Characterization of ampicillin resistance mechanisms in clinical *Haemophilus influenzae* strains isolated in Portugal between 2009 and 2012

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1. Introduction

*Haemophilus influenzae* (HI) is a major pathogen associated with community-acquired respiratory tract infections (RTIs). Even though antibiotic therapy is of great meaning in HI infections, its efficacy may be compromised by the emergence of resistant strains to β-lactams since this antibiotic class is the most used for HI and RTIs. The two well known mechanisms of β-lactam antibiotics resistance in *HI* are: β-lactamase production, which is responsible for the enzymatic inactivation of the antibiotic, and a non-enzymatic mechanism, that involves decreased affinity for β-lactams due to altered penicillin-binding proteins (PBPs)

The aim of this study is to characterize ampicillin resistance mechanisms in clinical strains of *HI* collected in our laboratory between 2009 and 2012.

2. Material and Methods

**Haemophilus influenzae** strains

Two hundred and thirty five isolates were chosen according to their ampicillin MICs: 140 BLNAR (MIC>1mg/L), 32 susceptible strains (BLNAS; MIC<1mg/L) and 63 β-lactamase producers (BLPAR) to be analyzed.

**Methods**

- Antibiotic susceptibility was determined by the broth micro dilution method, according to the CLSI guidelines;
- Beta-lactamase production was determined by a nitrocefin assay;
- Characterization of TEM-1/ROB-1 was performed by PCR Multiplex;
- Amplification of bexA gene and capsular serotype characterization were also performed by PCR;
- Amplification and sequencing of *ftsI* gene was performed as previously described.

3. Results

![Gender and Age Groups](figures.png)

**Phenotype**

BLNAR  59%  
BLNAS  14%  
BLPAR  9%  
BLPACR  2%  
Unknown  19%

**Genotype**

BLNAR  35%  
BLNAS  40%  
BLPAR  25%  Unknown  0%  

4. Discussion and Conclusion

Among gBLNAR and gBLPACR strains there were 40 different mutation patterns, that were included in the six previously described groups and subgroups (I, IIa, IIb, IIc, IIIa, III-like) (Table 1).

The most common amino acid substitutions were located near KTG motif: V547I (174/199, 87.4%), N526K (153/199, 76.9%) and N569S (143/199, 71.9%) (Table 1).

Strains with *ftsI* mutations were less susceptible to the β-lactam antibiotics studied (data not shown).

Comparing these results with previously ones, performed in our laboratory (between 2001 and 2008) we are assisting to an increase of susceptible strains (ampicillin MIC<2mg/L) as well as resistant strains (β-lactamase producers) with mutations in the *ftsI* gene, being so called gBLNAR and gBLPACR.

**CLSI breakpoints** alone can’t characterize these strains as susceptible or resistant in the susceptibility tests performed routinely in the laboratory. In this way, a continuous research on breakpoints and methodologies to better define strains of this kind is of crucial importance.

In conclusion, we emphasize the importance of continuing surveillance studies of this nature as essential tools to define trends in the antibiotic resistance of *HI*.

5. References