### Antigenic and Genetic Characterization of Bordetella pertussis recovered from Quebec, Canada, 2002-2014: detection of a genetic shift

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Antigenic and Genetic Characterization of *Bordetella pertussis* recovered from Quebec, Canada, 2002-2014: detection of a genetic shift

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Abstract

Despite vaccination, cyclical peaks of *Bordetella pertussis* incidence rates are still observed in Canada and other developed countries, making pertussis one of the most prevalent vaccine preventable bacterial diseases. In the post-acellular vaccine era, evolution of bacterial strains has resulted in strains with altered vaccine antigens. Previous Canadian studies have focused on isolates mainly from the provinces of Ontario and Alberta, with only small numbers of isolates from other provinces. Therefore, in this study, we examined a larger sample (n=52) of isolates from Québec, Canada between 2002 and 2014. Isolates were characterized by serotype, sequence type and prevalence of pertactin-deficiency. The Québec isolates shared similar characteristics with other Canadian isolates as well as isolates circulating globally. Although pertactin-deficient isolates were not present, a significant shift in sequence type was observed in more recent years. This study highlights the importance of continually monitoring disease causing isolates in order to track evolutionary trends and gain a better understanding of the molecular epidemiology of pertussis in Canada.

Keywords: *Bordetella pertussis*, pertactin, genotyping
Whooping cough, caused by the bacterium *Bordetella pertussis*, became a nationally notifiable disease in Canada in 1924 (Varughese 1985). Prior to the introduction of the vaccine, cyclical increase in incidence was occurring every 2 to 5 years and the average incidence rate was 156 cases per 100,000 in the five years before vaccination (Smith et al. 2014). Vaccination began in 1943 using a fluid whole cell vaccine, which was replaced in the 1980s by the adsorbed whole cell vaccine manufactured by Connaught. The introduction of this poorly protective vaccine was rapidly followed by a large resurgence of pertussis. This product was replaced in 1997-98 by the less reactogenic and more efficacious acellular vaccines. As a result of vaccination, pertussis incidence decreased dramatically, reaching a record low in Canada of 2 cases per 100,000 in 2011 (Smith et al. 2014). Despite better vaccine effectiveness and extensive coverage, periodic outbreaks still occur and the last peak in Quebec was observed in 2012 (Smith et al. 2014). During that year, the national rate of pertussis jumped seven fold (13.9 cases per 100,000) and 9 out of 13 Canadian provinces and territories reported increased disease activity with a large outbreak in the province of New Brunswick which accounted for 1/3 of the cases nationally (Smith et al. 2014).

Acellular vaccines have now replaced whole cell vaccines in many industrialized countries. They are made up of purified pertussis proteins and contain between 1 and 5 of the following components: pertussis toxin (Ptx), pertactin (Prn), filamentous haemagglutinin (Fha) and fimbriae types 2 and 3 (Fim 2 and Fim 3). In Quebec, the 5 (Ptx+Fha+Prn+Fim 2/3) components acellular pertussis vaccine has mostly been used in the past decade but the 3 (Ptx+Fha+Prn) component vaccine is also offered. The schedule consists of 3 primary doses given at 2, 4, and 6...
months, followed by booster shots given at 18 months, another between 4 and 6 years of age, and
again between 14 and 16 years of age. Since 2013, a single booster is recommended after 18
years of age (MSSS 2013). With vaccination coverage rates >95% with ≥3 doses at 24 months
of age (Boulianne et al. 2014), pertussis incidence rates have been relatively low in Québec in
the past 15 years. This is different from the pertussis resurgence (larger number of cases than
expected, given the periodic variability of pertussis disease, when compared to previous cycles in
the same setting) observed in several countries, including Australia, Portugal, USA and UK
(WHO 2015). For example, California noticed in 2010 the highest number of pertussis cases in
more than 60 years, including > 9000 cases, 809 hospitalizations and 10 deaths (Winter 2012). In
2012, a large UK outbreak saw nearly 10,000 cases, including 14 deaths in infants under the age
of 3 months (Sealey et al. 2015). This resurgence of pertussis has been linked to several factors:
1) waning immunity, especially in the adolescent/adult populations; 2) improved laboratory
methods, including the use of PCR for diagnosis; 3) lower efficacy of the acellular vaccines
compared to whole cell vaccines; 4) higher rates of non-vaccination in recent years and 5)
evolution of the \textit{B. pertussis} bacterium (Guiso 2014, Jakinovich and Sood 2014, Mooi et al.

Evolution of the \textit{B. pertussis} bacterium over the years has resulted in a divergence between
currently circulating strains versus the strains used in vaccine production (Bart et al. 2014). It has
also led to the emergence of strains with mutations in cell surface protein encoding genes,
resulting in strains with altered vaccine antigens (van Gent et al. 2015). Reports of strains with
increased fitness due to the \textit{ptxP3} type mutations in the \textit{ptx} promoter region emerged in the
2012, Schmidtke et al. 2012, Shuel et al. 2013, Pawloski et al. 2014). These isolates are thought
to have increased levels of pertussis toxin, however this has not been confirmed at the
transcriptomic level (King et al. 2013, de Gouw et al. 2014, Bouchez et al. 2015). Isolates
lacking cell surface proteins Fha, Ptx, Fim and Prn have also been identified (Bouchez et al.
lacking Ptx, Fha or Fim are relatively uncommon compared to those lacking Prn (Zeddeman et
al. 2014). The Prn-deficient strains are now a cause for global concern as they have emerged in
areas such as the US, Japan, Australia and several European countries (Pawloski et al. 2014,
Otsuka et al. 2012, Lam et al. 2014, Zeddeman et al. 2014). In addition to its prevalence, Prn-
deficient strains appear to have a selective advantage over Prn-positive strains, especially in the
context of acellular pertussis vaccination. Prn-deficient strains were found more often from
patients who have received acellular pertussis vaccine when compared to unvaccinated patients
(Martin et al. 2015). Also Prn-deficient strains caused longer colonisation than Prn-positive
strains in acellular pertussis vaccine immunised mice (Hegerle et al. 2012).

A previous study, focussing on Canada’s most populous province of Ontario, identified 1
predominant clone (ST-1) that was seen in 83.5% of isolates (Shuel et al. 2013). Another study
(Tsang et al. 2014) which included isolates from 8 out of 13 Canadian provinces and territories,
identified Prn-deficient isolates in only 2 provinces:Ontario (7/138 isolates) and Alberta (5/56
isolates). However, in that study, only 3 isolates recovered in 2008 from Québec were included.
Therefore, in this study we examined a larger sample (n = 52) over more than 10 years in order
to ascertain the prevalence or absence of Prn-deficient isolates in Québec. Here we present the
characterization of *B. pertussis* isolates collected between 2002 and 2014 from the province of Québec: Canada’s largest province in land area and second largest province based on population.

There were 52 *B. pertussis* isolates sent to the National Microbiology Laboratory (NML) for the purpose of this study. Isolates were collected by the provincial reference laboratory of Québec, Laboratoire de santé publique du Québec (LSPQ), between 2002 and 2014 and represented all years, with the exception of 2011 and 2013. There were 5 isolates for the years 2002, 2003, 2004, 2005, 2006 and 2009; 2 isolates for 2007 and 2010; 7 isolates for 2008, 10 isolates for 2012 and 1 isolate for 2014. Isolates were serotyped by indirect whole-cell ELISA (Tsang et al, 2005) and genotyped by partial sequencing of the following virulence factor genes: pertussis toxin subunit 1 (*ptxS1*), pertactin (*prn*), fimbriae 3 (*fim3*), filamentous haemagglutinin (*fhaB*) and the pertussis toxin promoter region (*ptxP*) in order to determine sequence type (ST) (Shuel et al, 2013). In order to identify Prn-deficient isolates, complete sequencing of the *prn* gene was performed on all 52 isolates and Western blot for the detection of pertactin was carried out on 51 isolates according to procedures previously described (Tsang et al, 2014).

All 52 isolates were found to belong to the Fim 3 serotype. Genotyping found that the majority of isolates belonged to ST-1 (n=34, 65.4%) characterized by *ptxS1A, prn2, fim3B, fhaB1* and *ptxP3*, followed by ST-2 (n=12, 23%) (*ptxS1A, prn2, fim3A, fhaB1, ptxP3*), ST-8 (n=4, 7.7%) (*ptxS1A, prn9, fim3B, fhaB1, ptxP3*), and the 2 remaining isolates belonged to ST-4 (*ptxS1A, prn1, fim3A, fhaB1, ptxP1*) and ST-7 (*ptxS1A, prn3, fim3B, fhaB1, ptxP3*). There were no Prn-deficient isolates identified. All isolates were found to contain an intact *prn* gene based on
complete prn gene sequence analysis and all 51 isolates tested by Western immunoblot were found to produce the Prn protein.

Sequence types 1 and 2 differ only in their fim3 allele type and were the most common STs identified in this study as well as in previous Canadian studies from the provinces of Ontario and Alberta (Shuel et al. 2013, Simmonds et al. 2014). Ontario had the highest frequency of ST-1 isolates at 83.5%, compared to 77% and 65.4% from Alberta and Québec, respectively. ST-2 was found in frequencies of 10.9%, 19.2% and 23% in Ontario, Alberta and Québec, respectively. In the Québec isolates, there was a significant shift from ST-1 (fim3B) to ST-2 (fim3A) in more recent years (p<0.01; Table 1). The percentage of ST-2 isolates increased from 4% (1/25) in isolates collected prior to 2006 to 40.7% (11/27) after 2006 while for the same period, ST-1 isolates decreased from 92.0% (23/25) to 40.7% (11/27).

Circulating B. pertussis clones from Australia, the US and Europe also share similar characteristics with Canadian isolates (Octavia et al. 2012, Pawloski et al. 2014, van Gent et al. 2015). A study on 466 B. pertussis isolates from 13 European countries found that the Fim 3 serotype and prn2 and ptxP3 allele types were dominant in all countries except Poland, where the whole cell vaccine is still being used (van Gent et al. 2015). In contrast, Polish isolates were mainly typed as serotype Fim 2, ptxP1 and either prn1 or prn2. A slight shift from ST-1 (fim3B or fim3-2) to ST-2 (fim3A or fim3-1) was also apparent, although not quite as pronounced as what was noted in Québec. Allele type fim3B was seen in 59% of European isolates between 2002 and 2006 and fim3A was observed in 41%. Between 2007 and 2012, 42% were fim3B and 56% fim3A (van Gent et al. 2015).
Strains lacking Prn expression arise from a multitude of different mutational events, including disruption of the prn gene with insertion sequences IS481 or IS1002; mutations/inversions/deletions in the promoter region or within the gene itself; single nucleotide polymorphisms leading to premature stop codons and complete deletion of the entire prn gene (Zeddeman et al. 2014, Lam et al. 2014, Pawloski et al. 2014, Tsang et al. 2014). First identified in the US in 1994, Prn-deficient strains have become increasingly more common, especially in Australia and the US (Lam et al. 2014, Pawloski et al. 2014). Prn-deficient B. pertussis increased rapidly in Australia, going from 5% in 2008 to 78% in 2012 (Lam et al. 2014) and in the US, Prn-deficient strains were responsible for >50% of the cases in 2012 (Pawloski et al. 2014). Prn-deficient strains are not quite as prevalent in Europe, ranging from 0% up to 25%, depending on country, with an overall average of 3.4% based on 414 isolates tested between 2007 and 2012 (Zeddeman et al. 2014). A previous Canadian report on 224 isolates from 1994 to 2013 found that 5.4% were Prn-deficient and all were isolated in either 2011 or 2012 from the non-neighboring provinces of Alberta and Ontario (Tsang et al. 2014). However, only very small numbers of isolates from the provinces of British Columbia (n = 12), Manitoba (n = 3), Saskatchewan (n = 8), Québec (n = 3), and Nova Scotia (n = 3) were included in that study; and therefore, would not be definitive in terms of prevalence of such isolates in these provinces. The present study includes 10 isolates from 2012 and no isolates from 2011.

It has been suggested that the lack of Prn production is a direct result of selection pressure mediated by the acellular vaccines; and genes encoding vaccine antigens have been shown to evolve at a significantly faster rate compared to genes encoding other cell surface proteins.
(Sealey et al. 2015). This supports the idea that selection pressure may be the driving force behind evolution of *B. pertussis*. Although Prn-deficient isolates present a better fitness in subjects primed or vaccinated with acellular pertussis vaccines (Hegerle et al. 2012, Martin et al. 2015), acellular vaccines do provide good protection in the short term especially when the coverage is high. In Australia and the US, where a resurgence of pertussis was observed (WHO SAGE pertussis Working Group 2014), an increasing proportion of strains are Prn-deficient. Interestingly, there is no strong evidence of pertussis resurgence in Canada (WHO SAGE Pertussis Working Group 2014, Smith et al. 2014) where Prn-deficient stains are uncommon. Although this does not represent any evidence of a causal association and some countries, such as the UK, observed pertussis resurgence without any increase in Prn-deficient strains (Sealy 2015). Thus further work regarding the impact of Prn deficiency on vaccine effectiveness is required. Another possible hypothesis for the absence of pertussis resurgence in Québec and most other Canadian provinces is the use of the poorly protective adsorbed Connaught whole cell pertussis vaccine (De Serres 1996, Bettinger 2007) and the extensive transmission of disease in the 1990s (Ntezayabo 2003), leading to some degree of protective immunity at the population level.

Surveillance of *B. pertussis* in Québec and Canada is hindered by the fact that there is no formal program in place and isolates are not routinely sent to the LSPQ nor to the NML for characterization. Consequently, one limit of this study is the number of isolates which represented only about 5% of culture positive samples in Québec from 2002-2014. Also, more and more laboratories are relying on PCR for diagnosis and not culturing for the bacterium,
rendering it increasingly difficult to document the evolving strain characteristics in pertussis cases in Canada.

Based on reports so far, many of the Canadian isolates appear to share similar characteristics with each other as well as with *B. pertussis* isolates circulating worldwide. However, this is the first report of a significant shift in ST over time. While Prn-deficient isolates were interestingly absent from the Québec isolates studied, the number of isolates examined was still small and the potential for rapid increase still exists, such as what has been seen in Australia, the US and other Canadian provinces (Pawloski et al. 2014, Lam et al. 2014, author’s unpublished data).

Therefore, it is important to continually monitor future isolates for both of these trends and maintain culture capability in Québec and Canada in order for strain characterization to be done either periodically or when required. It is only when strain characterization is coupled with epidemiological investigation and clinical information that a more comprehensive view of pertussis can be achieved in Québec and in Canada.
Acknowledgements

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References


Table 1. Sequence type distribution of 53 *Bordetella pertussis* isolates from Quebec, Canada.

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<th>Sequence Type (ST)*</th>
<th>Number of Isolates (%)</th>
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<tr>
<td></td>
<td>2002-2006</td>
<td>2007-2014</td>
<td>Total</td>
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<tr>
<td>ST-1</td>
<td>23 (92.0)</td>
<td>11 (40.7)</td>
<td>34</td>
<td></td>
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<tr>
<td>ST-2</td>
<td>1 (4.0)</td>
<td>11 (40.7)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>ST-4</td>
<td>0 (0)</td>
<td>1 (3.7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ST-7</td>
<td>1 (4.0)</td>
<td>0 (0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ST-8</td>
<td>0 (0)</td>
<td>4 (14.8)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>27</strong></td>
<td><strong>52</strong></td>
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*ST-1: ptxS1A, prn2, fim3B, fhaB1, ptxP3; ST-2: ptxS1A, prn2, fim3A, fhaB1, ptxP3; ST-4: ptxS1A, prn1, fim3A, fhaB1, ptxP1; ST-7: ptxS1A, prn3, fim3B, fhaB1, ptxP3; ST-8: ptxS1A, prn9, fim3B, fhaB1, ptxP3.*