Bordetella pertussis Strain Lacking Pertactin and Pertussis Toxin

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A Bordetella pertussis strain lacking 2 acellular vaccine immunogens, pertussis toxin and pertactin, was isolated from an unvaccinated infant in New York State in 2013. Comparison with a French strain that was pertussis toxin–deficient, pertactin wild-type showed that the strains carry the same 28-kb deletion in similar genomes.

Pertussis has resurged in the United States in recent decades; >48,000 cases were reported in 2012 (http://www.cdc.gov/pertussis/surv-reporting/cases-by-year.html). Suggested causes include improved surveillance and diagnostics, waning immune response to acellular vaccines introduced in the United States in the 1990s (DTaP [diphtheria, tetanus, and pertussis]; Tdap, [tetanus, diphtheria, and pertussis]), and changes to circulating B. pertussis strains, which led to a mismatch with vaccine strains (1). Components of acellular pertussis vaccines in the United States are pertactin (Prn), pertussis-toxin (Pt), filamentous hemagglutinin, and sometimes fimbrial proteins 2/3. Since 2010, multiple mutations have been documented in the Prn-encoding gene (prn), which have spread rapidly across the United States and other countries (2,3). Pt-deficient Bordetella pertussis isolates are rare, with 1 report from France (4). To our knowledge, B. pertussis that lacks Pt and an additional acellular vaccine immunogen has not been documented.

The Case

Prodromal pertussis symptoms developed on March 4, 2013, in an 11-month-old white, non-Hispanic infant from New York State while the family was traveling outside the state. Cough reportedly began on March 14, 2013, and 12 days later (March 26) he was brought to his healthcare provider (HCP) with symptoms consistent with pertussis.

Since the child’s birth, the diagnosing HCP had seen the child only once; no visits to other HCPs were known. Per parental report, the case-patient was experiencing paroxysmal cough, apnea, and posttussive vomiting. No thoracic radiograph was obtained. A 5-day course of oral azithromycin was prescribed; the parent reported that the infant received treatment for 3 consecutive days, beginning March 26, 2013. The infant was not reported to have any pertussis-associated complications (seizures, pneumonia, or encephalopathy) and had only light coughing as of April 11, 2013.

The infant was unvaccinated because the parents refused administration of all vaccines. Three siblings, ages 12, 10, and 8 years, lived with the infant and were undervaccinated; they had received 2, 1, and 3 doses, respectively, of pertussis-containing vaccines. No coughing illness was reported among the siblings. The mother reported that she received Tdap vaccine during her pregnancy with the case-patient, but receipt of vaccine could not be verified.

A nasopharyngeal swab specimen was collected from the infant on March 26, 2013, for testing at a commercial laboratory. The isolate was also forwarded to New York State’s public health laboratory, the Wadsworth Center, where it was found to be positive for B. pertussis by PCR targeting IS481 and BP283. Both laboratories yielded positive culture results for B. pertussis. No other testing was performed.

The Wadsworth Center forwarded the isolate, designated I979, to the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA) for confirmatory identification and molecular typing as part of the Enhanced Pertussis Surveillance program. PCR amplification of the gene encoding the first subunit of Pt (ptxA) was unsuccessful while the CDC multitarget real-time PCR diagnostic assay was performed. Amplification of the promoter region (ptxP) and ptxA was also unsuccessful during multilocus sequence typing targeting acellular vaccine component genes ptxA, ptxP, prn, and fim3.

Further characterization of 1979 and French strain FR3749 was undertaken by multilocus sequence typing, multilocus variable-number tandem-repeat analysis, pulsed-field gel electrophoresis, and whole-genome sequencing. Long sequencing reads were obtained with the Pacific Biosciences RS II (Menlo Park, CA, USA) at >120× coverage and assembled de novo into a single contig by using HGAP v3 and Quiver v1. Assembly structure was confirmed with a genome.

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optical map after restriction digestion with KpnI (OpGen, Gaithersburg, MD, USA). The final sequence was polished with short reads obtained with Illumina MiSeq and CLC Genomics Workbench v7.5.1 (QIAGEN, Valencia, CA, USA) with >90x coverage. Completed genomes were submitted to the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) with GenBank accession nos. CP010965 (FR3749) and CP010966 (I979). Basic genome metrics are listed in Table 1.

Prt production was determined by ELISA (2,11). Pt production was examined through Western blot analysis of cultures grown in cyclodextrin-modified Stainer-Scholte liquid medium to optical density (OD) 600 nm = 0.1 (12). Proteins precipitated with trichloroacetic acid were washed, reduced, and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Pt detection is performed after growth for 48 hours. Protein was transferred with the iBlot Dry Blotting system (Invitrogen, Carlsbad, CA, USA). The primary antibody consisted of a concentration of 1b7 anti-PTX S1 monoclonal antibody at a dilution of 20 µg/mL diluted in 0.01 M PBS/Tween with 5% milk. The secondary antibody was a FITC-conjugated goat anti-mouse pAb from AbCam, diluted 1:1,000 with 0.01 M PBS/Tween with 5% milk. The benchmark protein ladder, 6–180 kDa (Life Technologies, Grand Island, NY, USA): lane 1, benchmark protein ladder, 6–180 kDa (Life Technologies, Grand Island, NY, USA); lane 2, Pt positive control, 2 µg; lane 3, empty; lanes 4 and 5, J024 (pertactin (Prt)+/Pt+); lanes 6 and 7, I979 (Prt+/Pt-); lanes 8 and 9, Tohama I (Prt+/Pt+); lanes 10 and 11, I978 (Prt+/Pt+); lanes 12 and 13, FR3749 (Prt+/Pt+); lanes 14 and 15, I974 (Prt+/Pt+).

Comparison of assembled 1979 and FR3749 genomes with that of Tohama I (GenBank accession no. NC_002929.2) (10) indicated that the entire ptx/ptl operon is missing as the result of a putative deletion spanning 28,040 bp (Figure 2). Both genomes contain a conserved, truncated IS481 immediately upstream of the deletion and a single IS481 (FR3749) or 2 tandem IS481 sequences (1979) immediately downstream (Figure 2). Within Tohama I, the region absent from 1979 and FR3749 encodes 30 predicted genes bound by 2 NCATGN motifs, the target sequence for IS481 insertion (Table 2). The insertion element IS1002 is located within the 3’ end of this region, and shares a GCATGG motif with IS481 immediately downstream. The 3’ deletion boundary is between IS1002 and IS481 (Figure 2). Whole-genome alignment, using progressiveMauve (14), of 1979 and FR3749 with Tohama I revealed structural variation through genomic rearrangements and inversions. In particular, 1979 and FR3749 genomes differ by a single, large inversion, the coordinates of which correspond to 2 conserved insertions of IS481 in opposing orientations (online Technical Appendix Figure). 1979 and FR3749 differ by 31 single nucleotide polymorphisms, each differing from Tohama I by 204 and 173 single nucleotide polymorphisms, respectively. FR3749 contains wild-type prn at position 1613, whereas 1979 prn contains an IS481 insertion, the most common cause of Prn-deficiency, at position 1613 (2,11).

Table 1. Characterization of Bordetella pertussis strains I979 and FR3749 in comparison to strain Tohama I*

| Strain | GenBank accession no. | Length, bp | No. IS481 | prn type | Prm | Reference |
|--------|-----------------------|------------|-----------|----------|-----|-----------|
| FR3749 | CP010965              | 4,079,396  | 249       | 2        | +   | This study |
| I979   | CP010966              | 4,082,551  | 252       | 2        | –   | This study |
| Tohama I | NC_002929.2         | 4,086,189  | 238       | 1        | +   | (10)      |

*Prn, pertactin; + positive; – negative.
Conclusions

*B. pertussis* strain I979, identified in our study, is both Prn- and Pt-deficient. Loss of Pt in *B. pertussis* is a rare occurrence; only 2 isolates have been documented in 8 years. Both I979 and FR3749 were isolated from unvaccinated infants (11 months and 3 months old, respectively), who exhibited typical pertussis symptoms, although FR3749 had difficulty colonizing and multiplying in respiratory tracts of adult mice (4). *B. pertussis* isolates with deletions at other sites across the genome, including part or all of *prn*, were reported previously (4, 15). During the past 5 years, US *B. pertussis* isolates have become nearly 100% Prn-deficient (2, 3) (unpub. data), and Prn-deficient isolates have been obtained from vaccinated persons (11). The loss of Pt may represent a higher fitness cost to *B. pertussis* than the loss of Prn. In addition, the possibility that only the Pt-deficient isolates were recovered from patients who were co-infected with wild-type and mutant *B. pertussis* cannot be discarded. Further testing in models to understand the clinical relevance of Prn- and Pt-deficient strains in vaccinated and unvaccinated persons is warranted.

Although incidence of combined Pt- and Prn-deficiency in *B. pertussis* is rare, any increased mutation in these

| Protein ID, GenBank accession no. | Gene | Product |
|----------------------------------|------|---------|
| NP_882281.1                      | ptxA | Hypothetical protein |
| NP_882282.1                      | ptxB | Pertussis toxin subunit 1 |
| NP_882283.1                      | ptxD | Pertussis toxin subunit 2 |
| NP_882284.1                      | ptxE | Pertussis toxin subunit 4 |
| NP_882285.1                      | ptxC | Pertussis toxin subunit 5 |
| NP_882286.1                      | ptxD | Pertussis toxin subunit 3 |
| NP_882287.1                      | ptiA | Type IV secretion system protein PtiA |
| NP_882288.1                      | ptiB | Type IV secretion system protein PtiB |
| NP_882289.1                      | ptiC | Type IV secretion system protein PtiC |
| NP_882290.1                      | ptiD | Type IV secretion system protein PtiD |
| NP_882291.1                      | ptiI | Type IV secretion system protein PtiI |
| NP_882292.1                      | ptiE | Type IV secretion system protein PtiE |
| NP_882293.1                      | ptiF | Type IV secretion system protein PtiF |
| NP_882294.1                      | ptiG | Type IV secretion system protein PtiG |
| NP_882295.1                      | ptiH | Type IV secretion system protein PtiH |
| NP_882296.1                      | tRNA-Asn |
| NP_882297.1                      | Membrane protein |
| NP_882298.1                      | AraC family transcriptional regulator |
| NP_882299.1                      | Hypothetical protein |
| NP_882300.1                      | Membrane protein |
| NP_882301.1                      | Hypothetical protein |
| NP_882302.1                      | Peptide ABC transporter substrate binding protein |
| NP_882303.1                      | Transport system permease |
| NP_882304.1                      | Pseudogene |
| NP_882305.1                      | IS481 | Transposase |
| NP_882306.1                      | argJ | Bifunctional ornithine acetyltransferase/N-acetylglutamate synthase |
| NP_882307.1                      | Hypothetical protein |
| NP_882308.1                      | Hypothetical protein |
| NP_882309.1                      | IS1002 | Transposase |

*ID, identification; ptx, pertussis toxin gene; pti, pertussis toxin liberation gene.*
or other acellular vaccine immunogens may have serious implications for the efficacy of current vaccines. Global epidemiologic, culture-based, and molecular-based monitoring of B. pertussis is critical for understanding current trends of the disease it causes.

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Technical Appendix

![Genomic rearrangement in Bordetella pertussis strains I979 and FR3749 compared with vaccine strain Tohama I, aligned in progressiveMauve.](http://darlinglab.org/mauve/user-guide/progressivemauve.html) I979 and FR3749 differ by 1 large rearrangement. Location of the 28-kb deletion that includes the *ptx/ptl* operon is marked by a red vertical line.

**Technical Appendix Figure.** Genomic rearrangement in *Bordetella pertussis* strains I979 and FR3749 compared with vaccine strain Tohama I, aligned in progressiveMauve (http://darlinglab.org/mauve/user-guide/progressivemauve.html). I979 and FR3749 differ by 1 large rearrangement. Location of the 28-kb deletion that includes the *ptx/ptl* operon is marked by a red vertical line.