Molecular epidemiology and evolution of *Bordetella pertussis*: vaccine driven selection?

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Bordetella pertussis

- Gram negative coccobacillus
- Causes pertussis (whooping cough)
  - 50,000,000 infections worldwide
  - 300,000 deaths/year

- Protection → vaccination
Whole cell and Acellular vaccines

Whole cell vaccine (WCV) 1950s-1990s
- Fimbriae (FIM2/3)
- Pertussis Toxin (PT)
- Pertactin (PRN)
- Filamentous Haemagglutinin (FHA)

Acellular vaccine (ACV) 1997- present
- [Table showing antigenic components and dosages for Boostrix and Adacel]

<table>
<thead>
<tr>
<th>Antigenic component</th>
<th>Boostrix</th>
<th>Adacel</th>
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<tbody>
<tr>
<td>PT (µg)</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>FHA (µg)</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>PRN (µg)</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>FIM 2 + 3 (µg)</td>
<td>–</td>
<td>5</td>
</tr>
</tbody>
</table>
Incidences of pertussis in Australia

Multiple factors for re-emergence

- Increased awareness/better diagnostics
- Waning Immunity
- Adaptation of organism to vaccine selection
Analysis of Australian isolates from 1970s to present

• Multilocus Variable Number Tandem Repeat (VNTR) Analysis (MLVA)
  – 6/8 VNTR loci scheme
  – Rapid evolution

• Single Nucleotide Polymorphisms (SNPs)
  – Base changes between isolates - biallelic
  – Likely neutral if in housekeeping genes
  – Can be used to make inference of strain relationships

• Antigenic genes
  – Vaccine antigens (PT, PRN, FHA, FIM2 and FIM3)
  – Non-neutral evolution
Major Australian MLVA types in the last 40 years

SNP typing

- *B. pertussis* is very homogenous
- NimbleGen CGS SNP discovery: ~200 SNPs
- 65 SNPs typed
- 315 isolates (208 Australian isolates from 1970s to 2008) divided into 42 SNP profiles (SPs)
MT27
Australia (1994-2008) and 6 other countries

MT29
 Mostly Australian (1989-1996)

MT64
 Mostly Australian (1985-2002)

MT70
 Australian only (1977-2005)

MT186
 Australian but mostly Japanese isolates

No Australian isolates
Old isolates (1920-1954)

Correlation with ACV

- Clusters I and IV correlated with ACV period
- Cluster II correlated with WCV period

- Cluster I flourished since
- Disappearance of other clusters
  - Clusters III and IV have not been detected in Australia since 2008
What about changes to virulence (ACV antigenic) determinants?

Fimbriae (FIM2/3)

Pertactin (PRN)

Pertussis Toxin (PT)

Filamentous Haemagglutinin (FHA)
Changes in the pertussis toxin genes

ptxA1

GAC

ATA

D

I

ptxA2

..C

..G

D

M

Pre-vaccination

ptxA2

WCV

ACV

202

682
Higher toxin production from *ptxP3*

- Multiple alleles
  - *ptxP1* and *ptxP3* predominant

- *ptxP3* = 1.6x production of PT

ptxP3 specific to cluster I

ptxP3

Cluster I

Cluster II

Cluster III

Cluster IV

Cluster V

Cluster VI

Pertactin changes

**prn1** \(\rightarrow\) **prn2**

(ACV allele) \(\rightarrow\) (Non-ACV allele)

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He *et al.* 2003 *JID* 187 (8): 1200-1205

Prn2

• Allelic specific epitope
  – Prn type-specific antibodies (He et al. 2003)

• Mouse colonisation
  – Prn1>Prn2 and Prn3 (van Gent et al. 2011)
Advantage of double mutants

2008-2012 pertussis epidemic in Australia
2008-210 isolates and molecular typing

194 isolates

Molecular markers

- SNPs
- VNTRs
- prn and fim3
- ptxP
  - Mooi et al. 2009, ptxP1 & ptxP3 predominate
  - SNP typing ptxP3 ↔ non-ptxP3
SNP typing

- 11 SPs
  - 3 from cluster I (SP13, 14 & 16)
  - 2 from cluster II (SP17 & 37)
  - 6 from unclustered (SP1, 6, 7, 9, 11 & 18)
Identification of antigen non-expressing *B. pertussis* isolates
Summary

• Changeover of clones correlated with ACV
• Expansion of clones is associated with small changes
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