

## BRIEF COMMUNICATION

### PHENOTYPICAL AND GENOTYPICAL CHARACTERIZATION OF *Bordetella pertussis* STRAINS ISOLATED IN SÃO PAULO, BRAZIL, 1988-2002

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#### SUMMARY

Whooping cough or pertussis was a major cause of childhood morbidity and mortality in the world until the introduction of a whole-cell vaccine in the 1940's. However, since the early 1980's whooping cough cases have increased in many countries, becoming an important problem of public health. This increase may be due to accuracy of laboratory diagnosis and reporting of the disease, a decline in immunity over time, demographic changes, and adaptation of the bacterial population to vaccine-induced immunity. The purpose of this study was to analyze phenotypically and genotypically a collection of 67 *Bordetella pertussis* isolates recovered during the period 1988-2002 in São Paulo State, Brazil to determine their characteristics and relatedness. All isolates were submitted to susceptibility testing to erythromycin, serotyping, and 56 isolates were analyzed by Pulsed Field Gel Electrophoresis (PFGE). All isolates were susceptible to erythromycin and the majority of them belonged to serotype 1,3. The 56 isolates were classified into 11 PFGE profiles according to the differences in banding patterns. Although more than 60% of the isolates were recovered from patients aged less than three months, almost 15% of them were isolated from adolescents/adults evidencing the increase in the incidence of pertussis among this group of age.

**KEYWORDS:** *Bordetella pertussis*; Whooping cough; Serotypes; Genetic profiles.

Although a significant decline in the incidence of whooping cough was observed following the widespread use of pertussis vaccine in the mid-1940s<sup>3</sup>, the incidence of the disease has increased since the early 1980s in many countries even with high vaccination coverage<sup>3,5</sup>. Data concerning the prevalence of pertussis in Brazil have been published since late 1970's but more recently report shows that pertussis cases were substantially reduced after the public use of pertussis vaccine since the 1980's<sup>2,6,11,12</sup>. In Brazil, pertussis became a reportable disease only in 2001<sup>1</sup> making laboratory methods for pertussis diagnosis be implanted and or improved in all State health laboratories in the country. After 2001, laboratory data show that pertussis cases may be underestimated and that *Bordetella pertussis* strains are still circulating in São Paulo State.

Characterization of *B. pertussis* is important to understand the dynamic of the disease, to determine the relatedness of the isolates, and also to provide information related to the appropriated vaccine composition to be used in each region. In this study, we analyzed a collection of 67 *B. pertussis* isolated during the period of 1988-2002 in São Paulo State. Suspected colonies of *B. pertussis* on Reagan-Lowe medium (RL) were submitted to Gram staining, biochemical tests and

nutritional requirements assays following the standard methods<sup>10</sup>. Susceptibility of all isolates to erythromycin was determined by disk diffusion testing<sup>5</sup>. For serotyping specific antisera against antigen 1, 2 and 3 were prepared at Instituto Adolfo Lutz, using reference strains and following the methods previously described<sup>8</sup>. Molecular typing was performed using Pulsed Field Gel Electrophoresis (PFGE) as described by GAUTOM<sup>4</sup>.

All *B. pertussis* isolates were susceptible to erythromycin. The majority of the isolates belonged to serotypes 1,3 (86%) whereas strains belonging to serotypes 1, and 1,2 accounted for 10% and 4%, respectively. **PFGE using *Xba*I generated eighth to twelve bands ranging from approximately 90 to 700 kb. Although few differences were detected in banding patterns, it was possible to discriminate the 56 isolates into 11 genetic profiles (1-11); however, no genetic profile was associated with a particular geographic area, age of patients or serotypes.** Strains belonging to profile 8 were the most prevalent.

Four strains isolated in the city of Santos, exhibited the same banding pattern, and three of them were recovered from three infants

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**Table 1**  
Characteristics of *Bordetella pertussis* strains isolated in different cities in São Paulo State, Brazil, during the period 1988-2002

Strain	Age <sup>1</sup>	Serotype	PFGE	City <sup>3</sup>	Strain	Age <sup>1</sup>	Serotype	PFGE	City <sup>2</sup>
<b>168/88</b>	<b>07m</b>	<b>1,3</b>	<b>1</b>	<b>Santos</b>	13/01	05m	1,3	6	Rib. Preto
<b>35/89</b>	<b>09m</b>	<b>1,3</b>	<b>1</b>	<b>Santos</b>	17/01	68y	1	8	Rib. Preto
<b>45/89</b>	<b>12m</b>	<b>1,3</b>	<b>1</b>	<b>Santos</b>	21/01	01m	1,3	8	Rib. Preto
<b>57/89</b>	<b>01m</b>	<b>1,3</b>	<b>1</b>	<b>Santos</b>	26/01	01m	1,3	8	Rib. Preto
24/93	09m	1,3	5	Campinas	27/01	03m	1,3	8	Rib. Preto
143/96	02m	1,3	3	Rib. Preto	38/01	03m	1,3	9	Campinas
233/96	03m	1,2	5	Rib. Preto	40/01	01m	1,3	8	Campinas
32/97	02m	1,2	-	Rib. Preto	48/01	45d	1	8	Rib. Preto
106/97	09m	1,2	5	Rib. Preto	49/01	02m	1	5	Rib. Preto
58/98	03y	1,3	9	São Paulo	50/01	26y	1,3	8	Campinas
193/98	02m	1,3	7	Rib. Preto	57/01	01m	1,3	-	Sorocaba
216/98	22d	1,3	4	SJ.Campos	58/01	03m	1,3	-	Rib. Preto
170/99	UN	1,3	8	Rib. Preto	59/01	01m	1,3	-	Rib. Preto
375/99	02m	1,3	7	Rib. Preto	65/01	12y	1,3	-	Campinas
376/99	23y	1,3	8	Rib. Preto	66/01	01m	1,3	-	Rib. Preto
615/99	01m	1,3	2	Rib. Preto	70/01	01m	1,3	8	São Paulo
1261/99	02m	1,3	-	São Paulo	73/01	01m	1,3	-	Campinas
1342/99	01m	1,3	4	Rib. Preto	02/02	19y	1,3	9	Rib. Preto
1878/99	02m	1,3	10	Rib. Preto	03/02	03m	1,3	8	Campinas
1946/99	11m	1,3	5	São Paulo	07/02	02m	1,3	-	Rib. Preto
1947/99	2m	1,3	8	São Paulo	08/02	26y	1,3	8	Rib. Preto
1969/99	1m	1,3	10	Rib. Preto	16/02	32y	1	8	Campinas
2080/99	04m	1,3	10	São Roque	<b>24/02</b>	<b>01m</b>	<b>1,3</b>	<b>5</b>	<b>Rib. Preto</b>
16/00	07m	1,3	-	Rib. Preto	<b>25/02</b>	<b>Adult</b>	<b>1,3</b>	<b>5</b>	<b>Rib. Preto</b>
46/00	02m	1,3	8	Santo André	<b>26/02</b>	<b>Adult</b>	<b>1,3</b>	<b>5</b>	<b>Rib. Preto</b>
50/00	02m	1,3	11	São Paulo	27/02	01m	1,3	7	Campinas
51/00	24y	1,3	9	Rib. Preto	30/02	02m	1,3	9	Rib. Preto
55/00	01m	1,3	11	Santo André	39/02	03m	1,3	-	Rib. Preto
76/00	01m	1,3	9	Rib. Preto	42/02	02m	1,3	8	Rib. Preto
93/00	17d	1,3	9	Rib. Preto	44/02	06m	1,3	8	Rib. Preto
95/00	02y	1,3	9	Rib. Preto	48/02	04m	1,3	8	São Paulo
99/00	01m	1	8	Campinas	49/02	01m	1,3	8	Rib. Preto
11/01	02m	1,3	9	Campinas	55/02	01m	1,3	-	Campinas
12/01	07m	1,3	8	Rib. Preto					

<sup>1</sup> Age of individual. m: month ; y: year; d: day ; UN: unknown. <sup>2</sup> Rib. Preto: Ribeirão Preto; S.J.Campos: São José dos Campos; - : not tested.

between January-February 1989, suggesting the occurrence of a probable pertussis outbreak in that period. In another city, Ribeirão Preto, a genetically similar profile was also seen among strains isolated from one infant (strain 24/02) and his parents (strains 25/02 and 26/02) suggesting them as the possible source of infection.

Our data show that although more than 60% of the 67 isolates were recovered from patients aged < 3 months, almost 15% were isolated from patients > 12 years of age. This shows the important role of adolescents and adults as reservoir for infections in very young infants<sup>5,7</sup>. Laboratory data are important for improving surveillance programs and for accurately estimate pertussis cases, helping to set priorities for immunization strategies to control this re-emerging disease<sup>5</sup>.

## RESUMO

### Caracterização fenotípica e genética de cepas de *Bordetella pertussis* isoladas em São Paulo, Brasil, 1988-2002

A coqueluche ou pertussis foi a maior causa de morbidade e mortalidade infantil em todo o mundo até a introdução de uma vacina na década de 1940. Entretanto, desde a década de 1980, a coqueluche tornou-se, em muitos países, um importante problema de saúde pública. Este acontecimento pode ser atribuído à melhoria do diagnóstico laboratorial e da notificação da doença, declínio da imunidade no decorrer do tempo, mudanças demográficas ou adaptação da população bacteriana à imunidade induzida pela vacina. O objetivo deste estudo

foi analisar as características fenotípicas e genotípicas de uma coleção de 67 cepas de *Bordetella pertussis* isoladas no período 1988-2002 em São Paulo, Brasil. Todas as cepas foram submetidas à determinação do perfil de resistência à eritromicina, à sorotipagem e 56 cepas à eletroforese em campo pulsado (PFGE). Todas as cepas foram sensíveis à eritromicina e a maioria delas pertencia ao sorotipo 1,3. As 56 cepas foram classificadas em 11 perfis de PFGE com base nas diferenças no padrão de bandas. Embora mais de 60% das cepas tenham sido isoladas de crianças com menos de três meses de idade, cerca de 15% delas era de adolescentes/adultos evidenciando um aumento da incidência da coqueluche nesse grupo etário.

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#### REFERENCES

1. BRASIL. Ministério da Saúde - Portaria Número 1943, de 18 de outubro de 2001. Define a relação de doenças de notificação compulsória para todo o território nacional. **Diário Oficial da União, Poder Executivo**, 24 de outubro de 2001.
2. BRASIL. Ministério da Saúde - Situação epidemiológica da coqueluche no Brasil. Uma breve descrição. Relatório de Maria da Glória Vicente. Brasília, outubro de 2003.
3. CHERRY, J.D. - Pertussis in the preantibiotic and prevaccine era, with emphasis on adult pertussis. **Clin. infect. Dis.**, **28**(suppl. 2): S107-S111, 1999.
4. GAUTOM, R.K. - Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other Gram-negative organisms in one day. **J. clin. Microbiol.**, **35**: 2977-2980, 1997.
5. GUIDELINES FOR THE CONTROL OF PERTUSSIS OUTBREAKS. Atlanta, Centers for Disease Control and Prevention, 2000.
6. IARIA, S.T. - Isolamento de bactérias do gênero *Bordetella* e provas sorológicas, a partir de crianças com sintomas de coqueluche, atendidas no Hospital de Isolamento Emílio Ribas de São Paulo. **Rev. Saúde públ. (S. Paulo)**, **7**: 409-432, 1973.
7. MOOI, F.R.; HALLANDER, H.; WIRSING von KONIG, C.H.; HOET, B. & GUIISO, N. - Epidemiological typing of *Bordetella pertussis* isolates: recommendations for a standard methodology. **Europ. J. clin. Microbiol. infect. Dis.**, **19**: 174-181, 2000.
8. PRESTON, N.W. - Technical problems in the laboratory diagnosis and prevention of whooping-cough. **Lab. Pract.**, **19**: 482-486, 1970.
9. REGAN, J. & LOWE, F. - Enrichment medium for the isolation of *Bordetella*. **J. clin. Microbiol.**, **6**: 303-309, 1977.
10. SANDEN, N. & WEYANT, R.S. - Genus III. *Bordetella*. In: BRENNER, D.J.; KRIEG, N.R. & STALEY, J.T., ed. **Bergey's manual of systematic Bacteriology**. New York, Springer, 2005. v. 2.
11. UBATUBA, A. - Bacteriologia da coqueluche. Identificação das Bordetellas isoladas em crianças no Rio de Janeiro. **Hospital (Rio de J.)**, **75**: 97-107, 1969.
12. UBATUBA, A. & SALMITO, A.T. - Bacteriologia da coqueluche. II. Determinação dos fatores aglutinantes "major" nas amostras de *Bordetella pertussis* isoladas no Rio de Janeiro. **Hospital (Rio de J.)**, **78**: 1301-1310, 1970.

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