**Bordetella pertussis**

Clones Identified by Multilocus Variable-Number Tandem-Repeat Analysis

Jacob Kurniawan, Ram P. Maharjan, Wai-Fong Chan, Peter R. Reeves, Vitali Sintchenko, Gwendolyn L. Gilbert, Frits R. Mooi, and Ruiting Lan

Multilocus variable-number tandem-repeats analysis (MLVA) of 316 *Bordetella pertussis* isolates collected over 40 years from Australia and 3 other continents identified 66 MLVA types (MTs), including 6 predominant MTs. Typing of genes encoding acellular vaccine antigens showed changes that may be vaccine driven in 2 MTs prevalent in Australia.

Despite longstanding vaccination programs, pertussis remains endemic to many industrialized countries, including Australia, Canada, Italy, Japan, the Netherlands, Switzerland, and the United States, all of which have reported recent increases in incidence (1). Although pertussis is classically a disease of infants and children, this increase has been mainly among adults and adolescents (2,3). Factors contributing to pertussis resurgence remain unclear, but possible causes are waning immunity, suboptimal vaccine coverage, improved surveillance and diagnosis, the switch from whole cell vaccine (WCV) to acellular vaccine (ACV), and adaptation of circulating *Bordetella pertussis* strains (4–9). To determine the global epidemiology of pertussis, we analyzed an international collection of *B. pertussis* isolates collected mainly over the past 40 years.

**The Study**

We typed 5 genes, the products of which are used in ACVs (prn, ptxA, fim2, fim3, and fhaB), using the method of Chan et al. (12) to assess the effect of the switch from WCV to ACV on prevalence of the 4 predominant MTs (MT27, MT29, MT64, and MT70) in Australia. Isolates from these MTs have the same ptxA1 and fhaB1 alleles but vary in the other 3 genes investigated (complete list available from authors). The predominant ACV used in Australia is from GlaxoSmithKline (GSK) (Research Triangle Park, NC, USA), which contains pertussis toxoid, filamentous hemagglutinin, and pertactin but no fimbriae (FIMs). The strain used for GSK ACV contains the alleles prn1, ptxA2, and fhaB1 (10,13). However, ACV from Sanofi-Aventis (Pasteur, Lyon, France), which contains FIM2+3 in addition to pertussis toxoid, pertactin, and filamentous hemagglutinin with unknown allele types, is also licensed in Australia, complicating interpretation of variation in *fim* genes. On the basis of their frequencies and late appearance, *fim2*-2 and *fim3*-B are not likely to be the vaccine alleles. A significant increase (p<0.005) of *prn2* (36% vs. 3%), *fim2*-2 (34% vs.
8%), and fim3-B (24% vs. 0%) was observed in the ACV period in comparison to the WCV period.

This increase of allelic frequency is better reflected in changes in antigenic profiles. MT27 has 3 profiles (prn1, fim2-1, fim3-A; prn2, fim2-1, fim3-A; and prn2, fim2-1, fim3-B). The first profile was seen once in the WCV period, whereas the other 2 first appeared in the WCV/ACV transition period and increased in frequency in the ACV period; the third profile, which differed by 2 alleles from the first, was more frequent. The appearance of prn2 in the second profile and additional change from fim3-A to fim3-B in the third represent increases in prevalence of alleles absent from ACV. MT29 also has 3 profiles (prn1, prn2, or prn3), fim2-1, fim3-A), which differ in prn only. Most MT29 isolates carry prn3, and the profile is prevalent in both WCV and ACV periods, with no obvious increase in non-ACV alleles.

MT70 and MT64 both have uniform allelic profiles (prn1, fim2-2, fim3-A and prn1, fim2-1, fim3-A, respectively). However, MT70 (with fim2-2, not likely to be in ACV) increased significantly over the study period while MT64 with all alleles likely to be in ACV remained steady.

Overall, the frequency of MT27 and MT70, with non-ACV alleles, increased significantly (p<0.0001) and correlated with the introduction of ACV, suggesting that antigenic changes could be driven by selection pressure.

The 2 MTs predominant in Australia were also prevalent in other countries and possibly have a global distribution. MT27 (18% of isolates) was found in 8 countries and MT29 (17% isolates) in 5. However, absence of an MT in a country might result from the small samples used. MT27 and MT29 were the most common types in the Netherlands (10) and the United Kingdom (11). MT10, MT64, MT70, MT84, and MT186 were also relatively common. MT10 and MT186 were found predominantly in Japan, although each had been found elsewhere, in China (1957) and Hong Kong (2002), respectively. MT64 was predominantly from Australia with 1 isolate from Japan, and MT70 was only found in Australia. However, all of these frequent MTs (except MT186) have been observed before. MT10 was frequent in the United Kingdom in the prepertussis vaccine era, while MT70 was common during 1998–2001 (11).

Nine isolates, including Tohama I, identified in samples of pertussis strains collected during the 1920s–1950s

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. repeats</th>
<th>No. alleles</th>
<th>D</th>
<th>No. alleles</th>
<th>D</th>
<th>No. alleles</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>VNTR1</td>
<td>2–12</td>
<td>6</td>
<td>0.58</td>
<td>4</td>
<td>0.63</td>
<td>7</td>
<td>0.26</td>
</tr>
<tr>
<td>VNTR2</td>
<td>2–5</td>
<td>4</td>
<td>0.02</td>
<td>2</td>
<td>0.01</td>
<td>3</td>
<td>N/A</td>
</tr>
<tr>
<td>VNTR3a</td>
<td>2–8</td>
<td>4</td>
<td>0.40</td>
<td>4</td>
<td>0.43</td>
<td>10</td>
<td>0.18</td>
</tr>
<tr>
<td>VNTR3b</td>
<td>0–10</td>
<td>5</td>
<td>0.21</td>
<td>5</td>
<td>0.21</td>
<td>4</td>
<td>0.15</td>
</tr>
<tr>
<td>VNTR4</td>
<td>2–9</td>
<td>7</td>
<td>0.34</td>
<td>5</td>
<td>0.24</td>
<td>8</td>
<td>0.21</td>
</tr>
<tr>
<td>VNTR5</td>
<td>3–9</td>
<td>6</td>
<td>0.20</td>
<td>4</td>
<td>0.19</td>
<td>7</td>
<td>0.18</td>
</tr>
<tr>
<td>VNTR6</td>
<td>2–11</td>
<td>8</td>
<td>0.72</td>
<td>5</td>
<td>0.70</td>
<td>8</td>
<td>0.60</td>
</tr>
<tr>
<td>VNTR7</td>
<td>3–4</td>
<td>2</td>
<td>0.01</td>
<td>1</td>
<td>0.00</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>VNTR8</td>
<td>2–4</td>
<td>3</td>
<td>0.16</td>
<td>1</td>
<td>0.00</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*D, Simpson index of diversity; VNTR, variable-number tandem-repeat; NA, not applicable.
B. pertussis Clones

from 5 countries (China, France, Japan, United Kingdom, and United States) were distributed among 7 MTs: MT10, MT12, MT75, MT83, MT127, MT205, and MT206, 2 of which were also represented among recent strains: MT10, 6 isolates from Japan 1989–2007; and MT75, 1 isolate from France in 1993. The remaining 5 MTs were either unique or shared only among the 9 early isolates.

MLVA data were used to construct a minimum spanning tree (MST) (Figure 2). The 66 MTs were grouped into 2 clonal complexes and 9 singletons. Most MTs (54 of 66) belong to 1 clonal complex and 3 (MT186, MT187, and MT194) belong to another. Relationships between singletons with multiple allelic differences are not robust because they can be connected to other nodes equally. Thus, the MST cannot be rooted to infer the direction of change. Two internationally predominant MTs (27 and 29) are closely related with 1 allele difference. MT10, prevalent in Japan, is also closely related to MT29, with 1 allele difference. MT29, first isolated in the prevaccine era in the United Kingdom (11), has the highest number of SLVs and was found over 4 continents, which suggests that it arose early. Because MT10 and MT27 have a high frequency of SLVs, both likely emerged quite early. MT10 was isolated as early as 1957 in China and MT27 in 1950 in the Netherlands (10). Two high-frequency MTs (MT64 and MT70) were found in Australia only recently, with few SLVs, and may have contributed to the resurgence of pertussis in Australia.

Conclusions

Analysis of 208 isolates from Australia and representative isolates of common pulsed-field gel electrophoresis types from Canada, Japan, Finland, and the United States identified 6 predominant MTs (clones). Two (MT27 and MT29) were distributed worldwide, while 4 (MT10, MT64, MT70 and MT186) predominated in specific countries. Several MTs have persisted over long periods, including 3 that have circulated for at least half a century. Typing of genes encoding ACV antigens showed that use of ACV may have driven antigenic changes of 2 MTs now predominant in Australia.

Acknowledgments

We appreciate the generous donations of isolates by Kazunari Kamachi, Shane Byrne, Sullivan Nicolaides, John Tapsall, Ian Carter, Margret Ip, Qiushui He, Nicole Guiso, Raymond Tsang, and Lucia Tondella. We thank Sophie Octavia for technical assistance and the reviewers for helpful suggestions.

This research was supported by the National Health and Medical Research Council of Australia. J.K. was supported by an Australian Postgraduate Award.

Figure 1. Temporal trends of predominant multilocus variable-number tandem-repeat analysis (MLVA) types in Australia. Isolates of 4 major MLVA types (MT70, MT27, MT29, and MT64) obtained in Australia were divided into 3 periods: whole cell vaccine (WCV) (before 1997), transition from WCV to acellular vaccine (ACV) (1997–1999) and ACV (2000 onward).

Figure 2. Minimum spanning tree (MST) of multilocus variable-number tandem-repeat analysis (MLVA types for global Bordetella pertussis isolates. The MST produced in Bionumerics (Applied Maths, Kortrijk, Belgium) used categorical coefficient and the eBURST priority rule of the highest number of single-locus changes for the clustering. Each circle represents an MLVA type with the type number in the circle. Thick lines, types differing by a single MLVA locus; thin lines, double-locus variants; dotted lines, 2 types differing by >2 MLVA loci. The size of the circle reflects the number of isolates with a given MLVA type. The color codes for country of origin are shown, and pie charts within a circle are used to indicate the proportion of isolates.
Mr Kurniawan is a PhD student in medical microbiology at the University of New South Wales, Sydney, Australia. His research interests include molecular epidemiology and evolution of *B. pertussis*.

References


Address for correspondence: Ruiting Lan, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia; email: r.lan@unsw.edu.au