Multilocus Sequencing Typing of Invasive *Haemophilus influenzae* strains Isolated in Portugal in the Pre-vaccination Period (1989-2001)

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Aim of this work

- MLST characterization of Portuguese *H. influenzae* isolates (70 strains) from invasive infection, before introduction of Hib vaccine in NIP

STs from pre-vaccination period isolates (1989-2001) will be compared to STs from pos-vaccination period isolates (2002-2010)
Introduction / *H. influenzae*

- *Haemophilus influenzae* can cause life-threatening (invasive) infections in children and adults: pneumonia, bacteremia, meningitis...

- Six capsular types, a-f, have been identified. Non-capsulated *H. influenzae* (NCHi) have also been described.

- *H. influenzae* serotype b (Hib), has been a major cause of morbidity and mortality, especially in children (≤5 years old).
Introduction /Hib vaccine

- In Portugal, Hib vaccination was implemented in the National Immunization Program (NIP) in June 2000, for all children of pre-school age (≤5 years old).

- The vaccine schedule is three doses (2, 4, 6) months of age, and a booster dose at 18 months.

- The introduction of Hib conjugate vaccine has led to the nearly extinction of Hib disease.

- In turn an increase in non-Hib disease was observed, especially NCHi, in all age groups.

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Introduction /MLST

**H. influenzae MLST**: unambiguous characterization of both encapsulated and non-capsulated isolates

Initially, the database ([http://haemophilus.mlst.net](http://haemophilus.mlst.net)) contained the allelic profiles of 131 isolates published by Meats et al.. Further isolates have being added.

Recently: retirement of Brian Spratt - transfer of MLST databases from Imperial College London to Oxford: K. Jolley and M. Maiden have kindly agreed to host them at PubMLST.org ([http://pubmlst.org/hinfluenzae](http://pubmlst.org/hinfluenzae))
Methods

- DNA extraction by boiling procedure (pure culture of *H. influenzae* on PVX chocolate agar)

- Amplification of the 7 housekeeping genes using primers described by Meats *et al*, 2003

- Sequencing with only one primer for each allele (exception for new sequences types): *adk* (R), *atpG* (F), *frdB* (R), *fuK* (F), *mdh* (F), *pgi* (R), *recA* (R)

- *frdB* primers were substitute for the ones described by Schouls *et al*, 2005
Methods (Problems)

- Amplification protocol with HotStar Taq Polymerase (Quiagen): Frequently no amplification of atpG
- Amplification protocol with BIO-X-ACT Short DNA Polymerase (Bioline)

Amplification of the 7 loci
- gel agarose 2%

489bp-345bp

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Analysis

- The allelic profile of an *H. influenzae* strain was obtained after sequencing internal fragments of 7 house-keeping genes: *adk, atpG, frdB, fucK, mdh, pgi, recA*

- For each isolate, the alleles at each of the seven loci define the allelic profile or sequence type (ST)

- Two or more isolates with the same ST are characterized in the same clonal complex (CC)
Strain characterization

70 strains were randomly chosen among invasive isolates 1989-2001
Results
pre-vaccine vs pos-vaccine

1989-2001: 70 isolates

- 31 NC
- 29 ≠ STs
  - ST395 2 strains
  - ST396 2 strains
  - STNew 12 strains

2002-2010: 68 isolates

- 50 NC
- 47 ≠ STs
  - ST6 1 strain
  - ST3 2 strains
  - ST12 2 strains
  - ST103 2 strains

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Results
pre-vaccine vs pos-vaccine

1989-2001: 70 isolates

- ST159: 1 strain
- ST282: 1 strain
- ST1345: 1 strain
- ST117: 1 strain
- STNew: 1 strain
- 38 b: 1 strain

11 ≠ STs; 35 CC6

2002-2010: 68 isolates

- ST6: 10 strains; CC6
- ST474: 1 strain
- ST1149: 1 strain
- ST117: 1 strain
- ST206: 2 strains
- ST6: 14 strains; CC6
- ST474: 1 strain

18 b

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Pre-vaccination: 1989-2001
(n=70)

Phylogenetic estimations were conducted through MEGA5 by using the neighbor-joining method with bootstrapping (1000 replicates). The Kimura two-parameter method was employed to estimate the evolutionary distances.
Pos-vaccination: 2002-2010 (n=68)
Discussion

Our MLST results showed:

- **NCHi**: highly genetic diversity in both periods
- **Hib**: highly clonal, in both periods

Strains from pre and pos-vaccination periods:

- Higher percentage of antibiotic resistance strains, including (Bla+) in pre-vaccination period especially due to MR Hib strains
- Decline in serotype b with concomitant increase of Nc, in pos-vaccination period
- non-b serotypes (a, d, and f) were characterized in pos-vaccination period

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MLST: advantages and limitations

- automated technology
- highly unambiguous
- portable and reproducible

MLST combines advances in sequencing and bioinformatics to establish population genetics

- data can be used to investigate evolutionary relationships among bacteria

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MLST: advantages and limitations

焰 MLST is an expensive technique

焰 Although it has a good discriminatory power sometimes this is not enough to differentiate isolates, due to the sequence conservation in housekeeping genes

焰 Limitation of its use in epidemiological investigations

焰 This can be overcome by comparing MLST results with PFGE patterns (technique with a more powerful discriminatory power)

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Conclusions

The majority of episodes of invasive disease occurring in Portugal are now due to

- fully susceptible
- highly diverse
- non-capsulated strains

Given the evolving dynamics of this pathogen and the increase in non-type b capsulated isolates, continuous surveillance is needed
References


References


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References


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