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)[1,2].**
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

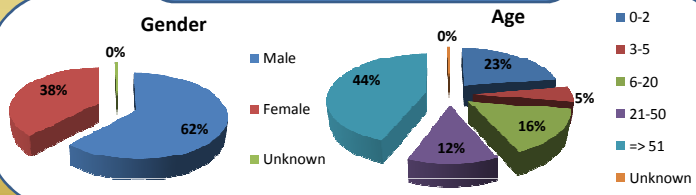
Haemophilus influenzae strains

- **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.**

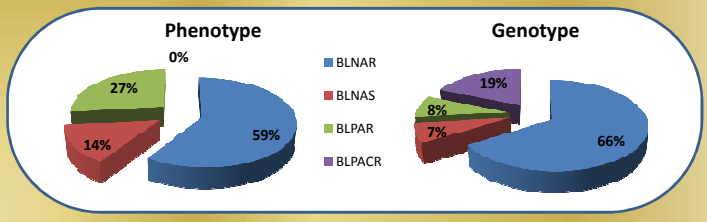
Methods

- **Antibiotic susceptibility was determined by the broth micro dilution method, according to the CLSI guidelines[3];**
- **Beta-lactamase production was determined by a nitrocefin assay;**
- **Characterization of TEM-1/ROB-1 was performed by PCR Multiplex[4];**
- **Amplification of *bexA* gene and capsular serotype characterization were also performed by PCR[5];**
- **Amplification and sequencing of *ftsI* gene was performed as previously described[6]**

3. Results



Figures I and II: Distribution of *H. influenzae* clinical isolates studied by Gender and Age Groups.



Figures III and IV: Phenotypes of *H. influenzae* clinical isolates collected between 2009 and 2012, and correspondent genotype (after sequencing).

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, IId, 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)[2] 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[7]. 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*.**

Group [a]	No. of isolates [b]		Amino acid substitution																
			near SSN motif									near KTG motif							
	Bla -	Bla +	Asp350	Ser357	Ala368	Met377	Ser385	Ala437	Ile449	Leu456	Gly490	Ala502	Arg517	Asn526	Ala530	Thr532	Val547	Ala554	Asn569
I	1																Val	Thr	Ser
	1												His				Val	Thr	Ser
		1						Ser					His						
II a	1		Asn								Glu			Lys	Ser				
	1										Glu			Lys	Ser		Val		Ser
		1									Glu			Lys	Ser				
	2	1												Lys					
	1		Asn			Ile								Lys			Val		Ser
		1	Asn											Lys			Val		
II b	16	1	Asn			Ile					Glu	Val		Lys			Val		Ser
	2	1	Asn			Ile						Val		Lys					
	1		Asn			Ile						Val		Lys			Val		
	3					Ile					Glu	Val		Lys			Val		Ser
	1					Ile						Val							
		1				Ile						Val		Lys			Val		Ser
	17	9	Asn			Ile						Val		Lys			Val		Ser
	3										Glu	Val		Lys			Val		Ser
	5	1										Val		Lys			Val		Ser
	4	1										Val		Lys					
	1											Val							
	4	2	Asn									Val		Lys			Val		Ser
	2		Asn			Ile		Ser				Val		Lys			Val		Ser
	1		Asn					Ser				Val		Lys			Val		Ser
	4		Asn			Ile				Val		Val		Lys			Val		Ser
	3	1	Asn			Ile				Cys		Val		Lys			Val		Ser
	1		Asn		Pro	Ile						Val		Lys			Val		Ser
II c	31		Asn									Thr		Lys			Val		Ser
	1		Asn									Thr		Lys			Val		
	4		Asn							Val		Thr		Lys			Val		Ser
	4											Thr		Lys			Val		Ser
	2	2										Thr		Lys			Val		
	4											Thr		Lys					
	1									Val		Thr		Lys					
	1				Thr							Thr		Lys					
II d	6	3							Val					Lys			Val		Ser
	1					Ile			Val					Lys			Val		Ser
	1								Val					Lys					
	1								Val	Val				Lys			Val		Ser
III-like	5		Asn	Ser		Ile	Thr						His	Lys		Ser	Val		
	1		Asn	Ser		Ile	Thr						His	Lys		Ser	Val		Ser
Misc	17	18																	

a The strains with mutations in the *ftsI* gene were classified into six groups: I and II (a, b, c and d) according to Dabernat *et al.* (8) and III-like according to García-Cobos *et al.* (9);
b Bla+, β-lactamase-producing strains; Bla-: β-lactamase-non-producing strains;

Table 1: Amino acid substitutions identified in the transpeptidase domain of the *ftsI* gene.

5. References

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