

Serotypes and genetic profiles of *Bordetella pertussis* strains isolated in the city of São Paulo, 2006-2008

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Abstract

Objective: Knowledge of *Bordetella pertussis* circulating in Latin America is limited. Therefore, the goal of this study was to use pulsed-field gel electrophoresis and serotyping to characterize *B. pertussis* strains isolated in the city of São Paulo, Brazil.

Methods: This study, conducted between 2006 and 2008, analyzed 652 nasopharyngeal swabs from suspected pertussis cases and contacts, collected from 37 sentinel hospitals in São Paulo. Randomized samples of 91 (70%) strains of *B. pertussis* were subtyped by pulsed-field gel electrophoresis and serotyping.

Results: Ninety-seven percent of strains from São Paulo were serotyped as Fim3. Fourteen pulsed-field gel electrophoresis profiles were identified; the most prevalent (57%) is also the most prevalent in the USA.

Conclusions: These data, in conjunction with surveillance activities, may impact strategies regarding prevention and control of pertussis in the region, providing useful information for introduction of new vaccination strategies and reduction of risk of transmission to infants less than 6 months of age.

J Pediatr (Rio J). 2012;88(4):357-60: *Bordetella pertussis*, pertussis, Latin America, whooping cough, pulsed-field gel electrophoresis, serotyping.

Introduction

Whooping cough, or pertussis, is an infectious respiratory disease caused by the bacterium *Bordetella pertussis*. *B. parapertussis*, *B. bronchiseptica* and *B. holmesii* also may be found in the human respiratory tract, causing a pertussis-like disease with milder symptoms. Despite mass vaccination programs and good vaccine coverage in many countries, *B. pertussis* continues to circulate worldwide with

48.5 million cases annually, 295,000 deaths and epidemics every 3-5 years.¹

High levels of efficiency have been obtained from both whole-cell (wP) or acellular (aP) pertussis vaccines. The duration of protection following the basic vaccination schedule with a booster dose of wP vaccine is estimated to be 6-12 years, the same period occurring after natural

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infection. Some studies show that the duration of protection with the use of aP vaccine is situated within the same time range.² In Brazil, a vaccination program was established in 1968 with diphtheria, wP and tetanus (DPT), and currently the Ministry of Health recommends three doses of DTP + Hib (tetraivalent vaccine) at ages 2, 4 and 6 months and two boosters of DTP, the first at 15 months and the second between ages 4 and 6 years.³

From 1980 to 1983 more than 40,000 cases of pertussis (incidence rate > 30/100,000) were reported in Brazil, but cases decreased substantially after 1983. In 1990, 15,329 cases (incidence rate 10.64/100,000) were reported, the highest rate observed in the decade. In 1995, 3,798 cases (incidence rate 2.44/100,000) were reported and, thereafter, the annual number of cases did not exceed 2,000 (incidence rate 1/100,000). In 2008, there were 1,344 cases (incidence rate 0.71/100,000).⁴

Pertussis remains endemic worldwide and its reemergence has been reported in many countries. Several explanations have been suggested for this resurgence including low vaccine efficacy, waning immunity without natural and vaccine boosters, improved clinical and laboratory recognition of pertussis, and changes in the circulating *B. pertussis* population.^{1,5}

Different methodologies have been described for studying the molecular epidemiology of *B. pertussis* including serotyping, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and multilocus variable number tandem repeat analysis (MLVA).⁶ Among the available methodologies that are capable of tracking genetic differences among isolates, PFGE has been chosen as a reference method for epidemiological surveys of pertussis as it has the greatest discriminatory power.^{7,8}

Serotyping is one of the oldest methods used for typing *B. pertussis* and based on reactions with specific antisera, *B. pertussis* can be divided into three fimbriae types: Fim2, Fim3 and Fim2,3.⁹ Although serotype identification is valuable for primary characterization of a strain, molecular methods with higher reproducibility and discriminatory power such as PFGE may be more useful in epidemiological investigations because PFGE provides a highly reproducible restriction profile of large bacterial DNA fragments.⁷

Information about circulating isolates of *B. pertussis* in Latin America is limited. Therefore, the goal of this study was to use PFGE and serotyping to characterize *B. pertussis* strains isolated in the city of São Paulo, Brazil.

Materials and methods

Between 2006 and 2008, 652 nasopharyngeal swabs from suspected pertussis cases and contacts, collected from 37 sentinel hospitals in the city of São Paulo, were cultured

at the Laboratory of Pertussis, Adolfo Lutz Institute, São Paulo, Brazil. Specimens were collected with sterile alginate swabs and transported in Regan-Lowe (RL, Oxoid) charcoal semi-solid agar, supplemented with 10% sheep blood and 40 µg/mL cephalaxin. The specimens were cultured on the collection day on RL agar with 10% sheep blood and 40 µg/mL cephalaxin, and incubated at 35-37 °C under ambient air with high humidity up to 10 days. Colonies suggestive of the genus *Bordetella* were confirmed by Gram staining and species were identified by biochemical tests. Detection of the specific *B. pertussis* O1 antigen was done by the slide agglutination test and O1 antiserum. During the study period, 132 *B. pertussis* strains were isolated and stored lyophilized. For the present study, randomized samples of 91 (70%) strains of *B. pertussis* were selected, according to age (Figure 1) and sentinel hospitals distribution.

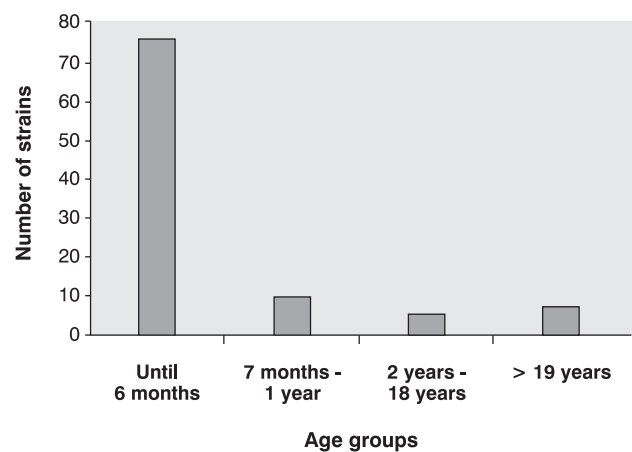


Figure 1 - Distribution of *Bordetella pertussis* strains isolated in the city of São Paulo, Brazil, by age groups during the period 2006-2008

Serotypes were determined by the microagglutination test¹⁰ with minor modifications. The test was performed using heat-inactivated (1 hour at 56 °C) and formalin-treated bacterial suspensions. Whole-cell enzyme-linked immunosorbent assay (ELISA) was performed as described previously¹⁰ using heat-inactivated suspensions. In both tests, *B. pertussis* control strains B222, B201 and D120, expressing, respectively, Fim2, Fim3 and Fim2,3 were added as positive controls. The anti-*B. pertussis* fimbrial 2 (04/154) and fimbrial 3 (04/156) monoclonal antibodies were obtained from the National Institute for Biological Standards and Control, UK.

We followed the PFGE method described by Hardwick et al.,¹¹ using *Xba*I as a restriction enzyme. The PFGE patterns were analyzed using the BioNumerics software package (ver. 4.0; Applied Maths, Inc., Austin, TX, USA).

This study was approved by the Research Ethics Committee of the Adolfo Lutz Institute (protocol number 065/2009).

Results and discussion

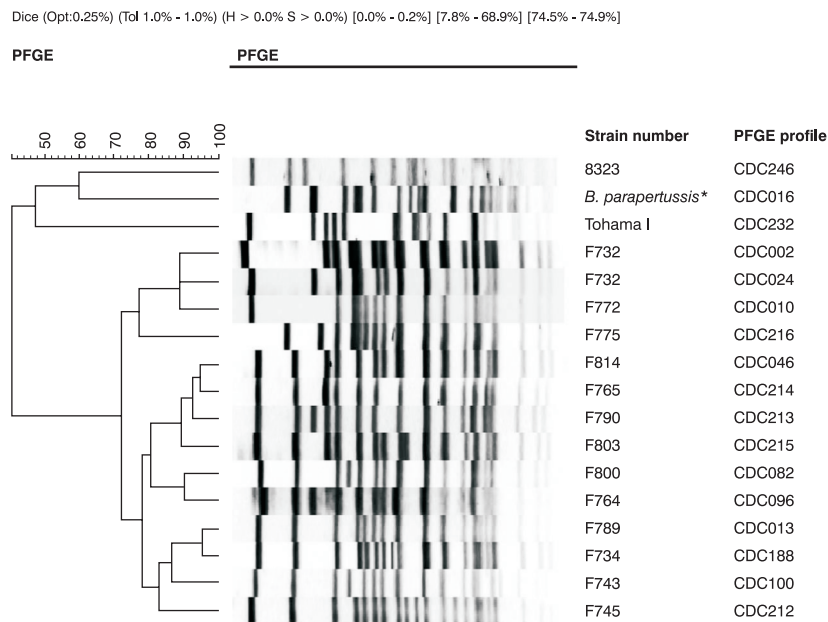
In the present study, most *B. pertussis* strains (97%, 88/91) were serotype Fim3 by both microagglutination test and ELISA. Two strains gave discordant results: one strain was Fim2,3 by microagglutination, but Fim3 by ELISA, and the other strain was Fim3 by microagglutination, but Fim2,3 by ELISA. One *B. pertussis* isolate was untypeable by both techniques.

PFGE was highly discriminatory, identifying 14 PFGE profiles, according to the nomenclature of the Centers for Disease Control and Prevention (CDC) (Figure 2). Profiles PFGE013 (57%, 52/91) and PFGE082 (15%, 14/91) were the most common types. Twelve other profiles were identified (1-6 strains each), and accounted for 28% (25/91) of the isolates.

As to distribution of cases by age group, the most prevalent was the group of children 1 year old or less, corresponding to 91.2% (83/91) of the strains studied. Only 8.8 % of the strains were isolated from adolescents and adults (Figure 1).

Monitoring the expression of fimbriae Fim2 and Fim3 by serotyping is important for strain differentiation and detection of changes in *B. pertussis* population. In this study, a predominance of serotype Fim3 strains was observed. Previously, in Brazil¹² 86% of 72 strains, collected between 1988-2002, were serotype Fim3, which suggests that this serotype has prevailed in the country for some decades. Similar results were also reported in other countries.¹³⁻¹⁵

PFGE has been used as a tool for epidemiological surveillance studies of genetic variation in *B. pertussis* strains over time and to identify outbreak-associated isolates.^{9,11} PFGE allows identification of strains which are epidemiologically linked and indistinguishable by other typing methods. PFGE has been used worldwide, but direct comparison of profiles from different laboratories is difficult because of variation in techniques and nomenclature. Like Gonçalves et al.,¹² we observed 14 related PFGE profiles with little genetic difference between them, demonstrating the relatively homogeneous or clonal structure of *B. pertussis*. Among the two most frequent profiles, PFGE 013 was the most prevalent and most frequent in Brazil between 1988 and 2002.¹² Moreover, it is the most prevalent profile currently circulating in the USA (data not shown). The fact that the same predominant PFGE profile is circulating in Brazil and the USA is interesting, considering that these countries have used different pertussis vaccination strategies over the past 19 years.



PFGE = Pulsed-field gel electrophoresis.
 * *B. parapertussis* isolated in Brazil during the study period.

Figure 2 - Relatedness of pulsed-field gel electrophoresis profiles of *Bordetella pertussis* strains isolated in the city of São Paulo, Brazil, 2006-2008

No relationship between age, PFGE profile and serotype was observed, although our data were limited by the small number of isolates from age groups older than 6 months (Figure 1).

A wide range of pertussis vaccines (aP or wP) has been produced, including aP containing one or more purified antigens pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM) type 2 and type 3 used separately or in different combinations. The efficacies of aP and wP vary depending upon the pertussis case definition used. Monitoring the serotypes and genetic profiles of strains collected over time and from different regions is important for a better understanding of the currently circulating *B. pertussis* population worldwide. These data give an overview of pertussis in eastern South America and in conjunction with surveillance activities may impact strategies regarding prevention and control of pertussis in the region, providing useful information to introduce new vaccination strategies aiming at the reduction of the risk of transmission to infants less than 6 months of age.

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