THE PENNSYLVANIA STATE UNIVERSITY
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IMPROVING THE DIAGNOSIS, TREATMENT, AND PREVENTION OF WHOOPING
COUGH: A CHARACTERIZATION OF THE CURRENTLY CIRCULATING STRAINS OF
BORDETELLA PERTUSSIS IN THE UNITED STATES

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Abstract

Prior to the advent of a whooping cough vaccine in the 1950s, hundreds of thousands of pertussis cases were reported annually in the United States. Although the vaccine era has seen a remarkable decline in cases, incidence has been steadily increasing since the 1980s, hitting a 50-year high of 48,000 cases in 2012. Several recent studies have demonstrated the continued genetic shift in pertussis strains since the pre-vaccine era, while other research endeavors have suggested the inadequacy of current acellular vaccines to prevent infection and transmission. In this study, we characterize 35 currently circulating strains of *Bordetella pertussis*, the main causative agent of whooping cough, collected through the Collaborative Pediatric Critical Care Research Network, the Centers for Disease Control and Prevention, and several U.S. children's hospitals. We have established a more comprehensive profile of the current strains by evaluating isolate-specific variations in susceptibility to competitive inhibition and clinically used antibiotics, genomic sequence, pulsed-field gel electrophoresis (PFGE) type, virulence factor production, *in vivo* growth, and acellular vaccine protective response. Our analysis indicates consistent sensitivity to clinical antibiotics and a lack of competitive inhibition. However, there is tremendous variation in PFGE types and virulence factor alleles, particularly filamentous hemagglutinin (FHA) and pertactin (PRN), the latter of which demonstrates strain-specific diversity in antibody recognition. Despite similar growth *in vivo*, strains also exhibited differences in host immunity and vaccine protective response, suggesting that vaccination may not protect as adequately against clinical isolates, particularly PRN-negative strains, as it does against a lab strain from the pre-vaccine era.

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Introduction

Whooping cough, also known as pertussis, is a highly communicable upper respiratory disease caused by a Gram-negative coccobacillus species of bacteria called *Bordetella pertussis*. Bacterial attachment to the respiratory tract and the production of toxins results in inflammation and the arrest of cilia activity, inhibiting the clearing of infectious pulmonary secretions.[1] Following airborne transmission and an incubation period usually ranging from seven to ten days, the disease proceeds through three separate stages.[1] The first stage, the catarrhal stage, involves symptoms such as a runny nose, a low-grade fever, and a mild cough; such close resemblance to the common cold and high communicability at this stage makes pertussis difficult to accurately diagnose and effectively treat early in the course of infection.[1] Progression into the paroxysmal stage, which usually lasts six weeks, is marked by paroxysmal coughing, posttussive vomiting, and the inspiratory whoop for which the disease is aptly named.[2] In the final stage of infection, the convalescent stage, recovery is gradual and symptoms may take months to completely subside.[1] For adults and teenagers, the disease symptoms are often mild or completely asymptomatic, making these individuals unknowing reservoirs for spreading the infection to much more susceptible populations such as infants and young children.

Medical management of pertussis involves treatments that are more supportive than curative, particularly after the onset of the paroxysmal stage.[1] First line treatment to combat symptoms involves a two-week course of erythromycin, or a shorter regimen with milder macrolide antibiotics, such as azithromycin.[3] Although these medications are often accompanied by unpleasant side effects, antibiotics may decrease transmission risk by eliminating the bacteria from mucosal secretions.[1] Yet, of notable concern are the complications that can arise in young, incompletely immunized individuals. These patients are often hospitalized due to apnea, secondary bacterial pneumonia, or seizure onset, eventually succumbing to respiratory failure,

sepsis, and death in critical cases.[1] Although nearly 63% of pertussis cases occur in individuals aged 10 years and older, 17% of cases occur in patients younger than six months, for whom the risk of hospitalization is 60% higher.[3] Furthermore, this young age group is 20-fold more susceptible to infection and accounts for more than 90% of pertussis-related deaths.[4] Thus, due to the higher risks associated with infant and neonate pertussis infections, as well as the high communicability of the disease from older individuals, preventative measures such as antibiotic prophylaxis and vaccination are of particular importance.

Prior to the availability of pertussis vaccines, whooping cough was a common cause of morbidity and mortality in young children.[1] In the first forty years following the introduction of the whole-cell pertussis vaccine (DTP), the incidence of whooping cough dropped nearly 99%![3] However, for safety reasons, the original whole-cell vaccine has since been replaced by an acellular vaccine, which contains two to five inactivated virulence factor proteins and is administered in combination with tetanus and diphtheria toxoids.[1] These virulence factors include pertussis toxin (PTX), which helps to immobilize respiratory cilia and delays leukocytosis, pertactin (PRN), an outer membrane protein that promotes adhesion to tracheal cells, filamentous hemagglutinin (FHA), which promotes biofilm formation and cellular adhesion, and fimbria types 2 and 3 (FIM2/3), which function as attachment pili. Furthermore, the decreased toxicity of acellular vaccines has facilitated the development of pediatric formulations (DTaP), as well as adolescent and adult formulations (Tdap), allowing for a more extensive vaccination schedule that builds strong immunity in young infants and children, while reinforcing herd immunity among the older population.[5] DTaP formulations (Infanrix from GlaxoSmithKline, Tripedia from Sanofi Pasteur, and Daptacel from Sanofi Pasteur) are administered at 2, 4, and 6 months of age, followed by a fourth dose around the fourth birthday

and a fifth recommended dose before pre-school; as part of a cocooning strategy, Tdap

formulations (Boostrix from GlaxoSmithKline and Adacel from Sanofi Pasteur) are given as

boosters to adolescents aged 11 to 18 years, adults aged 19 to 64 years, and adults aged 65 years

or more who anticipate contact with an infant.[1] As of 2011, approximately 96% of children aged

19 to 35 months, 78% of adolescents, and 13% of adults in the United States were vaccinated.[6]

However, despite such widespread vaccination coverage, pertussis remains the most frequently

reported vaccine-preventable disease in the United States and has been steadily increasing in

incidence since the 1980s (Figure 1).[2, 7]
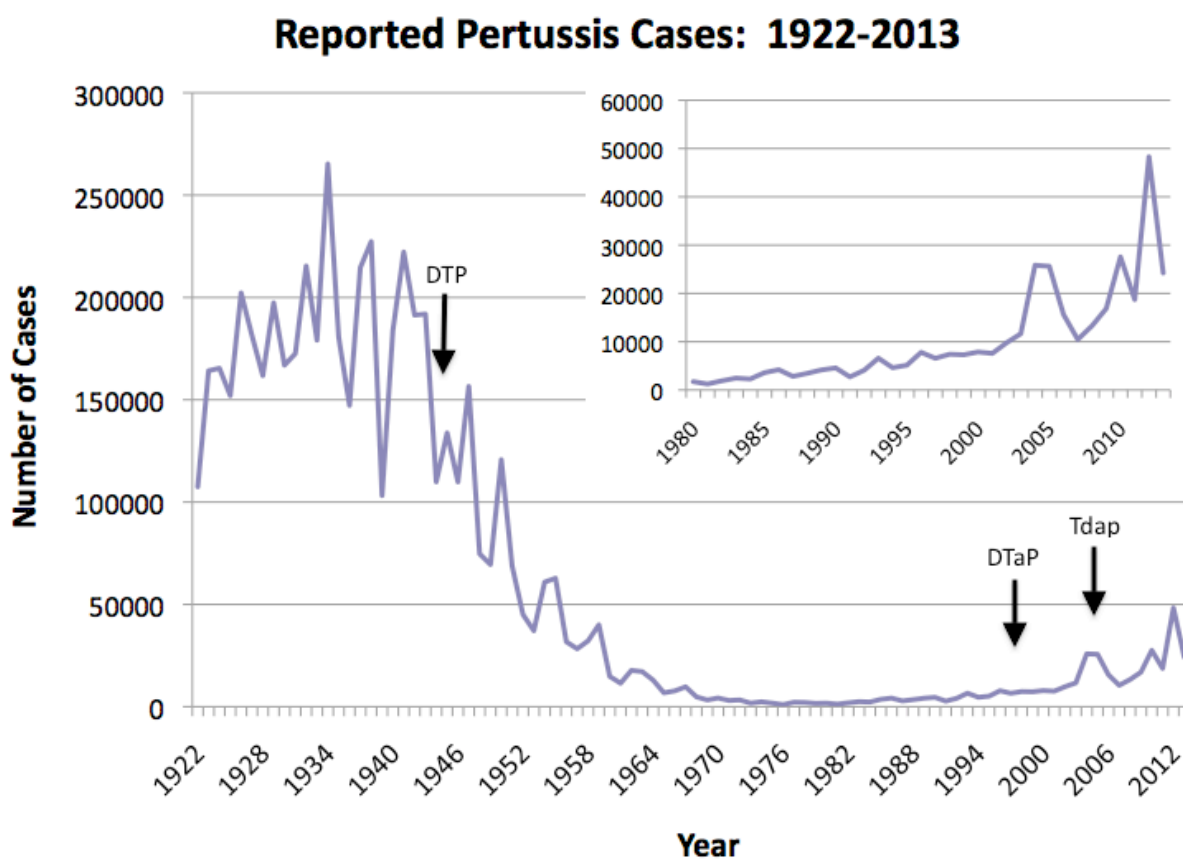

Figure 1: Pertussis cases reported to the CDC per the National Notifiable Diseases Surveillance System (NNDSS) from 1922 to 2013. Cases are defined as a cough illness lasting more than 2 weeks with paroxysmal coughing, inspiratory whoop, posttussive vomiting, or apnea; laboratory diagnostic criteria include isolation of and positive PCR for *B. pertussis* from clinical sample.[8]

Although national incidence still remains much lower than that reported in pre-vaccine era epidemics, whooping cough rates reached a 50-year high in 2012.[9] Some of this increase in incidence may be due to enhanced awareness and improved diagnostic techniques, but case-controlled studies following recent pertussis outbreaks have shed light on the dynamics of pertussis immunity over time. In recent years, 10 to 13-year-old adolescents have emerged as the primary population infected in pertussis epidemics, emphasizing the need for booster Tdap vaccines to bolster vaccine-induced herd immunity for under-immunized infants, the age group accounting for the second largest portion of epidemic pertussis cases.[10] This shift in the age structure of whooping cough outbreaks also suggests that vaccine-induced immunity is not life-long. In fact, studies have determined that immunity wanes as early as five years following a fifth DTaP booster, and that by age 14, over 90% of pertussis cases occur in previously vaccinated patients.[11] Acellular vaccine efficacy is estimated to range between 75% and 90% with a protective duration of about five years, mimicking the extent of natural pertussis immunity.[2, 11] Furthermore, a very recent study using a nonhuman primate model found that, although acellular vaccination induces large antibody responses and prevents infection symptoms, transmissible bacteria still colonize the respiratory tracts of aP-vaccinated test animals for up to six weeks.[12] In light of these findings and the continued increase in pertussis incidence in the United States, it has become imperative to answer the questions of why whooping cough has resurged and how to effectively minimize both infection and transmission.

In collaboration with the Collaborative Pediatric Critical Care Research Network, the Centers for Disease Control and Prevention, and several U.S. children's hospitals, we have studied 35 clinical isolates collected from pertussis patients. Evaluation of individual strain susceptibility to competitive inhibition and clinically used antibiotics, as well as a comparison of

genomic differences, PFGE type variation, virulence factor production, capacity for lung

colonization, and vaccine protective responses has revealed much about the types of whooping

cough currently afflicting American children. In characterizing the currently circulating strains

of *B. pertussis*, this study aims to provide insight regarding strain-specific differences in

infectivity and virulence to gain a better understanding of recent outbreaks and allow for the

improved prevention, diagnosis, and treatment of whooping cough.

Materials and Methods

Clinical Isolates and Bacterial Growth Conditions. Clinical *Bordetella pertussis* stains isolated from pediatric intensive care unit patients were provided by the Collaborative Pediatric Critical Care Research Network (CPCCRN); additional isolates were provided by Christopher Newth from the Children's Hospital of Los Angeles (CHLA) and the Centers for Disease Control and Prevention (CDC). *B. pertussis* lab strains Tohama I and 536, a streptomycin and nalidixic acid-resistant Tohama I derivative, were used as controls.[13] Each isolate was grown at 37°C on Bordet Gengou (BG) agar (HIMEDIA) containing 1% glycerol and supplemented with 10% defibrillated sheep's blood (HemoStat Laboratories). Liquid cultures were made in Stainer-Schölte media (prepared as described previously) supplemented with 4 mg/mL L-cystein, 1 mg/mL $FeSO_4 \cdot 7H_2O$, 0.4 mg/mL nicotinic acid, 15 mg/mL glutathione, 40 mg/mL ascorbic acid and heptakis (Sigma Aldrich) by suspending colonies taken from blood agar plates five days post-streaking; bacterial cultures were grown at 37°C overnight with shaking to mid-log phase.[14]

Genomic DNA Isolation, PFGE Typing, and Sequencing. Genomic DNA (gDNA) for each strain was isolated using a DNeasy Blood & Tissue Kit (Qiagen) according to manufacturer's instructions up to incubation with proteinase K; the remainder of the extraction was conducted using a modified phenol chloroform protocol in which Phase Lock Gel tubes were replaced with Eppendorf tubes and concentration by ethanol precipitation was performed prior to sodium acetate addition.[15, 16] Isolated gDNA was sequenced by the J. Craig Venter Institute (JCVI).[17] Isolate stocks were subjected to pulsed-field gel electrophoresis (PFGE) typing via *XbaI* endonuclease restriction by the CDC, as diagramed in Figure 2.[18, 19]
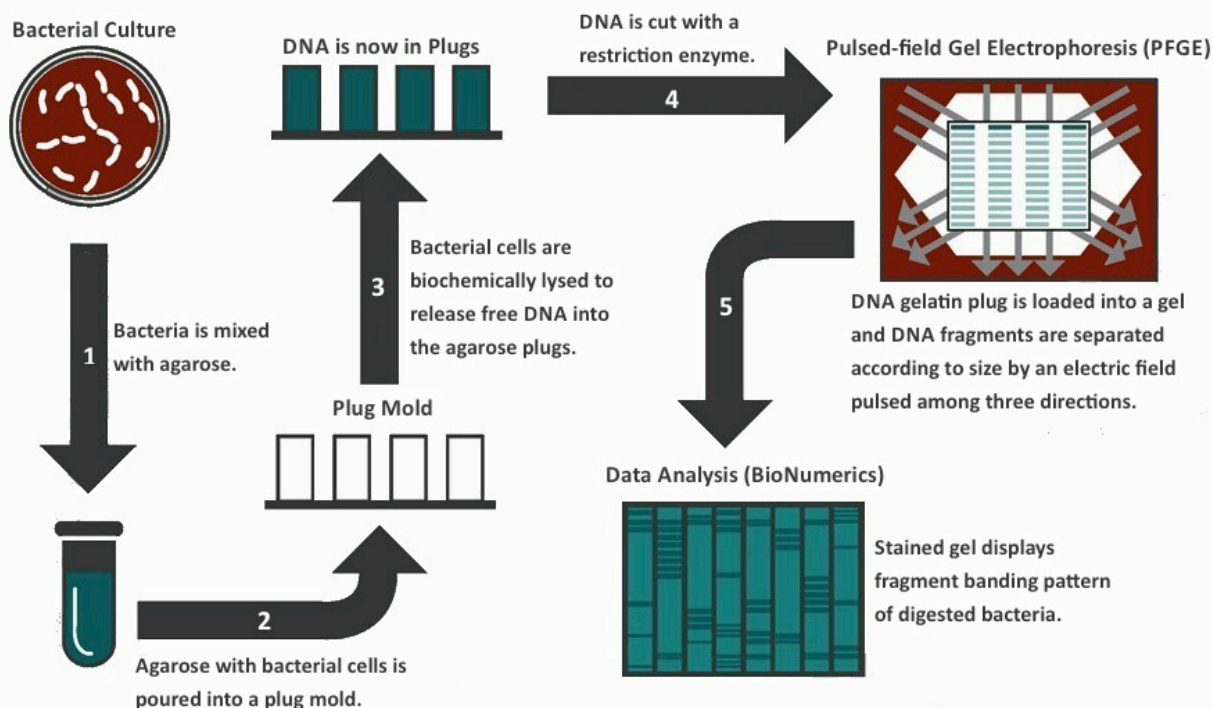
Figure 2: Schematic representation of the method behind PFGE for the profiling of bacterial specimens. (Adapted from *PulseNet*, Centers for Disease Control and Prevention).[19]

SNP Analysis. All *B. pertussis* clinical and reference strains were randomly shredded into 54 bp long reads and mapped onto the reference genome (Tohama I), using Ssaha v2.2.1.[20] High quality candidate single-nucleotide polymorphisms (SNPs) were identified using ssaha_pileup, and 1,496 SNP sites were identified in at least one strain based on Tohama I. Phylogenetic trees were constructed with RAxML v7.0.4 for all SNP sites in the reference genome, using a General Time Reversible (GTR) model with a gamma correction for among site rate variation and ten random starting trees.[21, 22]

Cross Streaking. A grid of cross-struck strains was generated on BG agar by linearly swabbing each randomly sampled strain across the appropriate plate. Plates were incubated at 37°C for

five days. Intraspecies competition was defined as regions of growth inhibition at the points of

intersection between different strains.

Polymerase Chain Reaction. Genomic DNA from each strain was extracted as described above.

Each reaction tube contained 17.6µL dH$_2$O, 2.5µL 10× ThermoPol Buffer (New England

BioLabs), 1.25µL DMSO (Sigma Aldrich), 0.4µL dNTPs (Sigma Aldrich), 0.25µL *Taq*

Polymerase (BioLabs), 1µL Reverse Primer, 1µL Forward Primer, and 1µL gDNA template;

PCR program conditions and pertussis toxin (*ptx*) gene primer sequences are listed in Table 1.

Amplified PCR product (20µL sample) prepared with 6x Loading Dye (5µL per sample; New

England BioLabs) was run on 1% agarose gels (IBI Scientific) at 150V with 5µL HyLadder 1Kb

(Denville Scientific Inc).

Table 1: PCR reaction conditions and primer sequences for *ptx* genes

Gene	5'-Forward Sequence-3'	5'-Reverse Sequence-3'
ptxA	ACT GCA ATC CAA CAC GGC ATG AAC	GGC ATG CTG TTC AAT TAC CGG AGT
	PCR Program: 94°C 5min; 94°C 30sec; 60°C 30sec; 72°C 1min; 72°C 8min; 4°C ∞	
ptxB	ATC GCG TAT TCG TTC TAG ACC TGG	TTC ACC AGC ACA TAA GGA ACG TCG
	PCR Program: 94°C 5min; 94°C 30sec; 58°C 30sec; 72°C 45sec; 72°C 8min; 4°C ∞	
ptxC	AAT CCG CTT GAG ACG ATC TTC CG	GAT TCA TTC GCG GTA TCC GTC AAG
	PCR Program: 94°C 5min; 94°C 30sec; 58°C 30sec; 72°C 45sec; 72°C 8min; 4°C ∞	
ptxD	TGA GGA CGC AAC GTT CGA GAC TTA	GAC AAC AGG ATG GAT GCG ATG GTA
	PCR Program: 94°C 5min; 94°C 30sec; 59°C 30sec; 72°C 30sec; 72°C 8min; 4°C ∞	
ptxE	GTT CTG CTT CGG CAA GGA TCT CAA	TTG ATC AGC ATG TTG CGG TGT TCC
	PCR Program: 94°C 5min; 94°C 30sec; 59°C 30sec; 72°C 30sec; 72°C 8min; 4°C ∞	
ptxP	CCC AAG ATA ATC GTC CTG CTC AAC	CTG TTC TTG CGG TTT GGC GAA T
	PCR Program: 94°C 5min; 94°C 30sec; 57°C 30sec; 72°C 15sec; 72°C 8min; 4°C ∞	

Immunoblot. Whole-cell lysates of each strain were extracted from mid-log phase bacterial

culture (0.3 < OD$_{600}$ < 1.2) normalized to 10^9 bacteria (1 OD unit = 10^9 bacteria); cultures were

centrifuged at maximum speed for 10min and each remaining pellet was re-suspended in 100µL

Sample Buffer (BIO RAD) prepared according to manufacturer's instructions and boiled in a water bath for 10 min. Bacterial lysates (10µL sample for PRN blots and 5µL sample for FHA blots) were run on 7% SDS-PAGE gels at 150V, with 5µL PageRuler Plus Prestained Protein Ladder (ThermoScientific); recombinant PRN (15.2ng/well) or FHA protein (15.2ng/well), provided by Sanofi Pasteur, and prepared Tohama I lysate (5µL for FHA blots and 10µL for PRN blots) were used as controls. Electrophoresed proteins were transferred to Immun-Blot PVDF membranes (BIO RAD) at 100V for 60min in a trans-blotting buffer (TBB) mixture (1:2:2 ratio of methanol:TBB:dH$_2$O). Prior to antibody probing, membranes were blocked with 5% non-fat milk in Tris Buffer Saline-Tween 20 (TBST) to minimize non-specific binding. Membranes were probed with anti-FHA sera (1:1,000 dilution with 5% non-fat milk in TBST) or anti-PRN sera (1:500 dilution with 5% non-fat milk in TBST) overnight with shaking; mouse serum for probing was collected on Day 24 following vaccinations with the appropriate protein and alum adjuvant on Days 0, 14, and 21. Membranes were washed three times with TBST and all probed with anti-mouse (immunoglobulin H) horseradish peroxidase-conjugated antibody (SouthernBiotech) at 1:10,000 dilution with 5% non-fat milk for 1hr. Following three more TBST washes, the membranes were visualized using SuperSignal West Pico Chemiluminescent Substrate (ThermoScientific).

Minimum Inhibitory Concentration (MIC) Assays. In 96-well plates, 100µL of mid-log phase bacterial culture ($0.3 < OD_{600} < 1.2$) was added to serial dilutions (0, 0.01, 0.1, 1, and 10 µg/mL diluted with PBS) of azithromycin (TCI) or erythromycin (Sigma Aldrich) for a total well volume of 200µL. Bacteria were incubated at 37°C while shaking at 210rpm, and OD_{600} were measured for each plate at approximately 0, 24, 48, and 72 hours. Each MIC_{50} value was

determined based upon the OD_{600} value recorded at the 48-hour time point for each strain, and was calculated as the antibiotic concentration at which the growth of the strain was decreased to at least half that of the strain's growth in PBS-only.

Animal Experiments. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC), and all animals were handled in accordance with institutional guidelines (IACUC Approval No. 40029). To determine bacterial burden after 3 or 7 days post-infection, groups of 3 to 4 mice (F2.129 wild type; Jackson Labs) were intranasally inoculated with 5×10^5 CFU in 50µL PBS of the appropriate strain on Day 0. Strains for this experiment were chosen to collectively represent all locations of isolate collection; *B. pertussis* strain 536 was used as a positive control *in vivo*. At 3 and 7 days post-infection, mice were euthanized and the lungs were excised and homogenized in 1mL PBS. Serial dilutions were used to enumerate bacterial burden in the lungs and plated on BG agar, incubating at 37°C for five days. To evaluate vaccine protectiveness, groups of 3 to 4 mice (C57BL/6J wild type; Jackson Labs) were intraperitoneally vaccinated with either 20µL Sham (Imject Alum (ThermoScientific) in 200µL PBS) or one fifth human dose of Adacel (Sanofi-Pasteur; 0.5 µg PT, 1 µg FHA, 0.6 µg pertactin, 5 µg fimbriae 2 and 3) with Imject Alum (ThermoScientific) on Days 0 and 14, and challenged on Day 28 post-vaccination as above. *B. pertussis* strain 536 was used as a positive control *in vivo*. At 3 days post-infection, the lungs were excised and homogenized in 1 mL sterile PBS. Serial dilutions were used for bacterial enumeration on Regan Lowe media supplemented with 40µg/mL cephalexin (Becton Dickinson).

Data Analysis. The means ± standard errors were calculated for all appropriate data. For animal studies, a one-way ANOVA unstacked with Tukey's comparison was used with a 95% confidence interval.

Results

Recent outbreaks have involved many different circulating PFGE types despite high strain similarity.

Pulsed-field gel electrophoresis (PFGE) analysis performed by the CDC revealed tremendous variation among currently circulating PFGE types. A compilation of all identified PFGE types, as well as the associated strains and information on the isolate source is presented in Table 2. There are currently six predominant PFGE types: 253, 13, 324, 321, 217, and 82. Figure 3A presents the PFGE data as organized by location and suggests no correlations between PFGE type and location or fatality of infection. Yet, the presence of newly identified PFGE types (324, 321, 338, 332, 330, 327, 325, 323, and 322), many of which appeared during the most recent 2012 outbreaks (Figure 3B), demonstrates that circulating strains are continually evolving; although the CDC isolates were selected for diversity, slightly biasing our sample, it is still significant to note the emergence of these new PFGE types.[18] Additionally, pair-wise comparison of genomic sequences reveals that isolates are very closely related (Figure 4), demonstrating $\geq 99\%$ similarity (Yury Ivanov, unpublished data); for example, isolate 2250905 is most closely related to the reference strain Tohama I, exhibiting 400 SNP differences.

Table 2: Full list of isolated strains, associated collaborators, year and location of collection, and PFGE type

Strain ID	Collaboration	Year	Location	PFGE Type
STO1-SEAT-0004	CPCCRN	2011	WA	253
STO1-SEAT-0006	CPCCRN	2012	WA	237
STO1-SEAT-0007	CPCCRN	2012	WA	13
STO1-CHOC-0008	CPCCRN	2010	CA	269
STO1-CHOC-0016	CPCCRN	2010	CA	324
STO1-CHOC-0017	CPCCRN	2010	CA	13
STO1-CHOC-0018	CPCCRN	2010	CA	338
STO1-CHOC-0019	CPCCRN	2010	CA	321
STO1-CHOC-0021	CPCCRN	2010	CA	217
STO1-CHOC-0026	CPCCRN	2012	CA	2
STO1-CNMC-0004	CPCCRN	2010	DC	230
STO1-CHLA-0006	CPCCRN	2010	CA	324
STO1-CHLA-0011	CPCCRN	2012	CA	253
STO1-CHOM-0012	CPCCRN	2010	MI	82
CHLA-11	CHLA	2010	CA	38
CHLA-13	CHLA	2010	CA	253
CHLA-15	CHLA	2010	CA	82
CHLA-20	CHLA	2010	CA	253
CHLA-25	CHLA	2010	CA	253
CHLA-26	CHLA	2010	CA	268
1996168	CHLA	2010	CA	170
2356847	CHLA	2010	CA	253
2370872	CHLA	2010	CA	82
2250905	CHLA	2010	CA	253
2371640	CHLA	2010	CA	13
H784	CDC	2012	OR	273
H897	CDC	2012	WA	242
H939	CDC	2012	WA	322
H921	CDC	2012	WA	324
H918	CDC	2012	WA	323
H934	CDC	2012	WA	217
H973	CDC	2012	WA	325
I002	CDC	2012	WA	321
I036	CDC	2012	WA	327
I176	CDC	2012	WA	330
Tohama I	LAB	1951	Japan	232
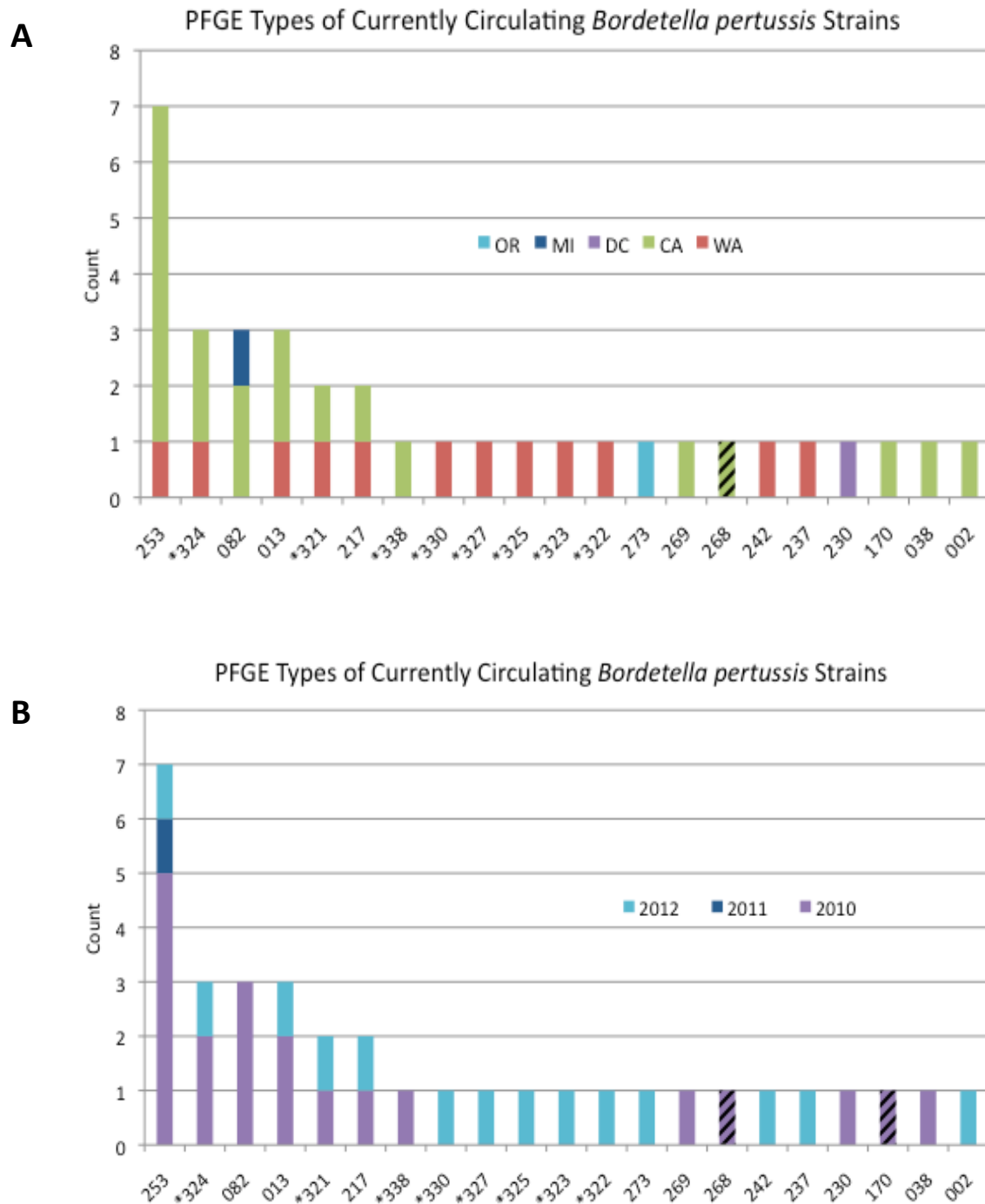
Figure 3: Frequency of PFGE types among currently circulating strains of *B. pertussis*.
PFGE analysis was performed on isolated strains. Each colored bar represents the
frequency of PFGE types in each location or year of isolate collection; striped bars
represent PFGE types associated with cases of infant fatality and * designates new PFGE
types. (A) Variation in PFGE types categorized by location. (B) Variation in PFGE types
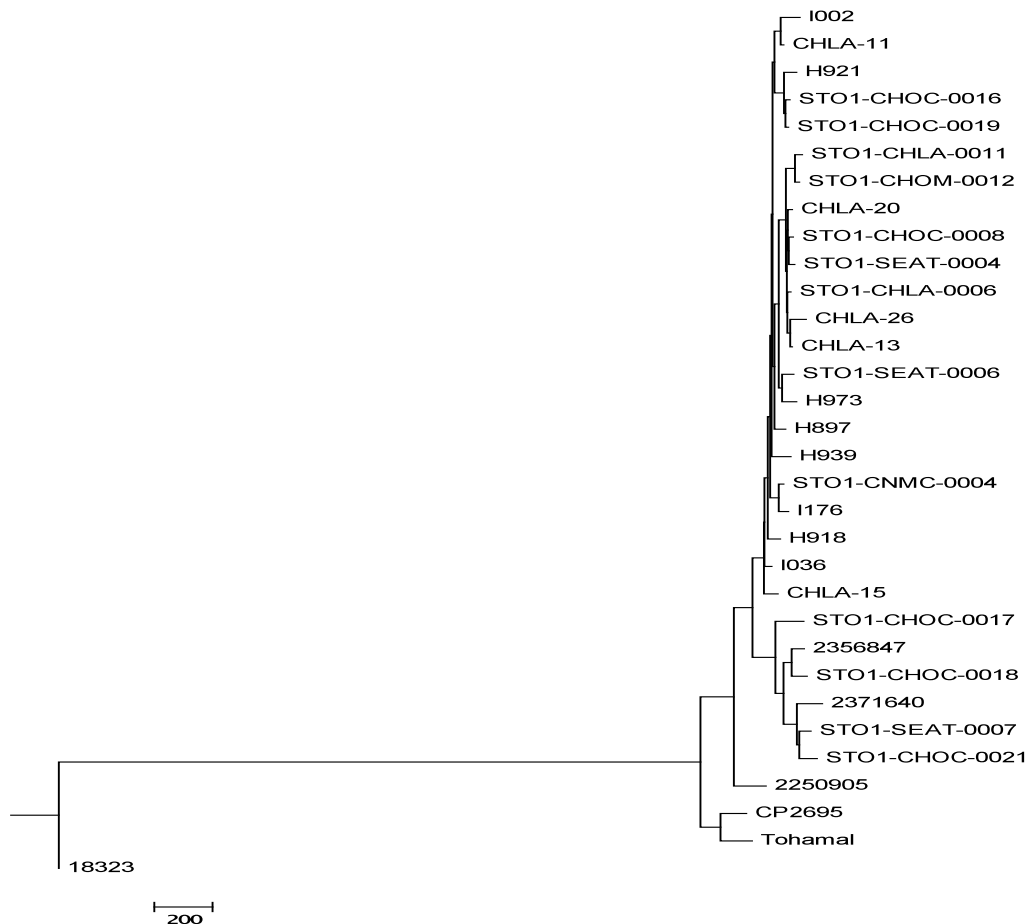categorized by year.

Figure 4: SNP tree suggesting the overall similarity of the *B. pertussis* genome. Genomic DNA from each strain was isolated via a modified phenol-chloroform protocol and concentrated by ethanol precipitation before being sent to JCVI for sequencing. (Jihye Park, unpublished data).

Intraspecies competition is absent among currently circulating strains.

Random cross-streaking of *B. pertussis* isolates (Figure 5) was conducted to determine if individual strains could engage in competitive inhibition. The lack of changes in growth patterns at points of strain intersection, demonstrates that currently circulating strains do not compete with each other. Despite the fact that certain strains (STO1-CHOC-0008 and STO1-CNMC-0004) are associated with cases of infant fatality, there do not appear to be any strains capable of outcompeting other *B. pertussis* strains.
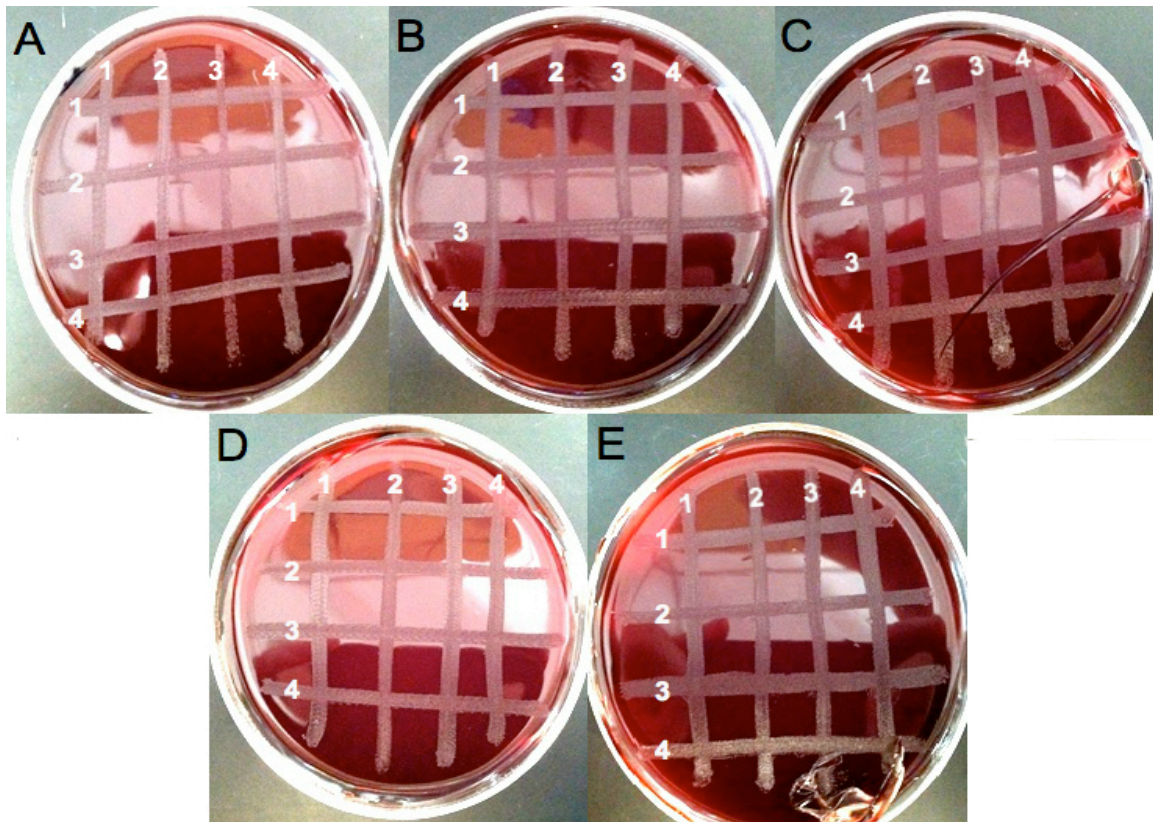
Figure 5: Cross-streaks exhibiting no intraspecies competition. Strains were struck on BG agar plates and incubated for five days at 37°C. Competition was defined as the presence of a zone of inhibition at strain intersections. (A) (1) STO1-CHOC-0006, (2) STO1-CHOC-0008, (3) STO1-CHOM-0012, (4) STO1-CHOC-0016. (B) (1) CHLA-13, (2) CHLA-15, (3) 2356847, (4) 2250905. (C) (1) 1996168, (2) 2230997, (3) CHLA-25, (4) CHLA-26. (D) (1) STO1-CHOC-0021, (2) STO1-CHOC-0026, (3) CHLA-11, (4) CHLA-20. (E) (1) 2371640, (2) 2370872, (3) STO1-CNMC-0004, (4) STO1-CHOC-0026.

Strains share the genes of the ptx locus.

PTX is a multimeric protein virulence factor found in all current acellular pertussis vaccines. It is composed of five subunits (S1 through S5) encoded by a polycistronic operon (Figure 6), which also codes for the *Ptl* machinery proteins required for toxin secretion via a Type IV secretion system (T4SS).[23] Shuttling of the toxin through the inner and outer membranes of the bacterial cell facilitates its extracellular action on a variety of target host cells, leading to a disruption in intracellular signaling and modulation of the adaptive immune response.[23]
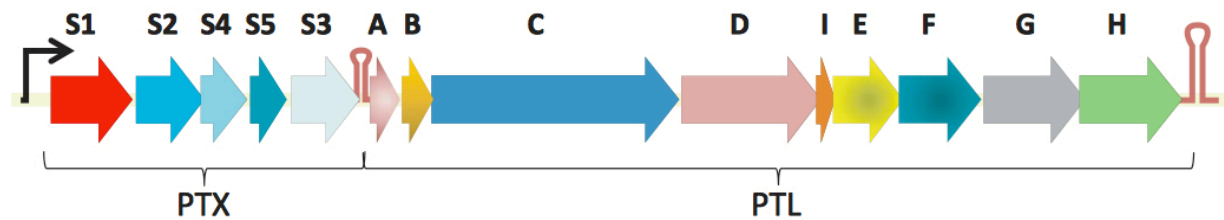
Figure 6: Diagram of the *ptx/ptl* locus. (Adapted from Locht et al.) Promoter (ptxP) is represented by the black arrow, and the hairpin structures designate transcriptional attenuation/termination sites.[23]

To determine the presence or absence of the PTX genes among currently circulating *B. pertussis* strains, PCRs were performed using isolated gDNA as template. In Figure 7, agarose gels of the resulting PCR products demonstrate that all of the randomly selected isolates, including those associated with cases of infant fatality (STO1-CHOC-0008 and STO1-CNMC-0004), exhibit the genes of the PTX locus. Further analysis to identify possible strain-specific genotypic differences in the PTX locus will be discussed in the following section.

Figure 7: Agarose gels of PCR product for genes of the *ptx* locus. Isolated genomic DNA for each randomly selected st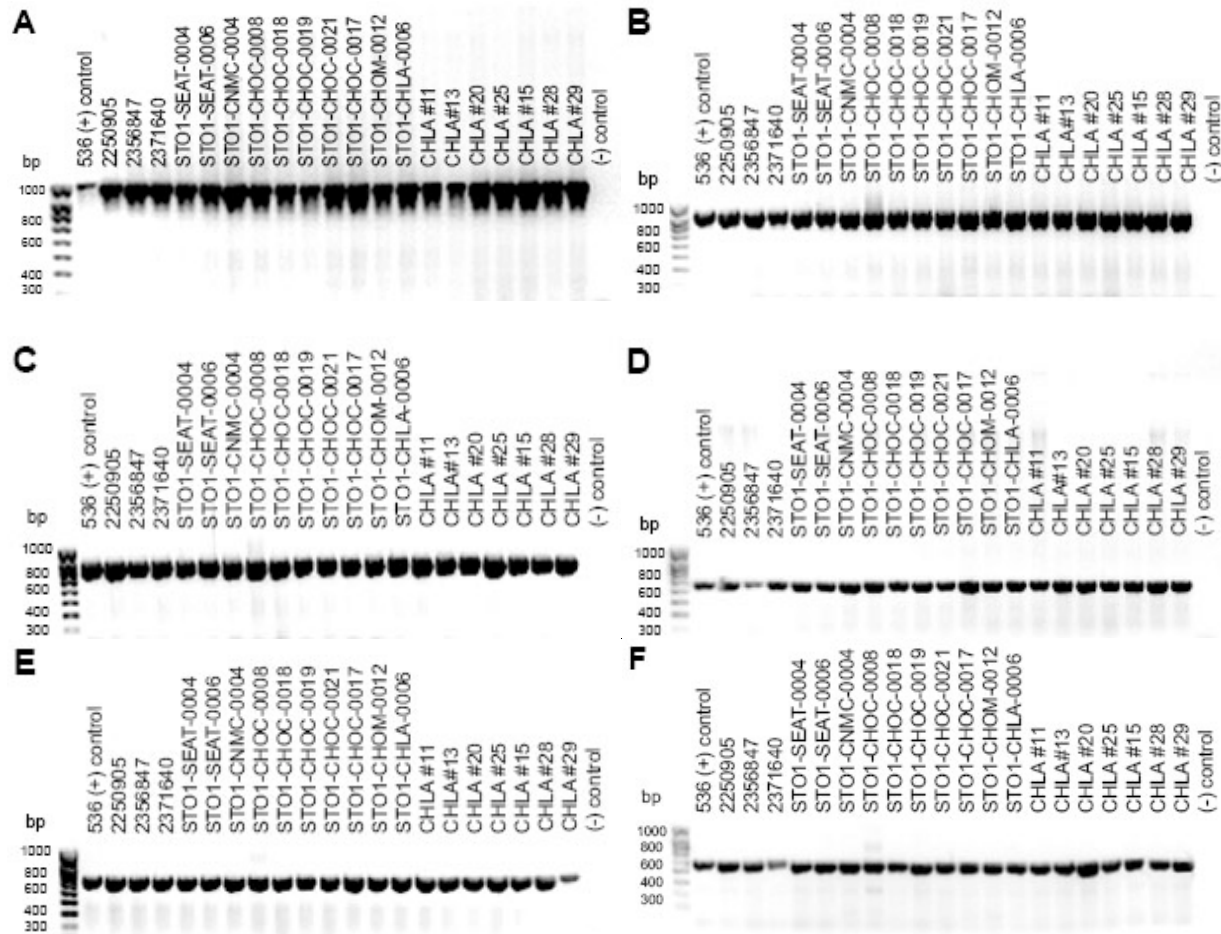rain was used as template for PCR reactions. Cistrons S1, S2, S3, S4, and S5 (Figure 5) are represented here as genes *ptxA, ptxC, ptxB, ptxD*, and *ptxE*, respectively. (A) Amplified *ptxA* gene. (B) Amplified *ptxB* gene. (C) Amplified *ptxC* gene. (D) Amplified *ptxD* gene. (E) Amplified *ptxE* gene. (F) Amplified ptxP sequence.

Strains differ in allele type and antibody recognition of vaccine-associated virulence factors.

DNA sequencing reveals strain-specific differences in the genes for and production of *B. pertussis* vaccine-component virulence factors. Table 3 presents each strain's alleles for vaccine-associated virulence factors, including pertussis toxin (PTX), fimbriae (FIM), pertactin (PRN), and filamentous hemagglutinin (FHA); six isolates (STO1-CHOC-0026, CHLA-25, 1996168, 2370872, H784, H934) are still in the process of being sequenced. This table also shows the pertinent PRN and FHA antibody-recognition status (positive or negative) of each

strain. All isolates contain the ptxP3, *ptxA1*, *fim2-1*, and *fim3-1*/*fim3-2* alleles, with the

exception of 2250905 (*ptxP1* allele) and H897 (truncated *fim2* allele). This suggests that the

ptxA and *fim2/3* genes are highly conserved across strains. In contrast, the lab strain Tohama I

exhibits the *ptxP2*, *ptxA2*, *fim2-1*, and *fim3-1* alleles. The prevalence of currently circulating

alleles that are different from that of Tohama I suggests a shift in gene content, which is

consistent with the strain evolution suggested by the PFGE type data and recent publications.[24]

Table 3: Vaccine-associated virulence factor alleles and FHA and PRN antibody recognition variation[a]

Strain ID	ptxP	*ptxA*	*fim2*	*fim3*	*fhaB*		*prn*	
					Allele	Ab	Allele (Group)[b]	Ab
STO1-CHOC-0026	UNK.[c]	UNK.	UNK.	UNK.	UNK.	+	UNK.	+
CHLA-25	UNK.	UNK.	UNK.	UNK.	UNK.	+	UNK.	-
1996168	UNK.	UNK.	UNK.	UNK.	UNK.	+	UNK.	+
2370872	UNK.	UNK.	UNK.	UNK.	UNK.	+	UNK.	+
H784	UNK.	UNK.	UNK.	UNK.	UNK.	+	UNK.	+
H934	UNK.	UNK.	UNK.	UNK.	UNK.	+	UNK.	+
STO1-CHOM-0012	ptxP3	*ptxA1*	*fim2-1*	Fim3-2	*fhaB1*	+	*prn2* (I)	+
STO1-CHLA-0011	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB3**[d]	+	*prn2* (V)	-
CHLA-13	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	frameshift (aa 2669)	+	*prn2* (V)	-
CHLA-26	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB9**	+	*prn2* (V)	-
STO1-CHLA-0006	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB7**	+	*prn2* (V)	+
STO1-CHOC-0008	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB6**	+	*prn2* (I)	+
2356847	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB8**	+	*prn2* (V)	+
STO1-SEAT-0004	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB5**	+	*prn2* (V)	-
CHLA-20	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	**fhaB8**	+	*prn2* (V)	-
STO1-SEAT-0006	ptxP3	*ptxA1*	*fim2-1*	Fim3-2	*fhaB1*	+	*prn2* (VI)	-
H973	ptxP3	*ptxA1*	*fim2-1*	Fim3-1	*fhaB1*	+	*prn2* (VI)	-
H939	ptxP3	*ptxA1*	*fim2-1*	Fim3-	*fhaB1*	+	**prn2 (Δ aa 907-910)**	-

				2			(III)	
CHLA-11	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
I002	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
I176	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (VI)	-
STO1-CNMC-0004	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
H897	ptxP3	*ptxA1*	*fim2* pseudo.	*Fim3-2*	*fhaB11*	+	***prn2* (IV)**	-
I036	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
H918	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	***prn9* (I)**	+
H921	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	***fhaB4***	+	*prn2* (I)	+
STO1-CHOC-0019	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	truncation (aa 2587)	+	*prn2* (I)	+
STO1-CHOC-0016	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
CHLA-15	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	*fhaB1*	+	*prn2* (I)	+
2371640	ptxP3	*ptxA1*	*fim2-1*	*Fim3-1*	*fhaB10*	+	*prn2* (I)	-
STO1-CHOC-0017	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	frameshift (aa 3402)	+	*prn2* (I)	+
STO1-CHOC-0021	ptxP3	*ptxA1*	*fim2-1*	*Fim3-1*	*fhaB1*	+	*prn2* (I)	+
STO1-SEAT-0007	ptxP3	*ptxA1*	*fim2-1*	*Fim3-2*	truncation (aa2587)	+	*prn2* (I)	+
STO1-CHOC-0018	ptxP3	*ptxA1*	*fim2-1*	*Fim3-1*	*fhaB1*	+	*prn2* (V)	-
2250905	**ptxP1**	*ptxA1*	*fim2-1*	*Fim3-1*	*fhaB1*	+	**Δ(aa 8-37) (II)**	-
Tohama I	ptxP2	*ptxA2*	*fim2-1*	*Fim3-1*	*fhaB1*	+	*prn1* (I)	+

[a] **Genomic DNA for each strain was isolated using a phenol chloroform protocol; gDNA isolates were sent to the J. Craig Venter Institute (JCVI) for full genome sequencing.**
[b] **PRN grouped by mutation type: (I) wild type, (II) signal peptide deletion, (III) translocator deletion, (IV) early stop codon, (V) Leu80::IS481 in outer membrane protein P.69, (VI) Leu537::IS481 in outer membrane protein P.69**
[c] **Strains that have yet to be sequenced are designated as unknown (UNK.)**
[d] **Bolded text designates newly recognized allele types**

Immunoblot analyses demonstrate the antibody-recognition patterns for the PRN (Figure 8) and FHA (Figure 9) virulence factors, which exhibited substantial allelic diversity (Table 3);

PTX and FIM were not assessed by immunoblot due to the minimal allelic variation in these

virulence factor genes among the strains. While all strains produced FHA, fourteen strains were

identified as PRN-negative by immunoblot (CHLA-25, STO1-CHLA-0011, CHLA-13, CHLA-

26, STO1-SEAT-0004, CHLA-20, STO1-SEAT-0006, H973, H939, I176, H897, 2371640,

STO1-CHOC-0008, and 2250905); antibody-recognition did not appear to be specific to location

of isolate collection. Additionally, comparing the variation in antibody recognition of PRN as

categorized by PFGE type (Figure 10A), it can be seen that PFGE profile 253 predominates the

PRN-negative isolates, while types 324, 082, 013, 321, and 217 predominate the PRN-positive

isolates. Nearly one third of the currently circulating strains in this study exhibit newly

identified PFGE types and, of these new strains, only 27.3% are PRN-negative; in contrast, 40%

of the older PFGE types are PRN-negative (Figure 10B).

Figure 8: Immunoblot demonstrating strain levels of PRN expression. Samples were run on 7% acrylamide gels; blots were probed with anti-PRN serum and exposed for 2 minutes.
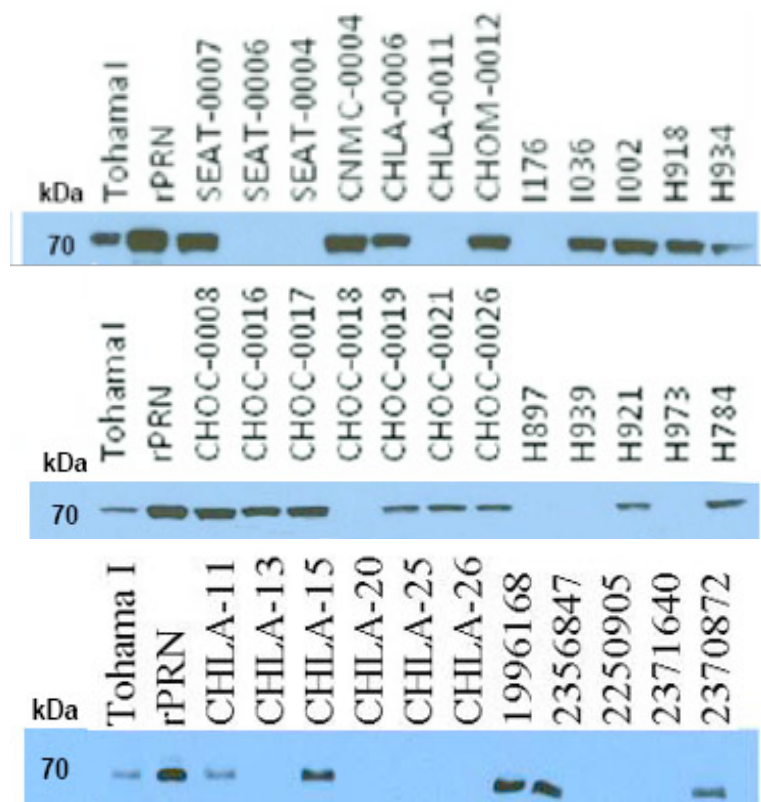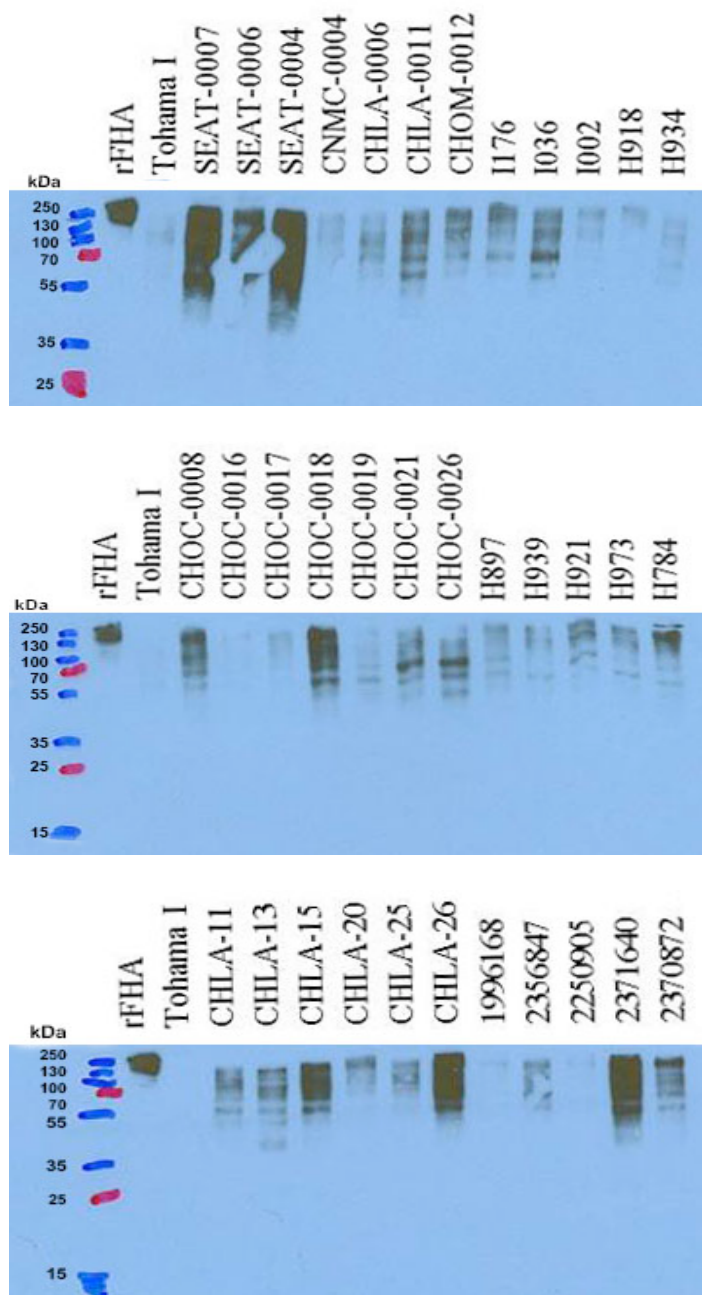
Figure 9: Immunoblot demonstrating strain levels of FHA expression. Samples were run on 7% acrylamide gels; blots were probed with anti-FHA serum and exposed for 5 minutes. Multiple banding pattern represents the SDS degradation of multimeric FHA protein.

A

B
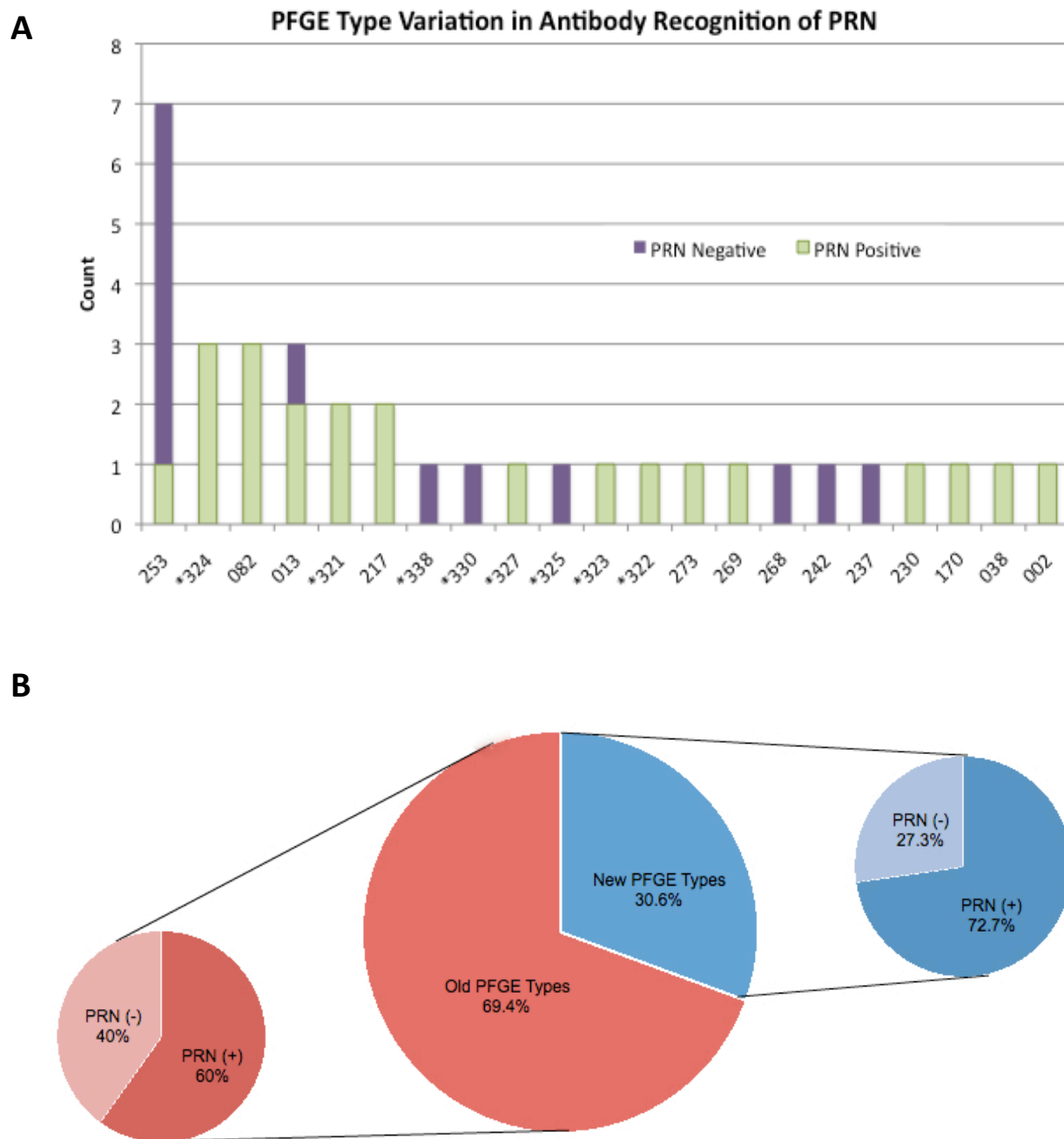
Figure 10: Variation in antibody recognition of PRN among the currently circulating strains of *B. pertussis* categorized by PFGE type. PFGE typing was performed on isolated strains by the JCVI, and antibody recognition of PRN was determined by immunoblotting. (A) Antibody recognition of PRN as categorized by PFGE type, where * designates new PFGE types. (B) Antibody recognition of PRN as percentages of previously and newly identified PFGE types.

Strains do not differ significantly in sensitivities to clinically used azithromycin and erythromycin antibiotics.

Incubation of bacterial cultures with varying concentrations of the clinically used antibiotics, azithromycin and erythromycin, demonstrates the relative sensitivities of each strain (Table 4). All strains were inhibited by antibiotic MIC50 concentrations between 1 and 0.1µg/mL. Thus, there are no markedly resistant strains (MIC50 > 1 µg/mL) or highly sensitive strains (MIC50 < 0.1 µg/mL). The strains known to be associated with infant fatality (STO1-CHOC-0008 and STO1-CNMC-0004) did not exhibit above-average resistance to either antibiotic.

Table 4: Minimum Inhibitory Concentration 50 (MIC50) values for Erythromycin and Azithromycin inhibition of strain growth

Strain ID	MIC50 (µg/mL)[a]		Strain ID	MIC50 (µg/mL)	
	Erythromycin	Azithromycin		Erythromycin	Azithromycin
STO1-SEAT-0004	1	1	CHLA-25	0.1	1
STO1-SEAT-0006	0.1	0.1	CHLA-26	1	0.1
STO1-SEAT-0007	0.1	0.1	1996168	1	0.1
STO1-CHOC-0008	0.1	1	2356847	0.1	1
STO1-CHOC-0016	1	0.1	2370872	ND[b]	0.1
STO1-CHOC-0017	1	0.1	2250905	0.1	1
STO1-CHOC-0018	0.1	1	2371640	0.1	1
STO1-CHOC-0019	1	0.1	H784	1	1
STO1-CHOC-0021	1	1	H897	1	0.1
STO1-CHOC-0026	1	1	H939	ND	1
STO1-CNMC-0004	1	0.1	H921	1	0.1
STO1-CHLA-0006	1	1	H918	0.1	1
STO1-CHLA-0011	1	0.1	H934	1	0.1
STO1-CHOM-0012	0.1	≥ 0.1	H973	1	0.1
CHLA-11	1	≥ 0.1	I002	1	0.1
CHLA-13	0.1	0.1	I036	0.1	1
CHLA-15	0.1	0.1	I176	0.1	0.1
CHLA-20	1	1	Tohama I	1	1

[a] Bacterial isolates were incubated with varying concentrations of antibiotics in a 96 well plate. Plates were incubated at 37°C while shaking at 210rpm and OD_{600} values were measured at 0, 24, 48, and 72 hours. MIC50 value was determined based upon the OD_{600} value recorded at the 48-hour time point for each strain, and was calculated as the antibiotic concentration at which the growth of the strain was decreased to at least half that of the strain's growth in PBS-only.
[b] ND, Not Determined.

There is no significant variation in murine lung colonization.

In vivo challenge of wild type mice with selected *B. pertussis* strains demonstrates the

relative capacity of isolates to colonize the lungs. Figure 11 shows that the selected strains,

including the lab strain derivative (536), colonize the lungs to similar levels. There are no

significant differences in infectivity of pertactin negative strains (STO1-CHLA-0011 and STO1-

SEAT-0004) compared to pertactin positive strains (STO1-CHOC-0008, STO1-CNMC-0004,

STO1-CHOM-0012, and 536); similarly, there are no significant differences in bacterial burden

upon infection with isolates associated with infant fatality (STO1-CHOC-0008 and STO1-

CNMC-0004) compared to non-fatal isolates (STO1-CHOM-0012, STO1-SEAT-0004, STO1-

CHLA-0011). Thus, in this geographically comprehensive sampling of currently circulating

strains, there are no outlier isolates that are especially effective at infecting *in vivo*. Additionally,

for all tested strains, the observed decrease in bacterial burden from day 3 to day 7 demonstrates

activity by the host immune system to begin controlling infection after day 3. Compared to mice

infected with 536, bacterial burden by day 7 was significantly reduced in mice infected with

STO1-CHLA-0011 (PRN-deficient strain); a similar trend was observed in mice infected with

STO1-SEAT-0004 (another PRN-deficient strain), though not to a statistically significant extent.
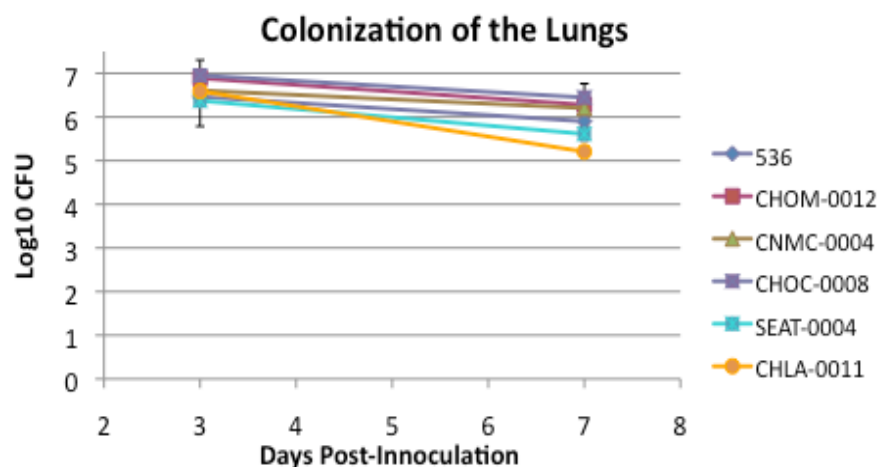

Figure 11: Murine lung colonization by clinical strains. For each strain, wild type F2.129 mice were inoculated with 5×10^5 CFU/50 µL bacteria and bacterial burden in the lungs on days 3 and 7 was enumerated.

Adacel vaccination reduces lung colonization, but protects less efficiently against currently circulating strains than against a lab strain.

Comparison of bacterial burden in the lungs following vaccination with a sham or Adacel (aP) vaccine demonstrates the *in vivo* protective response of the latter treatment. Figure 12A shows that Adacel vaccination significantly reduces lung colonization by day 3 post-infection with all tested strains. However, the differences in protection conferred by Adacel compared to the sham vaccine (Figure 12B) suggest that there is a significantly lower protective response against PRN-negative strains (STO1-CHLA-0011 and STO1-SEAT-0004) compared to the PRN-positive strains. There were no significant variations in vaccine-induced protection against strains associated with infant fatality (STO1-CHOC-0008 and STO1-CNMC-0004) compared to non-fatal strains (STO1-CHOM-0012, STO1-SEAT-0004, STO1-CHLA-0011); this suggests that the lethality of these cases was likely a feature of the immune conditions of the host rather than the virulence of the infecting strain. Furthermore, the observed treatment-specific differences in protective response demonstrate greater reductions in colonization by the lab strain than by the tested clinical isolates, suggesting a downward shift in the protectiveness of acellular vaccination since the early vaccine era.

A

Sham vs. Adacel Vaccination Reductions in Lung Colonization

B

Sham vs. Adacel Vaccination Differences in Protective Response
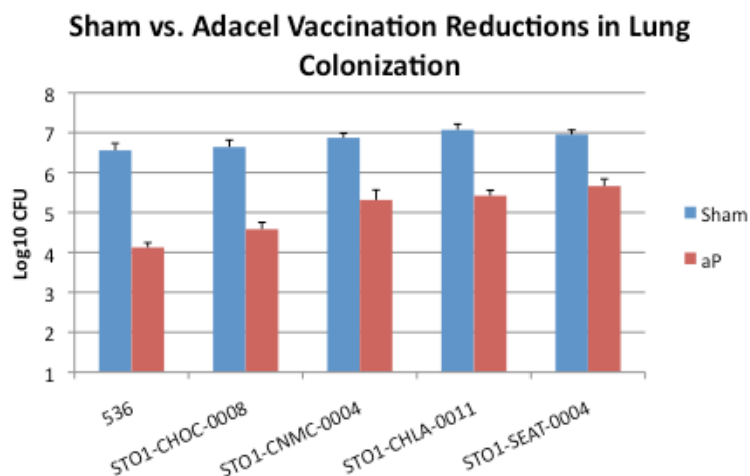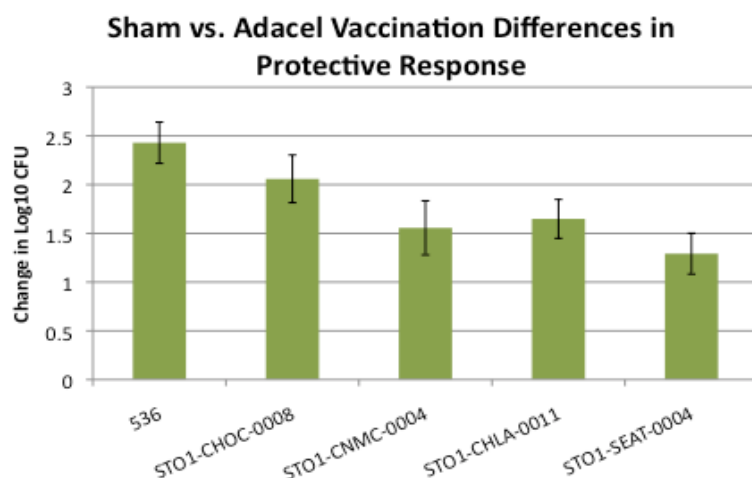
Figure 12: Differences in murine lung colonization by clinical strains following sham or Adacel vaccination. (A) Mice were either sham or Adacel vaccinated on Days 0 and 14, and challenged on Day 28 with the appropriate strain. At 3 days post-infection, lungs were homogenized and bacterial burden was enumerated. (B) Protective differences were calculated as the lung colonization following Adacel vaccination minus the lung colonization following sham vaccination.

Discussion

In the early 1900s, prior to the advent of a whooping cough vaccine, hundreds of thousands of pertussis cases were reported annually in the United States. Although the vaccine era has seen a remarkable decline in cases, incidence has been steadily increasing since the 1980s, hitting a 50-year high of 48, 000 cases in 2012.[9] This expansion of the disease despite widespread vaccination coverage has given rise to several questions about the cause of pertussis resurgence, as well as the severity of its clinical and epidemiological implications. Through our characterization of 35 clinical *B. pertussis* isolates collected from several U.S. hospitals we have established a better understanding of the currently circulating strains of pertussis; isolates collected from multiple sources during different outbreak years bolsters the scope of this study, allowing for a more accurate, representative sample. This analysis demonstrates both the similarities and differences among the currently circulating strains of pertussis, allowing for an assessment of the current clinical practices used to address whooping cough.

A comparison of clinical isolates demonstrates the importance of certain acellular vaccine components and the efficacy of vaccination itself. The lack of variation in the presence of the PTX locus genes, as well as the consistency in the corresponding alleles suggests that PTX is highly conserved across strains; this reinforces the conclusion that PTX is crucial to pertussis infectivity and remains an important virulence factor component of acellular vaccines.[23] Additionally, the conservation of the *fim2-1* and *fim3-1/2* alleles for pertussis fimbria indicate that this adhesin likely plays a critical role in pertussis infection, as well. Further analysis of fimbrial antigen potency would help to explain why this adhesin remains conserved in currently circulating strains. Additionally, from a sampling of isolates, vaccination with Adacel, a PTX and FIM-containing acellular vaccine, was found to reduce lung colonization by day 3; the

relative strain-specific reductions in lung colonization will be discussed later. This confirms the effectiveness of Adacel vaccination to induce a protective response, though the limitations of and variability in this response will be discussed later.

Characteristic similarities among currently circulating strains also provide insight regarding the clinical presentation of pertussis. All strains were sensitive to clinical antibiotics at concentrations between 1 and 0.1 μg/mL. In particular, the isolates associated with infant fatality (STO1-CHOC-0008 and STO1-CNMC-0004) demonstrated similar sensitivity to these antibiotics, suggesting that the lethality of these cases may have been due to factors other than failed antibiotic treatment; such host factors as age, immunocompetence, and acquired immunity are considered to greatly influence the clinical presentation of whooping cough.[2] Interestingly, our assessment of intraspecies competition demonstrated that none of the tested isolates were able to inhibit the growth of another *B. pertussis* isolate. To our knowledge, this suggests not only that these strains cannot outcompete other currently circulating strains, but also that there is potential for co-infection by multiple *B. pertussis* strains within the same individual. Perhaps such coinfection could contribute to more severe whooping cough cases, but additional studies of critical care pertussis patients would be needed to evaluate the potential role of comorbidity.

In vivo growth analysis similarly illustrates the clinical presentation of pertussis by highlighting the relative infectivity and immunogenicity of tested strains. In a geographically comprehensive sampling of isolates, there were no significant differences in murine lung colonization by day 3 post-challenge; this suggests that all of the strains are similarly capable of infecting. Interestingly, however, the *in vivo* growth of the strains did diverge by day 7, after the host immune system had begun to control infection. In particular, host immune response reduced lung colonization to the greatest extent in the mice infected with PRN-deficient strain

STO1-CHLA-0011; protective response in mice infected with STO1-SEAT-0004 (another PRN-deficient strain) also trended toward greater reduction in colonization by day 7, but not to a statistically signficant extent. This may suggest that strains lacking the virulence factor PRN are less fit, though studies using a larger sampling of *B. pertussis* strains, including those expressing and those deficient in PRN, would be necessary to draw more definitive connections between PRN and case severity; in very young or under-immunized individuals, *B. pertussis* may still be highly pathogenic even without a complete arsenal of virulence factors. Additionally, the lack of significant variation in *in vivo* growth of and host response to strains associated with infant fatality (STO1-CHOC-0008 and STO1-CNMC-0004) further suggests that the severity of these cases may likely be due to the immune conditions of the hosts rather than the specific pathogenicity of the infecting strains.

At the genetic level, variations exhibited by the studied isolates illustrate continued diversification in the profile of epidemic whooping cough strains. In particular, currently circulating strains exhibit tremendous variety in PFGE type, with six types predominating (253, 324, 082, 013, 321, 217) out of the 21 total types observed. This data did not correlate with fatality of infection, suggesting that no specific PFGE types are responsible for causing atypically severe disease. Additionally, a recent longitudinal analysis of PFGE types indicated that a historical sampling of *B. pertussis* isolates (collected from 1935 to 2009) was predominated by PFGE types 010, 013, and 082.[26] Our study indicates that two of these PFGE types continue to be circulating in high numbers during current epidemics, though the 010 PFGE type was not found in our sample at all. Additionally, the emergence of eight newly identified PFGE types further confirms that *B. pertussis* strains are continually changing.[18] Such change and diversity is mirrored in the genes of the vaccine-associated virulence factors, especially FHA

and PRN. Interestingly, a recent study of *B. pertussis* isolates from the prevaccine era to the present highlights the consistency of our findings. Prevaccine strains were predominated by the *ptxP1, ptxA4, fim3-1, prn1* profile, which matches that of the *B. pertussis* strain 10536 used in the United States aP vaccine (Sanofi-Pasteur).[7] This profile was, for the most part, replaced by a new profile (*ptxP3, ptxA1, fim3-2, prn2*) by the time a widespread acellular vaccination schedule was established.[7] Our findings are consistent with this trend, demonstrating the same *ptxP3, ptxA1, fim3-2, prn2* profile for the majority of strains in our sample, though isolate 2250905 exhibited the *ptxP1* allele and nearly half of the isolates exhibited the *fim3-1* allele. The divergence of allelic frequency from that of *B. pertussis* strain Tohama I (the Japanese vaccine strain) and from that of the 10536 US aP vaccine strain demonstrates a shift in gene content for circulating strains.[7] Although *B. pertussis* is highly monomorphic, exhibiting $\geq 99\%$ similarity relative to the reference lab strain Tohama I (Jihye Park and Yury Ivanov, unpublished data), small mutations, predominantly SNPs and some insertions and deletions, have been responsible for producing the allelic variation in virulence factor genes over the past few decades.[24] While the genetic shift has been suggested to be the result of vaccine selective pressure, this remains a speculative explanation as the limited availability and breadth of pre-vaccine era *B. pertussis* strains limits comprehensive comparison.[18] Nevertheless, such changes, and the fact that most circulating strains possess virulence factor alleles that no longer exactly match the strains used to make the vaccines, emphasizes the importance of regularly monitoring vaccine efficacy to ensure it remains as effective as possible against the current strains. Despite this divergence, the decreased incidence of whooping cough relative to the pre-vaccine era, lends credence to the overall effectiveness of vaccines, and supports their continued prophylactic use until they become no longer effective.[8]

One particular pertussis strain change that has recently incited new and growing public health concern is the recent emergence of PRN-deficient strains. Longitudinal surveys of *B. pertussis* strains have indicated that the first PRN-deficient strain emerged in 1994 and that, compared to the pre-vaccine era, PRN-deficient variants have expanded dramatically, particularly in the past few years.[26] Thus, the emergence of strains lacking PRN has been proposed to be a result of acellular pertussis vaccinations; however, the initial emergence of PRN deficiency at the time of early acellular vaccine implementation suggests that these variants were already circulating.[26] In our comparison of the current predominant PFGE types and antibody recognition of PRN, we found that PFGE type 253 predominates the PRN-negative strains while types 324, 082, 013, 321, and 217 predominate the PRN-positive strains; overall, the majority of PFGE types (72.7% of newly identified types and 60% of previously identified types) are still PRN-positive. Although our collection is not extensive enough chronologically to draw conclusions about the progression of PRN deficiency over time, it is important to note that most currently circulating strains still produce PRN, suggesting a selective advantage to maintaining this virulence factor adhesin. In particular, PRN is still categorized as a virulence factor by virtue of its ability to mediate eukaryotic cell binding, while its enhancement of protective immunity makes it an important vaccine target; some studies even indicated that PRN was most important to protective response, possibly participating in a synergistic relationship with PTX, and that the absence of PRN may be partially compensated for by the activity of another major adhesin, FHA.[24] As mentioned previously, the clinical significance of PRN deficiency still needs to be studied, as does the impetus behind the recent emergence of these variants. Nevertheless, the expansion of PRN-deficiency since the pre-vaccine era does emphasize the importance of re-evaluating acellular vaccines, many of which contain a PRN virulence factor component.

In light of recent whooping cough epidemics and the changes occurring in the currently circulating strains, another growing public health concern revolves around the epidemiology of pertussis infection and the potential for outbreaks of hyper-virulent strains. It has been demonstrated that pertussis is transmitted through aerosolized respiratory droplets, particularly following close or prolonged contact.[28] Another study demonstrated that parents (20 to 48%), siblings (16 to 21%), and non-household close contacts (18 to 29%) frequently served as reservoirs for transmitting the disease to infants.[29] In fact, in household studies of confirmed index cases, only 15 to 35% of unvaccinated and susceptible individuals escape infection.[27] These findings on *B. pertussis* local transmission dynamics support the characteristic patterns found in our study. Our analyses demonstrate no geographic trends in PFGE type, virulence factor alleles, or antibody recognition of virulence factors, suggesting a widespread, concurrent distribution of strains during outbreaks, rather than the clonal expansion of a single, hyper-virulent strain. This assertion was also demonstrated in a PFGE-based epidemiological study of isolates collected during outbreaks in Cincinnati, Ohio from 1989 to 1996 in which the authors concluded that PFGE types more prevalent pre-epidemic are thereby more readily transmitted by chance expansion.[30] The finding that, despite widespread vaccination coverage, outbreaks feature many different infecting strains brings into question the effectiveness of the current vaccination programs to provide broad protection against infection and transmission.

Our assessment of Adacel vaccine protection further stresses the need to evaluate current vaccine effectiveness. Although, compared to sham vaccination, acellular vaccination induced a significant protective response against all tested strains, we found that the vaccine-associated reduction in murine lung colonization was greater in mice challenged with laboratory *B. pertussis* strain 536 than in mice challenged with the clinical isolates. This finding suggests that

the current vaccines may not be as protective against currently circulating strains as they are against a strain isolated in the early vaccine era; alternatively, this may reflect lab adaptation of the *B. pertussis* strain 536. The aforementioned variations in currently circulating isolates, particularly in comparison to vaccine strains, may be limiting the effectiveness of current vaccines. Furthermore, the extent of vaccine protection was lesser against PRN-negative strains, compared to PRN-positive strains. Strains lacking PRN may be less susceptible to the adaptive immunity induced by PRN-containing vaccines. However, further study with a larger sample size and a comparison to vaccinations lacking a PRN component is necessary to corroborate this assertion. Collectively, the vaccine protection data illustrate several limitations of acellular vaccines, namely a failure to prevent host colonization and less efficient clearing of currently circulating stains and PRN-negative stains. This supplements published studies that demonstrate continued infection and contagiousness of aP-vaccinated non-human primates.[12]

The similarities and differences found in our study of 35 clinical *B. pertussis* isolates demonstrates the breadth of diversity and clinically relevant changes that are characteristic of currently circulating strains. The connection between these changes and the limited capacity of current vaccines to control disease, infection, and transmission are of particular concern and require further investigation to devise a more effective approach. We hope that these data will educate future studies and influence clinical practice to improve the diagnosis, treatment, and prevention of whooping cough.

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Education

The Pennsylvania State University, University Park, PA   Expected Graduation: May 2014
Schreyer Honors College
Bachelor of Science in Biochemistry and Molecular Biology--Biochemistry Option
Minors in Microbiology & Human Development and Family Studies

Honors and Awards

Gail Folena-Wasserman Undergraduate Research for Women Award, 2014
Schreyer Honors College Academic Excellence Scholarship, 2010, 2011, 2012, and 2013
Penn State Eberly College of Science Shigley Award, 2013
Penn State Eberly College of Science Bright Scholarship, 2012
Penn State Eberly College of Science Gilmore Memorial Award, 2011
Honor Society of Phi Kappa Phi Nominee, 2011, 2012, and 2013
Golden Key International Honor Society Nominee, 2011
Morrisville High School Valedictorian, 2010
Bausch & Lomb Honorary Science Award, 2010

Association Memberships/Activities

Teaching Assistant, Chem202, 2013-2014
Co-Founder, Pause for Paws, 2013
Secretary, Biochemistry Society, 2012-2013
Member, Alpha Epsilon Delta Pre-Med Honor Society, 2011-2013
Member, National Society of Collegiate Scholars, 2011-present
Volunteer, Schreyer Honors College Day of Service, 2010-present
Grader, PSU Chemistry Department, 2012-present
Co-Chair, Biochemistry Society THON Committee, 2011-2012
Caremate Volunteer, St. Mary Medical Center, 2011
Coordinator, Little Lions Day of Play, 2010

Professional Experience

Laboratory of Dr. Eric Harvill, University Park, Pennsylvania, Fall 2011—present
    <u>Undergraduate Honors Thesis Research</u>:
        Characterization of currently circulating strains of *Bordetella pertussis* collected from the
        Collaborative Pediatric Critical Care Research Network, the Centers for Disease Control and
        Prevention, and several U.S. children's hospitals

St. Mary Medical Center, Langhorne, Pennsylvania, Summer 2013
    Pre-Med Program:
        Clinical observation (~180 hours) with physicians of various disciplines including surgery, endocrinology, cardiology, neurology, anesthesiology, dermatology, emergency medicine, primary care, pediatrics, and underserved care

Penn State University College of Medicine, Hershey, Pennsylvania, Summer 2013
    Primary Care Scholars' Program:
        Medical student panels and physician presentations on primary care medicine, medical school education, the future of primary care, and the patient-centered medical home

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Publications and Papers

Harvill, E., Goodfield, L. L., Ivanov, Y., Meyer, J. A., Newth, C., Cassiday, P., Tondella, M., Liao, P., Zimmerman, J., Meert, K., Wessel, D., Berger, J., Dean, J. M., Holubkov, R., Burr, J., Liu, T., Brinkac, L., Kim, M., & Losada, L. Genome sequence of 28 *Bordetella pertussis* outbreak strains dating from 2010-2012. *Genome Announcements*, **1**, 1-2 (2013).

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Goodfield, L. L, Meyer, J. A., Ivanov, Y. V., Muse, S. J., Park, J., Kennett, M. J., Smallridge, W. E., Cassiday, P., Tondella, M., Moreau, M. R., Bendor, L., Karanikas, A. T., Harvill, E. T. Evaluating new *Bordetella pertussis* outbreak strains: Critical analysis of 2010-2012 outbreaks in the United States. Manuscript in preparation for *New England Journal of Medicine*.

Harvill, E. T., Meyer, J. A., Muse, S. J., Jacobs, N., Bendor, L., Smallridge, W. E., Goodfield, L. L., Ivanov, Y. V., Register, K., Cassiday, P., Tondella, M. L., Brinkac, L., Sanka, R., Kim, M., Losada, L. Genome sequence of 61 *Bordetella bronchiseptica* strains isolated in the Unites States. Manuscript in preparation for *Genome Announcements*.

Karanikas, A. T., Weyrich, L. S., Feaga, H. A., Goebel, E. M., Hester, S. S., Meyer, J. A., Harvill, E. T. Defining host specificity of the emerging pathogen *Bordetella holmseii*. Manuscript in preparation for *PLoS One*.