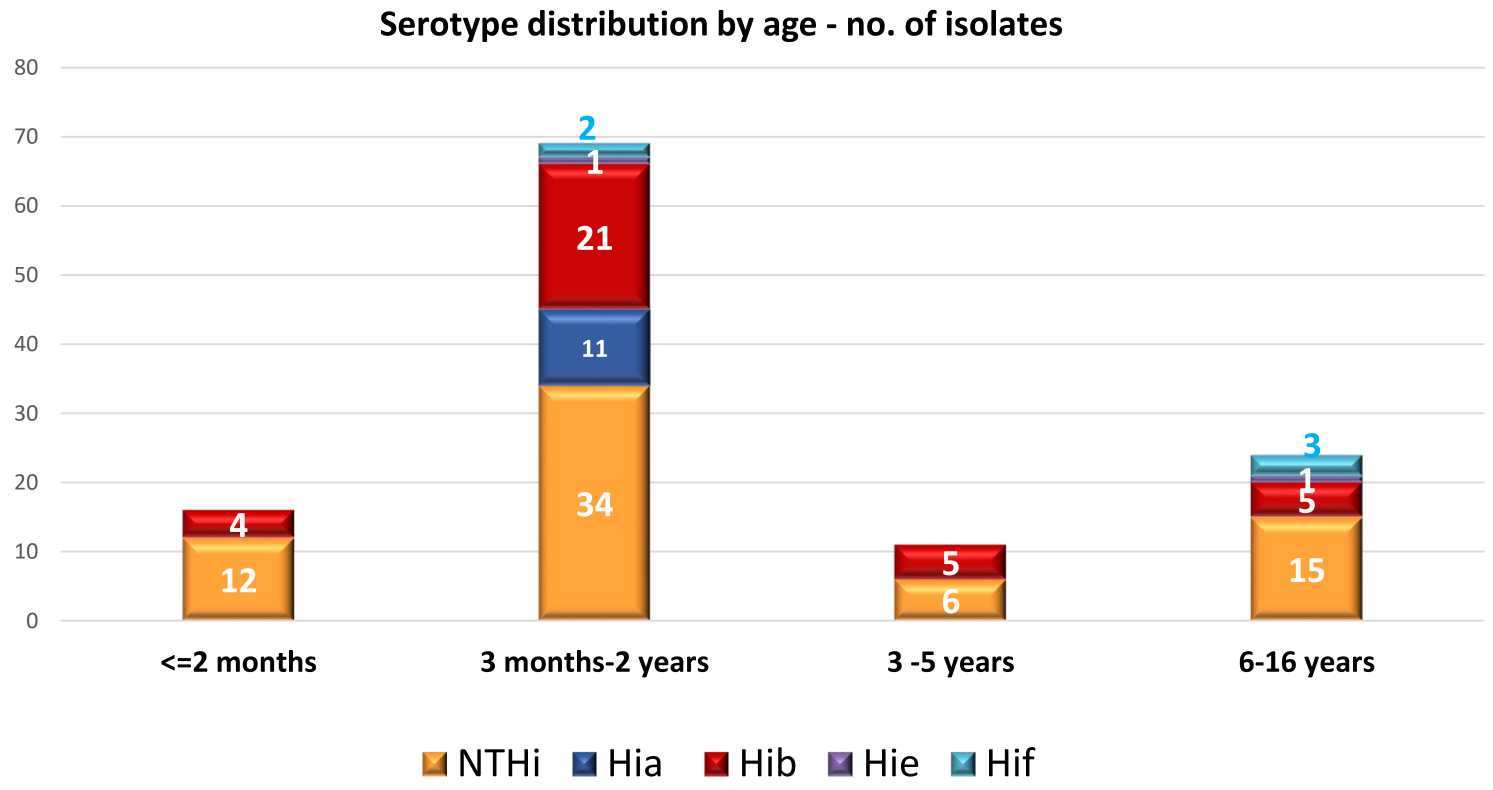


Most isolates were from blood (**66.7%**); CSF isolates were **16.7%**

Invasive disease is predominant among children between 3 months and 2 years old (**57.5%**)

20% of invasive disease in older children (6-16 years old)

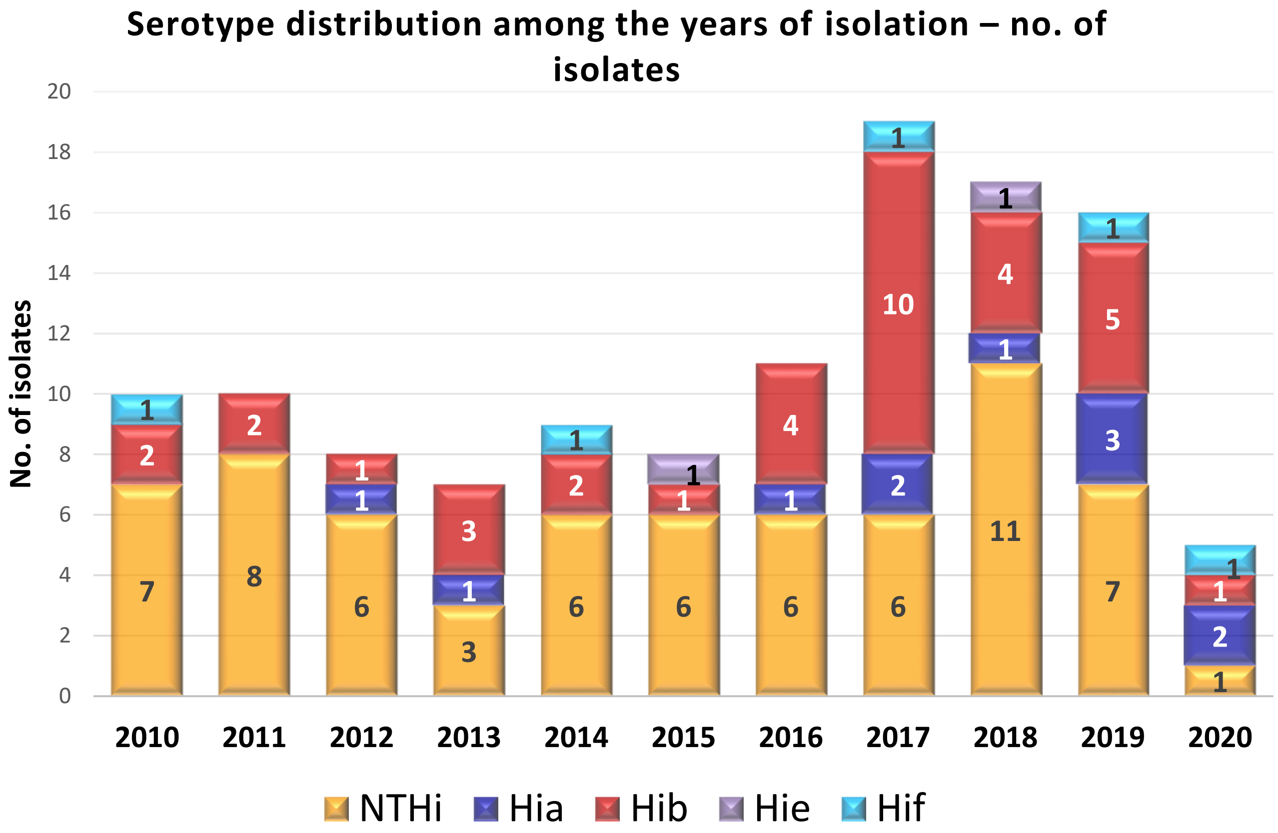
Haemophilus influenzae serotypes in children according to age



All of **serotype a** isolates were characterized in children between 3 months and 2 years old

Serotype b isolates are distributed among all age groups, with higher incidence in children from 3-5 years old (**45.5%**)

Haemophilus influenzae serotypes in children according to isolation year



Invasive disease due to **serotype b** was higher in **2017** (**52.6%**)
Although the number of non-b serotypes isolates is small, we notice an increase of **serotype a** isolates in last years

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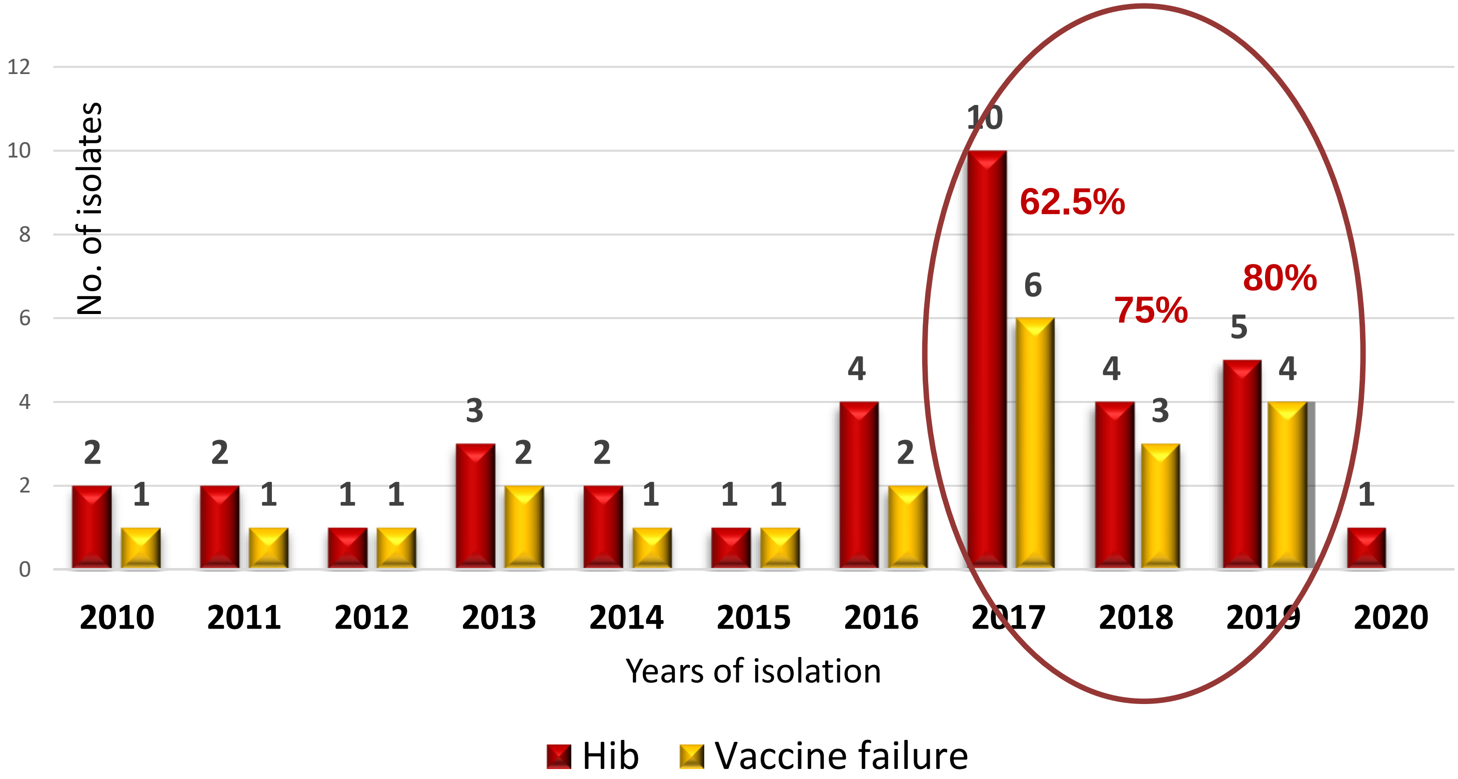
Vaccine failures



"A case was designated as true vaccine failure (TVF) if invasive Hib disease occurred ≥2 weeks after one Hib vaccine dose, given after the first birthday, or ≥ 1 week after ≥ 2 doses, given at <1 year of age".

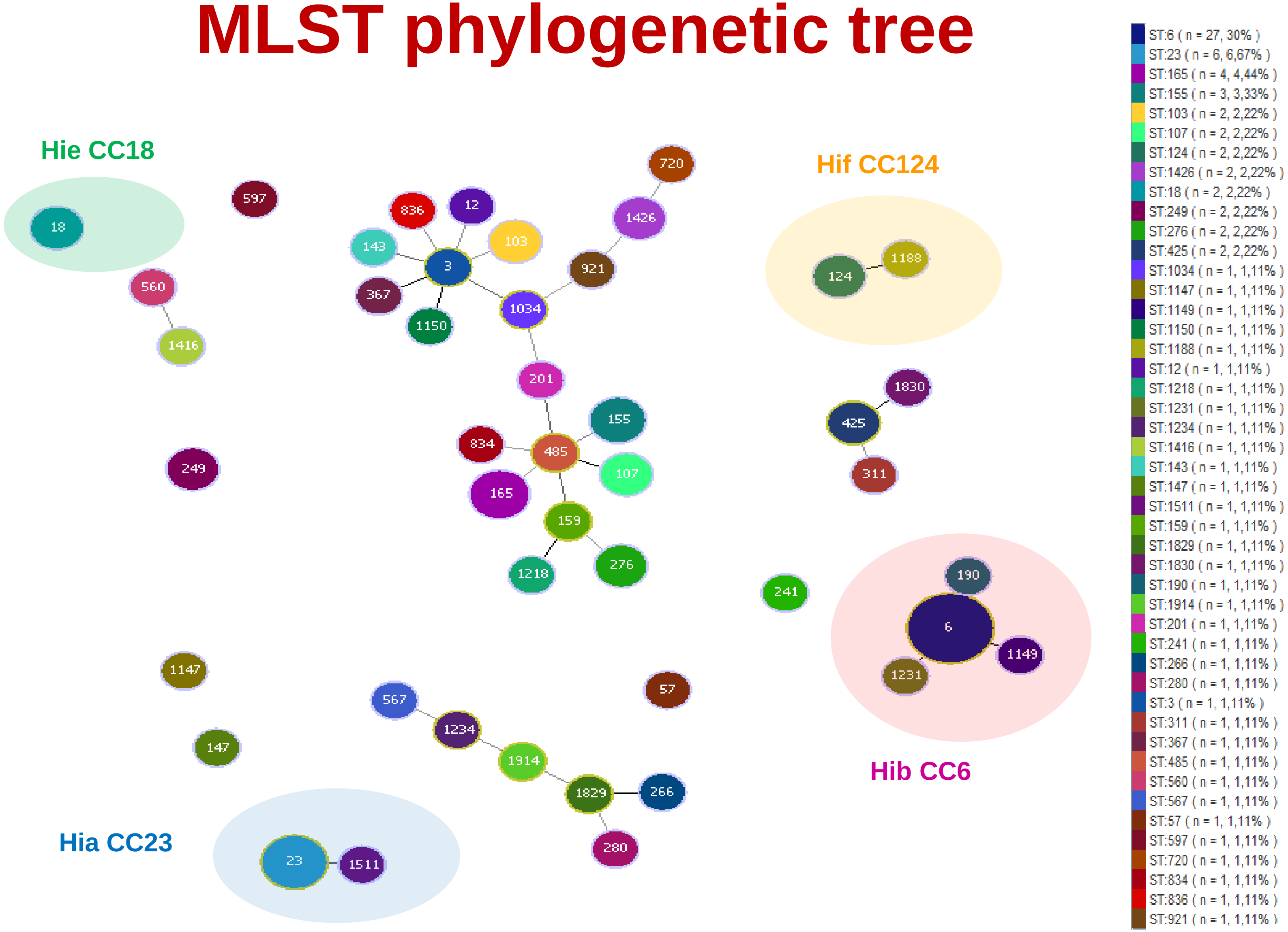
Heath P, Booy R, Griffiths H, Clutterbuck E, Azzopardi H, Slack MP, *et al*. Clin Infect Dis 2000; 31:973-80.
Ladhani S, Heath PT, Slack MP, McIntyre PB, Diez-Domingo J, Campos J, *et al*. Clin Microbiol Infect 2010; 16:948-54.

Hib invasive disease 2010-2020

120 children - total of 35 Hib cases – 22 Vaccine Failures (VF)

- **17 cases of VF among children up to 5 years old**
- **5 cases of VF among children between 6 and 16 years old**- loss of immunity?
- **2 cases of non-vaccinated children**
- **9 cases of children with one dose, or too young to be vaccinated**
- **2 children without information** - 10 and 20 months years old

MLST phylogenetic tree



Minimum spanning tree representation of multilocus sequence typing results for **84 *H. influenzae*** invasive disease isolates. Numbers indicate sequence types. Black lines connect single locus variants and grey lines connect isolates with more than one locus variant. The size of the node is proportional to the number of isolates included in each sequence type

Conclusion

- Our data suggests that after vaccine implementation, invasive disease among Portuguese children is mainly due to highly genetically diverse NTHi isolates

- Nevertheless, we are concerned with Hib disease (~30%) despite the higher vaccine coverage observed in our country

- Ongoing surveillance should be continued, in order to monitor the burden of the disease, especially serotype b, and develop additional public health prevention strategies

Acknowledgements

Pathologists and medical staff at Hospitals that send *Haemophilus influenzae* isolates to our Surveillance Project

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Thanks very much for your attention