



Prevalence and characterization of pertactin deficient *Bordetella pertussis* strains in Brazil, a whole-cell vaccine country

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ABSTRACT

Many countries have reported antigenic divergence among circulating *Bordetella pertussis* strains, mainly in those countries which introduced the acellular pertussis (aP) vaccine. This phenomenon can be seen, for example, with the recent rise of pertactin (Prn)-deficient *B. pertussis* strains, one of the antigens included in aP vaccine formulas. The whole cell pertussis (wP) vaccine has been used in Brazil since 1977 for the primary pertussis, diphtheria and tetanus immunization series. In 2014, the aP vaccine was recommended for women during pregnancy to protect infants in the first months of life. Our objective was to determine the prevalence of Prn-deficiency in 511 isolates of *B. pertussis* collected in Brazil during 2010–2016. All isolates were characterized, through PFGE and serotyping, and screened for the loss of Prn by ELISA. Prn-deficiency was confirmed by immunoblotting, and identification of the possible genetic markers was performed with PCR and Sanger sequencing. Results indicate that 110 PFGE profiles are currently circulating, with five profiles representing the majority, and the predominant serotype 3, has been gradually replaced by serotype 2 and serotype 2,3. ELISA screening and immunoblotting identified three Prn-deficient isolates. Genotypic characterization by PCR and sequencing indicated that one isolate had a promoter mutation in *prn*, while the other two did not have an obvious genetic explanation for their deficiency. While the lack of Prn was identified in a few isolates, this study did not detect a relevant occurrence of Prn-deficiency, until 2016, confirming previous observations that Prn-deficiency is likely aP vaccine-driven.

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1. Introduction

Bordetella pertussis causes whooping cough or pertussis, a highly contagious respiratory disease that is more severe in young unimmunized infants that are at highest risk of severe complications and death [1]. Despite a significant reduction in global burden of disease, with the introduction of the pertussis vaccines in several countries, the disease remains endemic worldwide, with outbreaks and epidemics peaks occurring usually every 3–5 years [1,2]. It is an important global public health problem with more than 151,000 cases of pertussis globally, estimated in 2018 by the World Health Organization -WHO [3].

In Brazil, since the beginning of the 19th century, vaccines have been used as a measure of disease control. In 1977, Brazil published the first national vaccination schedule, standardizing the childhood vaccination series with Bacillus Calmette-Guerin BCG vaccine, monovalent measles vaccine, oral polio vaccine (VOP) and DTwP (tetanus toxoid, diphtheria toxoid, and whole cell pertussis) [4].

The Brazilian National Immunization Program (NIP) in 2002, added the component *Haemophilus influenzae* type B to the DTwP vaccine (tetraivalent vaccine, DTwP-Hib) and in 2012, this vaccine was replaced by the pentavalent vaccine, DTwP + *H. influenzae* type B + hepatitis B (HBV). Currently, the pentavalent vaccine is administered with three primary doses to children at ages 2, 4 and 6 months followed by two booster doses with DTwP at 15 months and 4–6 years, in the public sector. Both wP vaccines confer efficacy between 75 and 85%. The DTaP (diphtheria-tetanus-acellular

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pertussis) are available only in private vaccination clinics to children who have developed serious side effects after the use of wP vaccines [5–7].

The wP vaccine has been used successfully in Brazil since 1977 and as the vaccine coverage increased to values close to 95–100% for DTP3 vaccine in the first year of life, the incidence rates were reduced (Fig. 1A). Data from the Brazilian Ministry of Health – MoH, showed cyclic epidemic peaks of pertussis over the time (1997–1998, 2004–2005 and 2007–2008) [5], 2014 being considered an epidemic year during the period from 2011 to 2015 (Fig. 1B) [6,8].

Maternal vaccination with aP vaccines was introduced in November 2014 as an additional protective measure for infants less than six months of age, a group at higher risk of hospitalization, health complications, and death. This vaccine, Tdap, is formulated with reduced quantities of tetanus toxoid, diphtheria toxoid, and pertussis antigens (inactivated pertussis toxin (Pt), formaldehyde-treated filamentous hemagglutinin (Fha) and pertactin (Prn)) (BOOSTRIX, GlaxoSmithKline) [9].

Although maternal vaccination was introduced as a protective measure for infants, coverage rates for maternal immunization was low (9% in 2014, 40.4% in 2015, and 34% in 2016) and varied from region to region, never exceeding 51% (Fig. 2) [10].

Despite pertussis vaccines (wP or aP) being included in all immunization programs; whooping cough has re-emerged and remains one of the less well-controlled vaccine-preventable diseases in the world [11,12]. The reasons for this situation are multiple, and relate to (i) varied vaccine efficacy, compliance and coverage for different wP formulations; (ii) rapid waning of aP vaccine-induced immunity; (iii) antigenic mismatch between vaccine and circulating strains; (iv) inability of current vaccines to impact nasopharyngeal carriage of this pathogen; (v) lack of protection against *B. pertussis* transmission; and (vi) increased clinical awareness and/or better diagnostic tests that could have increased the recognized cases [12,13].

Genetic changes in *B. pertussis* strains could also be playing a role in the resurgence of disease by allowing the organism to evade vaccine-induced immunity or be more virulent. An example of this is the recent rise of Prn-deficient *B. pertussis* strains in countries that use aP vaccines, for their primary vaccination series [14]. Prn, one of the 3–5 antigens included in aP vaccine formulas, is an autotransporter protein involved in bacterial adhesion to the respiratory tract and resistance to neutrophil-induced bacterial clearance [14,15]. A variety of mutations causing Prn deficiency (IS481 insertions at multiple locations, premature stop codons and deletions) have been identified, suggesting strong selective pressures exist to remove Prn [15].

In countries where wP vaccines in the primary series of vaccinations are still in use, detection of Prn-deficient *B. pertussis* strains has been rare [14,16] or completely absent [17–19]. Therefore, the objectives of this study were to (i) determine, between 2010 and 2016, the prevalence of Prn deficiency in *B. pertussis* strains in Brazil, a wP vaccine country, and (ii) characterize, by serotyping and pulsed-field gel electrophoresis (PFGE), the *B. pertussis* circulating strains in this same period.

2. Materials and methods

2.1. *B. pertussis* isolates collection

Since 1973, pertussis is a notifiable disease in Brazil, and all cases of pertussis should be reported to the National Notifiable Diseases Information System (SINAN) of MoH. The confirmation of suspected cases of pertussis is based in clinical, epidemiologic and laboratory criteria. Confirmation of laboratory criteria is based

on the isolation of *B. pertussis* using a culture of nasopharyngeal secretion or positive real time polymerase chain reaction (PCR).

Isolates of *B. pertussis* are sent routinely to the Centre of Bacteriology at Instituto Adolfo Lutz (IAL), the Brazilian National Reference Laboratory for Pertussis. These isolates are collected from Central Public Health Laboratories (LACENs), located in each of the Brazilian states, and are forwarded to IAL for storage in the National Biobank.

Between 2010 and 2016, IAL kept in its collection, 3,557 strains of *B. pertussis* either freeze-dried [20] or frozen in TSB (trypticase soy broth) with 15% glycerol [21].

Isolates were selected in a time range when countries were reporting the emergence and predominance of Prn deficiency to optimize the chance of observing Prn deficiency in Brazil.

Sample size (N) was calculated using the formula; $N = Z^2 P(1-P)/D^2$, where is P an expected proportion of isolates deficient in Prn ($P = 1.23\%$), D is the precision/margin of error (1%) and Z is the value from the normal distribution for a 95% desired confidence interval ($Z = 1.96$) [22], which gave us a sample size of 413 out of a pool of 3,557 isolates from the five geographic regions of Brazil. A total of 511 isolates were randomly selected from 19 Brazilian states based on the proportional distributions by geographical area and calendar year (Table 1).

All 511 *B. pertussis* strains were cultured on charcoal agar plates (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with sterile, defibrinated horse blood (10%) under humid conditions at 35–37 °C for up to five days.

2.2. Determination of circulating profiles of *B. pertussis*

2.2.1. Serotyping

The 511 strains were tested for the species-specific agglutinogen (factor 1) and two other fimbrial agglutinogens (factors 2 and 3). The specific O1 antiserum and anti-agglutinogen 2 and anti-agglutinogen 3 sera were prepared at IAL, using reference strains (GL353, 360E, H36) kindly supplied by the Pertussis Reference Laboratory, University of Manchester, and following the methods previously described [23–25].

2.2.2. Pulsed field gel electrophoresis

PFGE analysis was performed as described by Advani et al. [26], using *Xba*I as a restriction enzyme, with the following modifications. Electrophoresis was performed at 6 V/cm for 24 h at 14 °C and pulse times of 5–6 s for 11 h and 8–35 s for 13 h. The band profile analysis was performed using the program BioNumerics version 7.1 (Applied Maths, Keijkstraat, Belgium) for the construction of the dendrogram of genetic similarity by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on the band pattern of the *Salmonella* Braenderup DNA (H9812) as a normalization standard. The PFGE profiles were determined following the nomenclature standardized by IAL [27], starting at BpXba0001, BpXba0002, etc.

2.3. Detection of Prn protein production

2.3.1. Anti-Prn ELISA

All 511 isolates were initially screened by a qualitative anti-Prn ELISA, performed according to the methodology described by Martin et al. [28], to identify Prn production. Bacterial density was standardized for plate coating, based on the optical density (OD) of 1.6–1.8 readings by spectrophotometer at 620 nm in all isolate preparations. Any isolates that showed potential loss or absence of protein were further tested by immunoblotting for confirmation of deficiency. Prn-producing reference strains, H921 and H936, and Prn-deficient strains, H920 and H939, were kindly provided by the Pertussis and Diphtheria Laboratory of the Centers for Disease

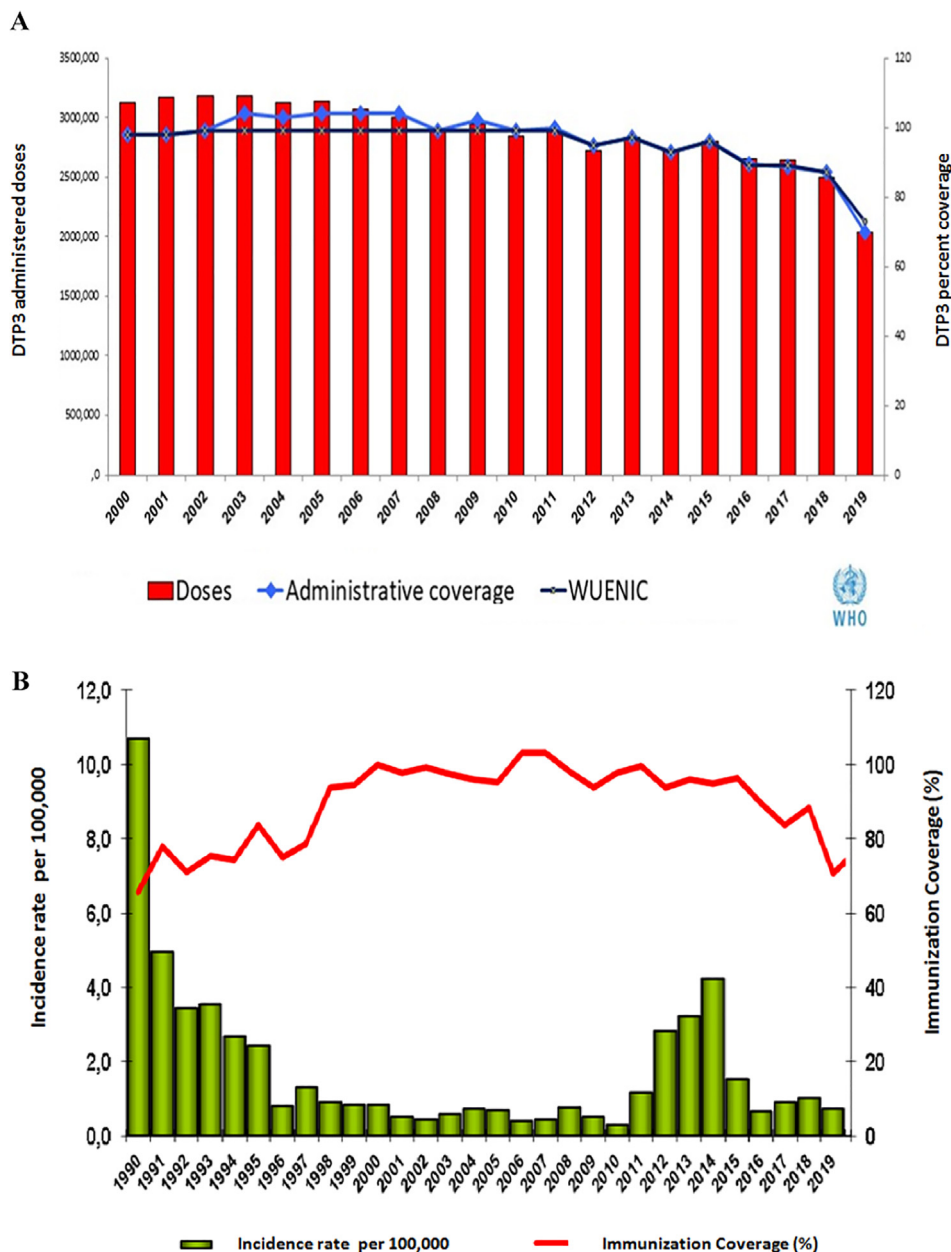


Fig. 1. Pertussis incidence rates and vaccine coverage, in Brazil, over years. (A) DTP3 Coverage Brazil, 2000–2019. (B) Incidence rates and DTWP/DTwP-Hib/DTwP-HBV-Hib immunization coverage, Brazil, 1990–2019.

Control and Prevention (CDC), Atlanta, GA, USA. Isolates that showed OD values in the range 0.6–2.5 were considered positive for Prn production while the isolates that showed OD values between 0.2 and 0.5 were considered Prn-deficient. Reaction controls (PBS only) had to show $OD \leq 0.06$ for a valid plate.

2.3.2. Conventional PCR

Following ELISA screening, Prn-deficient isolates were tested by PCR as described by Pawloski et al. [29] to detect the IS481 insertion within *prn* in one of three potential sites, in the 5' (nt 240 from the *prn* transcriptional start site), middle (nt 1613), and 3' (nt 2735) regions, or the mutation in the promoter region of this gene (Table 2). PCRs were run, as follows, in a 25 μ L reaction: 12.5 μ L HotStar Taq[®] Master Mix Kit (Qiagen, CA, USA), 0.5 μ L forward/reverse primers, 2 μ L dimethyl sulfoxide (DMSO), and 7 μ L of PCR grade water (Roche Diagnostics, IN, USA) were added with 2.5 μ L

of bacterial DNA. Cycling conditions were 95 °C for 15 min, 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 2.5 min. Isolates that did not have the IS481 insertion sequence produced the expected size amplicon of 1.4 kb and 2.2 kb for the 3' and 5' regions, respectively, and 450 bp for the promoter region. The expected amplicons for mutants were 2.4 kb and 3.2 kb for the 3' and 5' regions, respectively, and 0.7 kb for the promoter region.

2.3.3. Immunoblotting

Isolates that were identified as Prn-deficient by ELISA were also tested by immunoblotting as another confirmatory test of the results. The methodology was performed according to Green & Sambrook [32] with some modifications as follows.

Sample preparation. Confluent cultures were gently harvested from agar surface and resuspended in 0.01 M PBS, pH 7.2 in order to obtain 0.4 OD₅₄₀. Following centrifugation, pellets were resus-

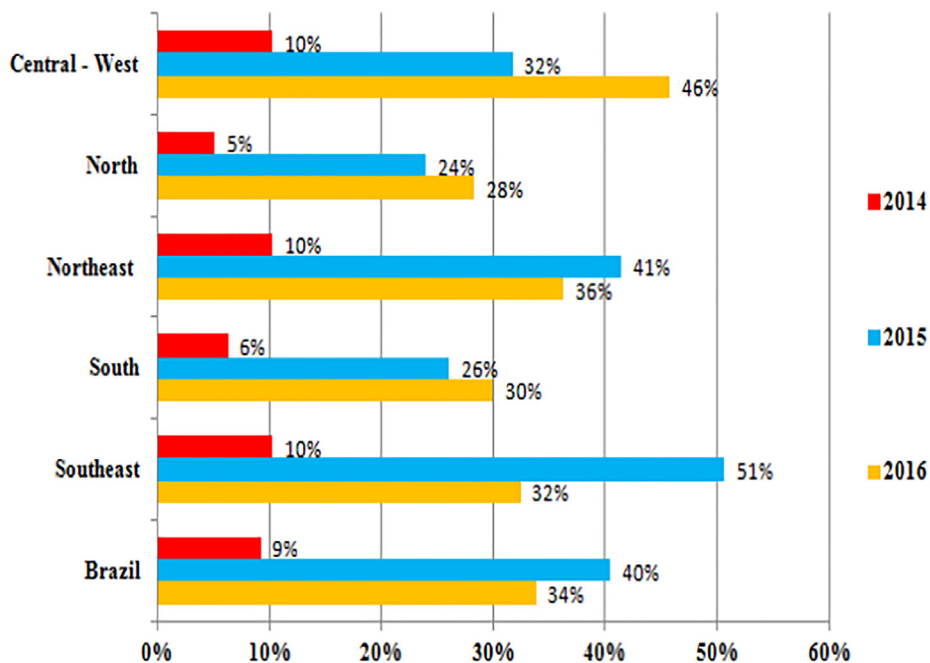


Fig. 2. Tdap vaccination coverage, Brazil and regions, 2014–2016.

Table 1
Bordetella pertussis isolates by region, state and year of isolation, Brazil, 2010–2016.

Region	State	2010	2011	2012	2013	2014	2015	2016	Total N°
South (n = 116)	Rio Grande do Sul	5	2	4	6	3	0	1	21
	Paraná	2	1	0	20	6	5	0	34
	Santa Catarina	3	4	4	21	13	4	12	61
Southeast (n = 211)	São Paulo	7	16	16	44	25	18	39	165
	Rio de Janeiro	0	0	0	0	1	1	1	3
	Espírito Santo	0	0	0	1	0	0	0	1
	Minas Gerais	1	1	1	21	4	4	10	42
Northeast (n = 61)	Bahia	4	0	2	5	5	0	0	16
	Alagoas	3	3	0	3	0	0	0	9
	Pernambuco	0	1	4	8	10	4	2	29
	Paraíba	0	0	0	0	3	3	0	6
	Ceará	0	0	0	0	0	1	0	1
North (n = 19)	Tocantins	0	0	0	0	0	1	0	1
	Amapá	0	0	1	4	1	0	0	6
	Acre	0	0	0	0	4	0	0	4
	Rondonia	0	0	0	0	6	2	0	8
Midwest (n = 104)	Mato Grosso do Sul	2	3	0	10	9	5	0	29
	Goiás	0	0	0	2	0	3	0	5
	Distrito Federal	3	0	15	23	23	4	2	70
	TOTAL N°	30	31	47	168	113	55	67	511

pended in 50 µL PBS and protein concentration estimated at A280 reading with NanoDrop® spectrophotometer (Thermo Fisher Scientific Inc., NY, USA). Fifty micrograms of protein were mixed with reducing sample buffer (v/v) and boiled for 10 min at 100 °C. Lysates of Prn-producing and deficient reference strains of *B. pertussis* were prepared under the same protocol and added in each gel.

Electrophoresis and immunoblotting. Samples were resolved in 12% SDS-PAGE and transferred to nitrocellulose membrane using a semi-dry blotting system. Protein transfer efficiency was assessed by staining the membrane with Ponceau S. The membrane was blocked with TBS (1.6 mM Tris-HCl, 153.9 mM NaCl, pH 7.5) containing 5% skim milk (w/v) for 2 h at room temperature. After washing 6x with TBS pH 7.5 with 0.05% Tween 20 (TBST), sheep serum anti-69kD *B. pertussis* protein (1:1000 in TBST plus 1% skim milk) (NIBSC 97/558, UK) was added and the membrane was incubated overnight at 4 °C, then probed with rabbit

anti-sheep IgG-horseradish peroxidase conjugate (1:2000 in TBST 1% skim milk) (KPL 14-23-06, MA, USA) for 1 h at room temperature protected from light. Bands were developed with 16 mM 4-chloro-1-naphthol in 0.2% methanol mixed with hydrogen peroxide diluted in TBS pH 7.5 at final concentration of 0.025%. Prn characterization by Western blot was performed twice for each sample. Two positive reference strains (H921 and H936) and two negative reference strains (H920 and H939) were included in each assay, as well as a lane of molecular weight of bands (190–11 kDa). Strains that presented bands of molecular weight equal to 69 kDa were considered positive for Prn production.

2.3.4. Sequencing analysis

Sequencing analysis was performed to check for possible genetic markers of loss for Prn in those isolates that were screened as deficient by ELISA. The isolates were sequenced using primers that covered the entire region (Table 2) and the 3010 XL analyzer

Table 2
Primers used for *prn* PCR and sequence analysis.

Primer	Sequence 5'-3'	Position on Tohama I relative to <i>prn</i> start codon*	Reference
BF	AGC TGG GCG GTT CAA GGT	1,398 to 1,415	[30]
BR	CGG ATT CAG GCG CAA CTC	1,915 to 1,932	[30]
AF	GCC AAT GTC ACG GTC CAA	505 to 522	[30]
AR	GCA AGG TGA TCG ACA GGG	1,073 to 1,090	[30]
PRN-F	CGT ACT TTT GCT GCG CCC AT	-79 to -60	[31]
PRN-R	CCA AGC TCC AGG AAA ACC TC	2,761 to 2,780	[31]
PRN-P-F	TGC CAA GAC GGT ATC TGT	-331 to -314	[29]
PRN-P-R	GAC TGG TTG TTC CAG TCG	102 to 119	[29]
1627R	TAT CGA CCT GCG CGT CCT T	1,627 to 1,645	[29]

prn = pertactin gene.

* *prn* lies in the region between nt 1,098,091 and 1,100,823 of the Tohama I complete genome (GenBank accession no. NC_002929.2). Primer sequence positions are in reference to the *prn* start codon, and may thus differ from previously published nucleotide positions.

(Applied Biosystems - Foster City, CA, EUA) - with a previously described method [33] and analyzed using DNASTar - SeqmanII and Bio Edit (Biological Sequence Alignment Editor Platform). The alignment with *prn* alleles was performed based on sequence identity of 18 *prn* alleles deposited in GenBank.

The study followed the recommendations provided by Resolution no. 466 of 2012 - National Health Council for Clinical Research in Humans; and it was approved by the Ethics Committee and being registered at the Plataforma Brazil under ID number CAAE: 42590415.8.0000.0059.

3. Results

3.1. *B. pertussis* strain characterization

All 511 *B. pertussis* isolates tested positive for the species-specific O1 antigen, confirming the identification of the species. One hundred and seventy-five isolates were characterized as serotype 2,3; 170 strains as serotype 3; 161 as serotype 2; and five strains were non-typeable. The dominance of each serotype varied over time (Fig. 3).

In the period between 2010 and 2012, there was a predominance of the serotype 3, which was gradually replaced by the serotype 2 predominating in 2014. In the years 2015 and 2016, there was a replacement of the predominant serotype, with the serotype 2,3 prevailing in approximately 75% of the strains compared to the other serotypes.

A total of 110 PFGE distinct profiles were identified, with types BpXba0008, BpXba0039, BpXba0040, BpXba0088 and BpXba0098 being the five prevailing profiles in our study population representing 59% of all tested isolates (Fig. 4).

The assessment of the most predominant profiles performed year by year shows differences in order of frequency and predominance (Fig. 5). None of the five types circulated in all study years, although BpXba0039 has been prevalent in the country since 2012, accounting for 27% of 511 isolates studied in the period. BpXba0008 had greater circulation in the early years of the study, being replaced by BpXba0039 and BpXba0098 in the final period. BpXba0040 and BpXba0088 remained constant for all evaluated years except 2011 and 2012, respectively.

3.2. Pertactin deficiency

All strains were initially screened for Prn production by a qualitative ELISA, and any that showed potential loss or absence of protein were followed up with the more sensitive and specific

immunoblotting technique for confirmation. A total of 503/511 strains were found to produce Prn by ELISA. Three of the eight isolates were confirmed by immunoblotting (Fig. 6) to be Prn-deficient and one presented an inconclusive result (very weak band profile) after repeated testing. Among the three Prn-deficient isolates, PCR identified the genetic mutation in only one isolate, a mutation in the promoter region of *prn*. The other two strains did not have an obvious genetic explanation in the *prn* gene for their Prn deficiency revealed by PCR and *prn* sequencing. The eight strains carried the *prn2* allele, the most prevalent type in recent isolates (Table 3).

4. Discussion

Over the past decade, the world has experienced a resurgence of pertussis, despite high vaccine coverage in many countries. In fact, the disease has not yet been globally controlled, and the vaccination has only a minimal herd immunity effect, insufficient to interrupt the circulation of *B. pertussis* in the population, increase the inter-epidemic time intervals or abolish the regular cycles altogether [2]. Fine & Clarkson [34,35] and Broutin et al. [36] pointed that pertussis periodicity may be associated with the accumulation of susceptible individuals, namely, susceptible births and waning natural immunity or waning vaccine-acquired immunity, providing favorable conditions for epidemics to occur.

Besides the disease cyclic behavior, the vaccine coverage and type of vaccine used in Brazil probably also contributed to replacement of predominant *B. pertussis* immunogenic profiles, since there were multiple manufacturers for the different vaccines used over the years.

Preston [37] has previously observed the relationship between the fimbrial serotype present in wP vaccines and the predominant fimbrial serotype isolated from pertussis patients. The serotype of the strains included in the wP vaccines has shifted the serotypes of circulating strains throughout the years, suggesting serotyping could play an important role in studying shifts in *B. pertussis* in relation to population immunity [37,38].

Since the 1990s, PFGE has been used for the typing of *B. pertussis*, because of its highly discriminatory power, that can observe genomic variation among strains circulating in aP or wP immunized populations. With this tool it is possible to identify isolates that are epidemiologically related or outbreak-associated isolates [26].

In this study, 110 profiles for PFGE were identified, with BpXba0039 and four others profiles making up most of the isolates tested from 2010 to 2016. Rocha, et al. [39] presented similar findings, with 14 circulating profiles in the central region of Brazil and an increase in the prevalence of BpXba0039 between 2012 and 2014. Other studies like Martins, et al. [40] in the central region of the State of São Paulo and Cassidy, et al. [41] in the US, also showed that a small number of PFGE profiles generally make up most of the circulating *B. pertussis* strains in defined time periods.

PFGE has been used worldwide, and although global comparison of profiles from different laboratories is difficult due to variation in techniques and nomenclature, this technique allows us to observe *B. pertussis* strains variation over time, the most prevalent profile in each country and their change between intervals of time.

The allelic *prn* profile of all strains analyzed was *prn2*. This profile is the most prevalent type circulating in the world and their increased prevalence has been associated with pertussis resurgence in many countries [42,43]. Regarding the detection of Prn-deficient strains in Brazil during the time Prn deficiency was predominating in aP vaccine countries, our study revealed three of 511 strains as possibly deficient in their production. Our results are consistent with studies presented in countries where wP vacci-

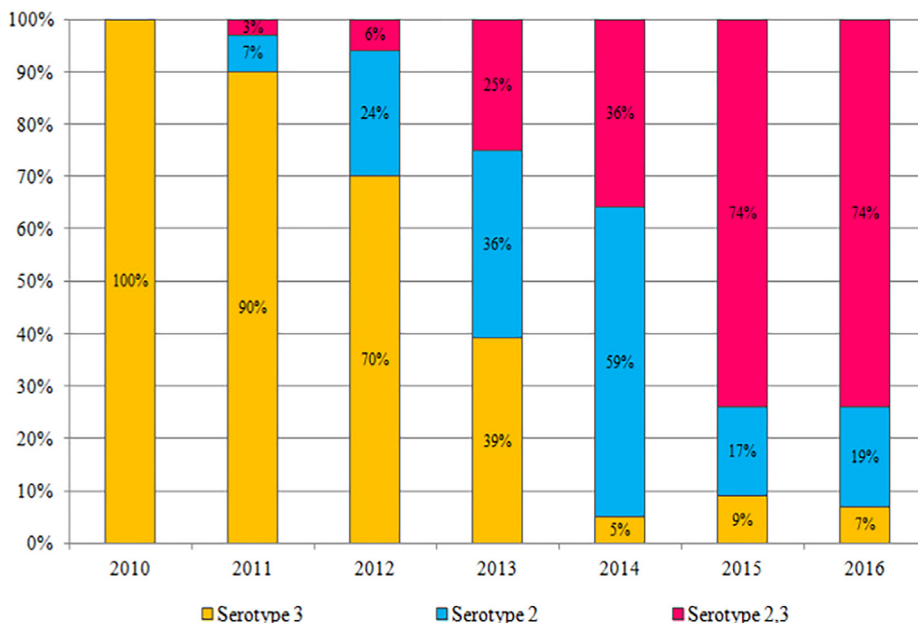


Fig. 3. Circulating serotypes among 511 strains of *B. pertussis* studied in Brazil, 2010 to 2016.

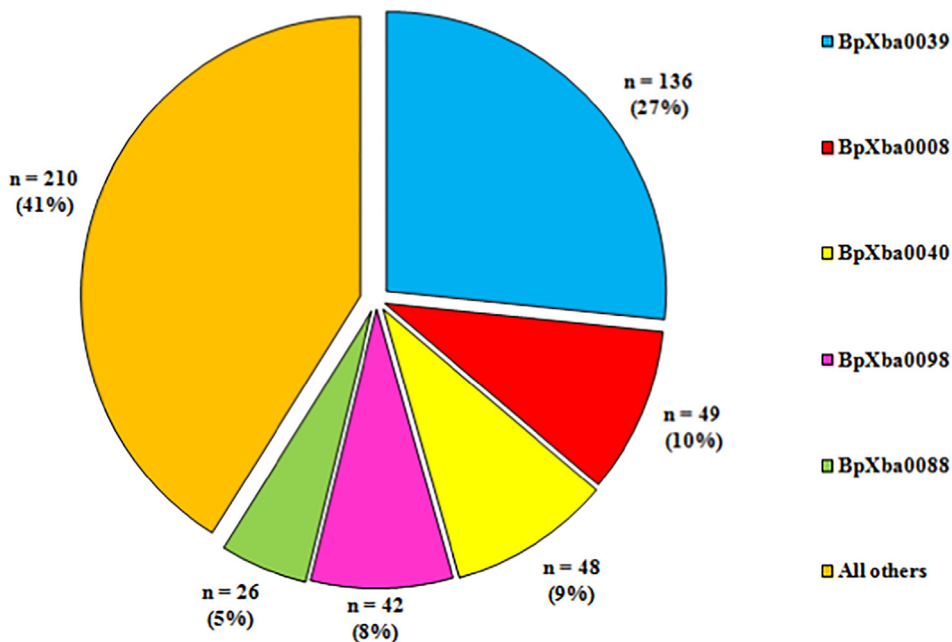


Fig. 4. Prevalent pulsed-field gel electrophoresis (PFGE) types of 511 isolates of *B. pertussis*, Brazil, 2010–2016.

nes are still in use. Countries like Tunisia, Russia and Iran [17–19] have not detected any Prn-deficient strains, and Argentina [16] and Poland [14], where wP vaccines have been in place for many years, few Prn-deficient strains have been detected.

A possible limitation of this study is regarding the difference in the numbers of strains analyzed between the country states. Brazil is a country of varying continental dimensions and for this reason it differs in population, environmental, socio-political and economic issues, which can directly impact the timely diagnosis and isolation of *B. pertussis*. Several states have (i) a limited surveillance system, (ii) a limited laboratory infrastructure for

carrying out culture, and (iii) an insufficient number of health professionals trained in the clinical identification of pertussis that could account for the lack of representative strains in some states.

Another limitation was the inability to find the genetic explanation for the loss of Prn in two of three Prn-deficient isolates. Because sequencing was restricted to the *prn* gene in this study, we cannot rule out the possibility of mutations elsewhere in the genome or epigenetic changes that affect the transcription or translation of Prn. Whole genome sequencing or transcriptional analysis would be needed to complement the characterization.

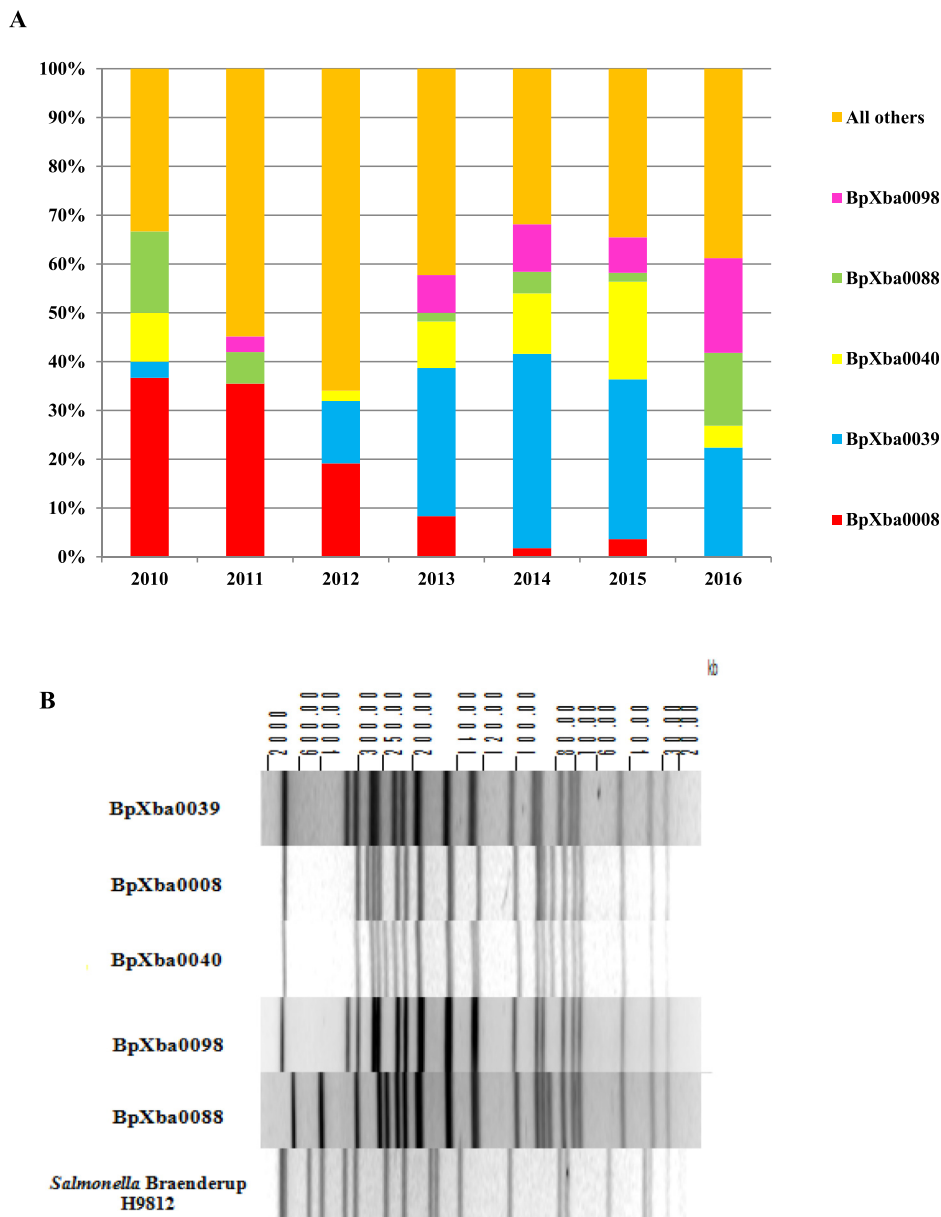


Fig. 5. Predominant pulsed-field gel electrophoresis (PFGE) profiles of 511 *B. pertussis* isolates, by year of collection, Brazil, 2010–2016. (A) Predominant *B. pertussis* PFGE profiles. (B) Banding patterns of the predominant *B. pertussis* PFGE profiles.

In Brazil, aP vaccines are available only to pregnant women and children who have developed serious side effects after the use of wP vaccines. Thus, the proportion of Brazilian children who have been immunized with these vaccines is unknown. This combined with low maternal vaccination rates, suggests these aP vaccines likely did not influence isolates obtained between 2010 and 2016. As was observed in aP countries, the predominance of Prn-deficiency did not occur until several years after the introduction of aP vaccines. Thus, the appearance of Prn-deficient strains in Brazil could happen in a few years from now, with an increase in Tdap

vaccination coverage and a greater number of vaccinated pregnant women. This is why surveillance in the country must continue, with monitoring of the circulating strains of *B. pertussis*, using serotyping and PFGE to study bacterial population dynamics and the evolution of the bacterial population under vaccine pressure.

5. Conclusion

While Prn deficiency was identified in a few isolates, this study did not detect a relevant occurrence of this phenomenon, confirm-

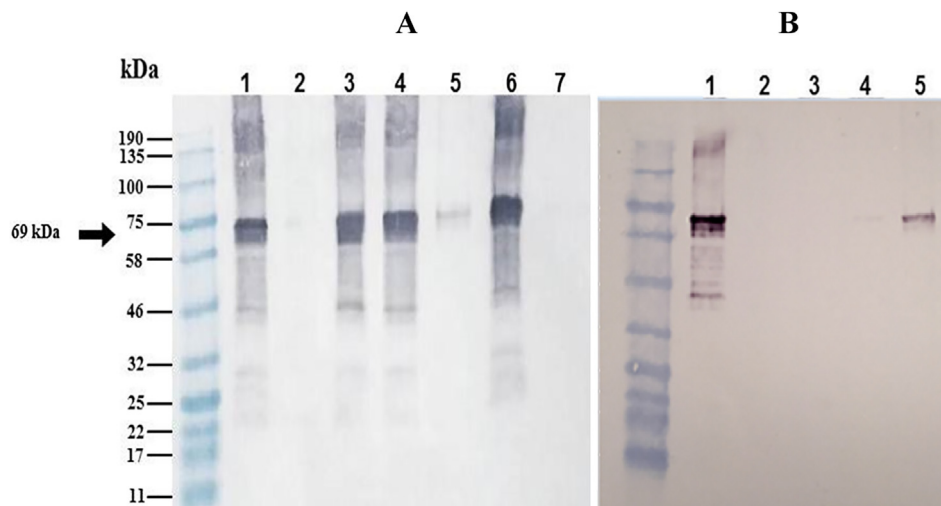


Fig. 6. Representative Immunoblots to detect the Prn production in *B. pertussis* isolates in Brazil. (A) Lane 1, *B. pertussis* H936, positive control; lane 2, *B. pertussis* H920, negative control; lanes 3–7, test samples (lanes 3, 4 and 6 show positive results for Prn, lane 7 shows the absence of Prn, while lane 5 shows an inconclusive result for Prn. (B) Lane 1, *B. pertussis* H936, positive control; lane 2, *B. pertussis* H920, negative control; lanes 3–5, test samples (lanes 3 and 4 show the absence of Prn, lane 5 shows a positive result for Prn production). Molecular mass markers (in kilodaltons) are listed to the left. The position of the 69-kDa band is indicated by the arrow.

Table 3
Compiled results of the eight putative Prn-deficient *B. pertussis* strains.

Id sample/year	State of Isolation	Serotype	PFGE	ELISA	PCR	Immunoblotting	Pertactin allele
IAL 16349/2012	Bahia	3	BpXba0104	PrnD	PrnP	PrnP	<i>prn2</i>
IAL 20140/2013	Distrito Federal	3	BpXba0040	PrnD	PrnD	PrnD	<i>prn2</i>
IAL 52857/2013	Pernambuco	2,3	BpXba0004	PrnD	PrnP	PrnD	<i>prn2</i>
IAL 22946/2014	Pernambuco	2	BpXba0071	PrnD	PrnP	Inconclusive	<i>prn2</i>
IAL 24416/2015	Distrito Federal	2,3	BpXba0039	PrnD	PrnP	PrnP	<i>prn2</i>
IAL 73228/2015	Pernambuco	2,3	BpXba0108	PrnD	PrnP	PrnP	<i>prn2</i>
IAL 73230/2015	Pernambuco	2,3	BpXba0039	PrnD	PrnP	PrnD	<i>prn2</i>
IAL 73243/2015	Pernambuco	2,3	BpXba0088	PrnD	PrnP	PrnP	<i>prn2</i>

prn = pertactin gene; PFGE = pulsed field gel electrophoresis; Prn P = pertactin producing; Prn D = pertactin deficient.

ing previous observations that Prn-deficiency is likely aP vaccine-driven. These data provide an overview of circulating *B. pertussis* in the country and, in conjunction with surveillance activities, can provide useful information to support discussions about the best vaccination strategies, with a focus on reducing transmission risk in our most vulnerable population, infants less than six months of age.

Author contributions

Drs Leite, Pawloski, Tondella, Oliveira and Vaz-de-Lima conceptualized and designed the study, drafted the initial manuscript and edited the final version of the submitted paper.

Dr Leite, Dr Vaz-de-Lima, Dr Kashino, Dr Martins, Polatto and Pereira performed the laboratory assays.

Drs Leite, Camargo, Kashino, Pawloski, Tondella, Oliveira and Vaz-de-Lima analyzed the data and reviewed and revised the manuscript.

Drs Oliveira and Vaz-de-Lima coordinated the study.

All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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