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Molecular epidemiology, antigenic profile and macrolide resistance of *Bordetella pertussis* clinical isolates circulating in Barcelona from 2007 to 2014

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Introduction

Whooping cough, a major acute respiratory infection resulting in severe childhood illness and infant death, is nowadays a public health problem in industrialized countries despite extensive vaccination campaigns and high immunization rates. In Spain, the number of cases has increased from 1998, reaching 7'2 and 7'45 cases/100.000 people reported in 2011 and 2012, respectively. The main causes postulated that may be involved in pertussis reemergence include the waning vaccine-induced immunity associated with acellular pertussis vaccines (ACV), currently administrated, and the adaptation of *Bordetella pertussis*, the causative agent, to the immunity induced by the vaccine. ACV, which were introduced in Spain in 1998, contain various combinations of different antigens: pertussis toxin (PT), pertactin (PRN), type 2 and 3 fimbriae (FIM2 and 3) and filamentous haemagglutinin (FHA). The objectives of the study are to determine the molecular epidemiology, the antigenic profile and the presence of the macrolide resistance genetic marker of *B. pertussis* producing whooping cough in patients from the Barcelona's metropolitan area since the introduction of ACV.

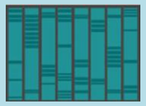
Conclusions

- From 2007 to 2014, two *B. pertussis* populations coexisted in the Barcelona's metropolitan area. The first population (clade I), was the most prevalent until 2010. Since then, it was progressively replaced by the second one (clade II).
- The encoded alleles of pertussis toxin and pertactin differed in both populations of the antigens contained in the currently used ACV (100% *ptxA1*, 96.12% *prn2).*
- The pertussis toxin gene promoter type 3 (*ptxP3*) was the most prevalent in both populations (96.33%).
- The type 3-2 fimbriae was the most prevalent until 2010. Then, it was gradually displaced by the type 3-1.
- Overall, the characterization of the antigenic profile of *B. pertussis* producing whooping cough in Barcelona, suggests that it may have adapted to the immunity induced by the ACV.
- Regarding the presence of the macrolide resistance genetic marker, we did not detect any isolate carrying the A2047G substitution associated to azithromycin and erythromycin resistance. Therefore, macrolides can still be considered as the first choice agents for treatment of whooping cough in our area.

Methods

109 *Bordetella pertussis* clinical isolates collected between 2007 and 2014 at Hospital Vall Hebron (Barcelona, Spain)

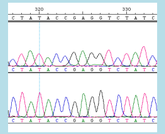
Molecular epidemiology ➔ PFGE



- Chromosomal DNA digestion with the restriction enzyme *Xba*I.
- Isolates with a DNA band pattern differing by ≥1 band were defined to be a distinct PFGE profile.
- PFGE profiles were grouped into different clades at a level of relatedness of 82%.

Advani A. *et al.* JCM. 2004.

Antigenic profile ➔ PCR and sequencing



- Subunit A pertussis toxin (*ptxA*)
- Pertussis toxin promoter (*ptxP*)
- Pertactin (*prn*)
- Type 3 fimbriae (*fim3*)

Macrolide resistance ➔ Allele specific PCR

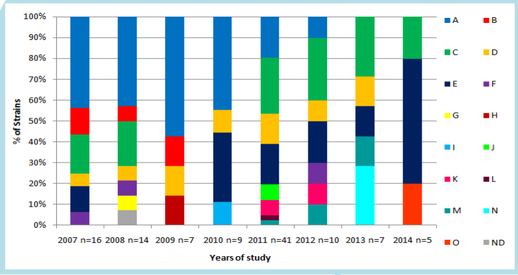
Based on the molecular mechanism previously identified, the isolates were tested by an allele specific PCR for rapid detection of the A2047G mutation in *23S rRNA* of *B. pertussis*, associated to macrolide resistance. The primers used were:

Primer	Sequence (5'-3')
FP	GTGATGGGGTGCAAGCTCTT
RP	TCTGGCGACTCGAGTTCTGC
MP	ATCTACCCGCGGCTAGACAGG

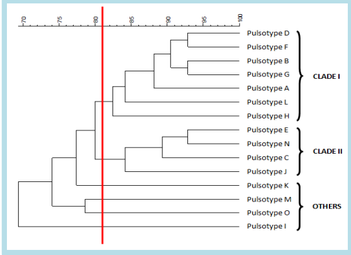
Wang Z. *et al.* JCM. 2015.

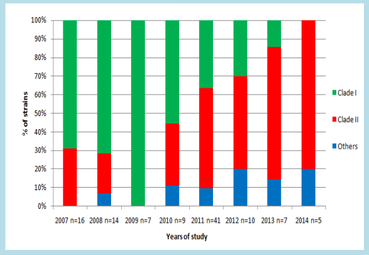
Results

Molecular epidemiology



- The 109 isolates studied were distributed in 15 pulsotypes.
- The pulsotypes more prevalent (66.05% of strains) were **A** (27.52%), **C** (21.1%) and **E** (17.43%).



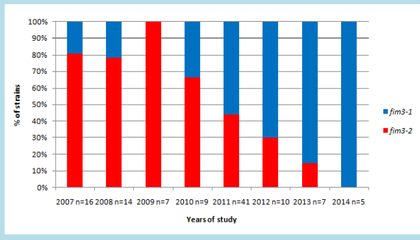


Clade I (47.71% of strains) ➔ 57.69% pulsotype **A**, 23.08% pulsotype **D**, 7.69% pulsotype **B**, 5.77% pulsotype **F**, 5.77% pulsotypes **H**, **G** and **L**

Clade II (43.12% of strains) ➔ 48.94% pulsotype **C**, 40.43% pulsotype **E**, 10.64% pulsotypes **J** and **N**

Antigenic profile

- Pertussis toxin: 100% ***ptxA1***
- Pertactina: 0.97% ***prn1***, 96.12% ***prn2***, 1.94% ***prn3*** and 0.97% ***prn9***
- Type 3 fimbriae: 45.87% ***fim3-1*** and 54.13% ***fim3-2***



ACV protein variants: *ptxA2/ptxA4, prn1/prn7* and *fim3-1*

Characterization of the pertussis toxin promoter

- Promoter of pertussis toxin: 96.33% ***ptxP3***

The promoter *ptxP3* has been associated with a major expression of the pertussis toxin. Whooping cough resurgence has coincided with the emergence of strains that carry the promoter *ptxP3*, which have replaced the previously dominant *ptxP1* promoter.

Clade I: associated to **virulent profile A** ➔ *ptxA1-ptxP3-prn2-**fim3-2*** (88.46%) ➔ most prevalent **from 2007 to 2010**

Clade II: associated to **virulent profile B** ➔ *ptxA1-ptxP3-prn2-**fim3-1*** (87.23%) ➔ most prevalent **from 2011 to 2014**

Macrolide resistance genetic marker

The 109 isolates studied were negative for the mutation A2047G in *23S rRNA*.