Isolate-Based Surveillance of *Bordetella pertussis*, Austria, 2018–2020

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Pertussis is a vaccine-preventable disease, and its recent resurgence might be attributable to the emergence of strains that differ genetically from the vaccine strain. We describe a novel pertussis isolate-based surveillance system and a core genome multilocus sequence typing scheme to assess *Bordetella pertussis* genetic variability and investigate the increased incidence of pertussis in Austria. During 2018–2020, we obtained 123 *B. pertussis* isolates and typed them with the new scheme (2,983 targets and preliminary cluster threshold of ≤6 alleles). *B. pertussis* isolates in Austria differed genetically from the vaccine strain, both in their core genomes and in their vaccine antigen genes; 31.7% of the isolates were pertactin-deficient. We detected 8 clusters, 1 of them with pertactin-deficient isolates and possibly part of a local outbreak. National expansion of the isolate-based surveillance system is needed to implement pertussis-control strategies.

Bordetella pertussis is the main causative agent of the reemerging respiratory disease commonly known as whooping cough (*1*). *B. pertussis* infection usually affects infants, toddlers, and children of school age, although adolescents and adults also can get infected and have symptoms. In addition, because transmission of pertussis can go unnoticed, asymptomatic carriers are considered an important source of infection (*2*). Despite its low sensitivity, culturing pertussis from nasopharyngeal swabs remains the standard diagnostic technique, although today it is scarcely performed (*3*).

To some extent, pertussis can be prevented by vaccination with either cellular or acellular vaccines (*4*). In Austria, cellular pertussis vaccines were replaced in 1998 by acellular vaccines (ACVs), and ever since, either 2 (filamentous hemagglutinin [FHA] and pertussis toxin [PTX]) or 3 (FHA, PTX, and pertactin) component vaccines have been used for primary vaccination. Booster immunizations have been recommended since 2003 for children of school age and adolescents. These vaccines include either 3 (FHA, PTX, and pertactin) or 5 (FHA, PTX, pertactin, FIM2, and FIM3) components.

Despite vaccinations, the incidence of pertussis has been increasing in the past few decades (*5*–*7*). In Austria, 579 pertussis cases were reported in 2015; the number increased to 1,274 in 2016, 1,411 in 2017, 2,198 in 2018, and 2,246 in 2019. The increase in the incidence of pertussis worldwide can be explained partially by the loss of the protective effect after immunity wanes; this loss is strongly associated with ACV use (*8*). Another factor that contributes to the resurgence of pertussis is the emergence of vaccine-evasive *B. pertussis* strains that differ genetically from the vaccine strains (*9*,*10*).

A molecular study conducted on pertussis cases from 3 different cities in Austria assessed the genetic variability of *B. pertussis* nationwide (*5*). However, the study used respiratory samples to perform PCR, followed by Sanger sequencing; therefore, typing was not based on whole-genome sequencing (WGS) of *B. pertussis* isolates.

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Because of the rise in the incidence of pertussis in Austria in recent years, we investigated pertussis cases from 3 states in Austria to assess the genetic variability of their *B. pertussis* isolates through WGS-based typing. The first objective was to set up a national isolate-based surveillance system, complementary to the case-based surveillance system in Austria, for collecting isolates from patients with suspected pertussis. Second, we aimed to characterize and to compare *B. pertussis* isolates with the vaccine strain Tohama I and other isolates from different geographic regions outside Austria.

Methods

Setup of the Surveillance System and Sequencing

For 2 years (May 2018–May 2020), hospitals, general practitioners, and pediatricians using clinical laboratories located in 3 states in Austria (Salzburg, Tyrol, and Styria) were asked to collect ≥1 nasopharyngeal swab containing transport medium (ESwab; Copan, https://www.copangroup.com) from patients with suspected *B. pertussis* infection (Figure 1). When possible, a second nasopharyngeal swab containing charcoal-based medium (Transystem Amies medium with charcoal; Copan) was collected.The swabs were then sent to the clinical laboratory of each state participating in the study. For each suspected case, PCR was performed using the swab containing the transport medium with a commercial kit (BD MAX, Becton Dickinson, http://bd.com; or BORDETELLA R-gene, bioMérieux, https://www.biomerieux.com). When PCR results were positive, either the same swab used for PCR or, if available, the charcoal swab was stroked on Oxoid *Bordetella*–selective medium (Thermo Fisher Scientific, https://www.thermofisher.com) or Bordet Gengou agar with 15% sheep blood (Becton Dickinson), followed by cultivation at 37°C under aerobic and humid conditions for 48–120 hours. Colonies compatible with *B. pertussis* were tested by MALDI Biotyper software version 3.0 (Bruker, https://www.bruker.com) or VitekMS software version 3.2 (bioMérieux). Colonies indentified as *B. pertussis* were sent to the Austrian Agency for Health and Food Safety in Vienna for further DNA extraction and 300-bp paired-end WGS using an Illumina Miseq device (https://www.illumina.com), as described in Appendix 1 (https://wwwnc.cdc.gov/EID/article/27/3/20-2314-App1.pdf). Additional information on the sequencing process, de novo assembly, and sequence quality checks also are found in Appendix 1. The Illumina reads of the 123 isolates in Austria have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive repository under project number PRJNA642701.

Generation of a *B. pertussis* cgMLST Scheme

A stable, ad hoc, core-genome multilocus sequence typing (cgMLST) scheme and accessory genome scheme were created by using Ridom SeqSphere + version 4.1.9 (Ridom, https://www.ridom.de; Appendix 1). In brief, 15 genomes (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/27/3/20-2314-App2.xlsx) were used as query genomes and the Tohama I vaccine strain genome (GenBank accession no. NC_002929.2) as a seed genome. Afterwards, 263 taxonomic and quality outliers were discarded, leaving a total of 2,983 core genome targets (Appendix 2 Table 2) and 179 accessory genome targets (Appendix 2 Table 3). We considered as core genes only those targets (i.e., genes) that were present in 100% of the genomes. Further validation of the scheme was based on a selection of *B. pertussis* genomes available in NCBI (n = 391), many of which were associated with outbreaks (Appendix 2 Table 4), and an old collection of clinical *Bordetella* sp. strains from Austria (Appendix 2 Table 5).

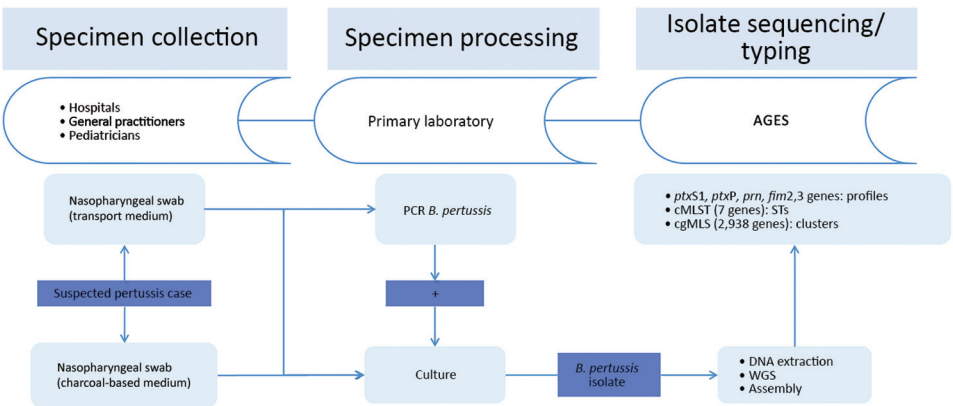

Figure 1. Flow chart of the *Bordetella pertussis* isolate–based surveillance system, Austria, May 2018–May 2020. AGES, Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety); cgMLST, core-genome multilocus sequence typing; ST, sequence type.

Typing of *B. pertussis* Isolates and Comparative Analysis

During the 2-year study period, all the clinical isolates collected within the isolate-based surveillance system were typed with our newly implemented cgMLST scheme. Allelic differences among the isolates from Austria and the vaccine strain Tohama I were visualized by generating minimum spanning trees with a preliminary cluster threshold established at ≤6 alleles (Appendix 1). We extracted the sequence types (STs) from the WGS data corresponding to the classical multilocus sequence typing (*11*), the variants and mutations present in each of the genes used as vaccine antigens (*ptx*S1, *ptx*P, *prn*, *fim*2, *fim*3), and their combination (genetic profiles).

To be certain that our scheme could be applied beyond our set of *B. pertussis* isolates from Austria, we used a selection (n = 106) of *B. pertussis* genomes, including outbreak strains used in the validation of the cgMLST scheme, to perform a genomic comparative analysis (Appendix 1; Appendix 2 Table 4). We compared the gene content obtained for our cgMLST scheme with the cgMLST scheme developed by the Pasteur Institute (Paris, France) (*12*). In addition, we compared the results obtained when applying our cgMLST with those derived from a single-nucleotide polymorphism (SNP)–based analysis on the 123 isolates from Austria (Appendix 1).

Table 1. Demographic characteristics of the 123 pertussis case-patients in the *Bordetella pertussis* isolate–based surveillance system, Austria, May 2018–May 2020

Characteristic	No.
Age group, y	
<1	8
1–4	15
5–9	31
10–14	31
15–19	7
20–29	3
30–39	7
40–49	11
50–59	3
≥60	7
Sex	
F	69
M	54
State	
Salzburg	86
Tyrol	21
Styria	12
Upper Austria	4
Clinical symptoms	
Coughing fits	66
Cough >4 weeks	64
Medical whooping cough diagnosis	37
Missing data	7
Post-coughing vomiting	7
Inspiratory whooping	5
Asymptomatic	2
Vaccination status	
Vaccinated	53
1st booster	1
2nd booster	22
3rd booster	16
4th booster	7
Unknown doses	7
Not vaccinated	39
Unknown	31

Statistical Analysis

Personal information and vaccination status were obtained for each pertussis culture-positive case-patient from the national electronic reporting system. We calculated odds ratios with Stata software version 13 (StataCorp, https://www.stata.com) to measure for associations between pertactin deficiency and vaccination status. In the analysis, we included all case-patients who had received ≥1 dose of pertussis vaccine and those reported as unvaccinated. Case-patients with an unknown vaccination status (n = 31) were excluded from the analysis. Statistical significance was defined as $p<0.05$ by using the Pearson χ^2 test or Fisher exact test.

Results

Culture-Positive Cases

At the Austrian Agency for Health and Food Safety, we received 123 *B. pertussis* isolates, collected from 123 pertussis case-patients (Table 1), through our newly implemented isolate-based pertussis surveillance system during May 2018–May 2020. Fewer than 20% of the total pertussis cases reported in Salzburg state (n = 310) in 2018 were estimated to be culture-positive, and no information on the proportion of cases with a positive pertussis culture was available for the other 8 states in Austria.

A total of 119 *B. pertussis* isolates belonged to patients with PCR-positive confirmed pertussis from Salzburg, Tyrol, and Styria (Figure 2), and 4 isolates belonged to pertussis case-patients identified in the state of Upper Austria, provided by a clinical microbiology laboratory located in Salzburg (MB-LAB Clinical Microbiology Laboratory). Overall, 15 *B. pertussis* isolates belonged to culture-positive pertussis case-patients who lived in the same household with ≥1 other culture-positive case-patient. Additional metadata for the 123 pertussis cases are presented in Appendix 2 Table 6.

Sequence Types and Typing of Vaccine Target Genes

The *B. pertussis* isolates obtained from the 123 pertussis patients in Austria differed in sequence type and in the vaccine antigen genes from the vaccine strain Tohama I (Table 2). We detected ST2 for all but 1

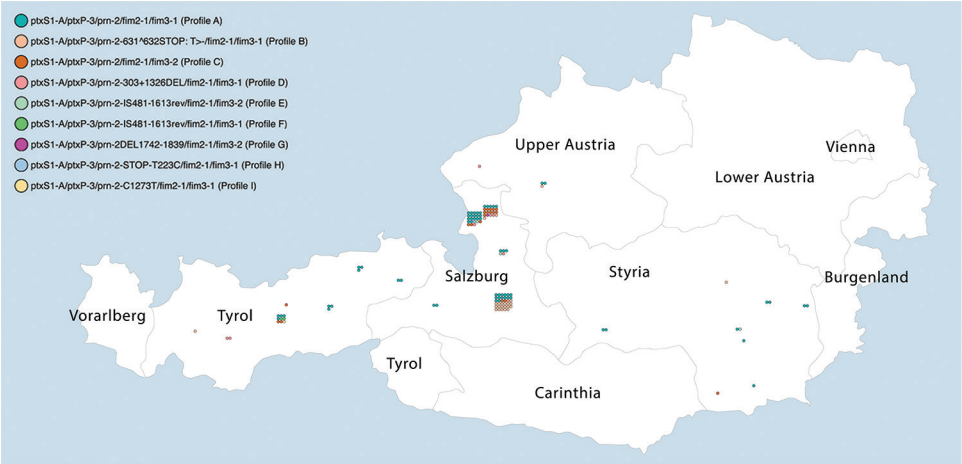

Figure 2. Pertussis cases by district of residence of case-patient and genetic profile of the corresponding *Bordetella pertussis* isolate identified in a *B. pertussis* isolate–based surveillance study, Austria, May 2018–May 2020. Each dot represents 1 case. Cases grouping next to each other belong to the same district. To protect patient confidentiality, only states and not districts are labeled.

isolate, which was of ST83. We found 9 different genetic profiles (A–I), 1 of which was new (profile G) (Table 2; Figure 3; Appendix 2 Table 7).

We found 7 pertactin-deficient profiles (B and D–I), representing 31.7% (n = 39) of the isolates, and 6 different known pertactin inactivation mechanisms (*13–16*; Table 2; Appendix 2 Table 7). Pertactin-deficient isolates were mostly of profile B (n = 23 [18.7%]). Twenty case-patients (51.3%) with pertactin-deficient isolates had been vaccinated, 11 (28.2%) case-patients were unvaccinated, and for 8 (20.5%) case-patients, vaccination status was unknown. Case-patients having received ≥1 dose of pertussis ACV were 1.5 times more likely to have a pertactin-deficient *B. pertussis* isolate (of any genetic profile) compared with unvaccinated case-patients, although this relationship was not statistically significant (unadjusted odds ratio 1.5, 95% CI 0.6–3.8). Persons living in the district of St. Johann in Pongau (Salzburg state) were 21.17 (95% CI 6.7–81.1) times more likely to have profile B, and this association was significant (p<0.001). Stratifying by vaccination status, vaccinated persons from St. Johann in Pongau were 13.3 (95% C: 2.9–99.1; p<0.001) times more likely to present profile B, whereas unvaccinated ones had 58.5 (95% CI 5.6–1876; p<0.001) times more chances to present profile B. No association was seen between the different age groups or having a pertactin-deficient profile and having profile B.

cgMLST and Comparative Analysis

The 123 *B. pertussis* isolates were closely related, differing by a maximum of 38 alleles (≤44 alleles when including the accessory genome), and they seemed to cluster in groups (Figure 3). We observed that we could separate *fim*3-1 and *fim*3-1 isolates into 2 branches (Figure 4) and that pertactin-deficient isolates of genetic profile B grouped together. Isolates of profile B (n = 23) differed by a maximum of 9 alleles when including the only isolate from Tyrol of that profile, and by ≤6 alleles, excluding the isolate from Tyrol. All other isolates were from Salzburg (n = 20), Styria (n = 1), and Upper Austria (n = 1). Isolates from profile D also clustered together (≤6 alleles), differing by ≥8 alleles with isolates of profiles A and B.

With the preliminary cluster threshold of ≤6 alleles, we distinguished 8 clusters (Figure 3; Appendix

Table 2. Genetic profiles of the 123 *Bordetella pertussis* isolates obtained through the *B. pertussis* isolate–based surveillance system, Austria, May 2018–May 2020*

		B. pertussis vaccine antigen genes					
Profile	No. (%)	*ptx*S1	*ptx*P	*prn*	*fim*2	*fim*3	Reference
Profile vaccine strain Tohama I	0	*ptx*S1-D	*ptx*P-1	*prn*-1	*fim*2-1	*fim*3-1	NA
Profile A	64 (52.3)	*ptx*S1-A	*ptx*P-3	*prn*-2	*fim*2-1	*fim*3-1	(*13,14*)
Profile B	23 (18.7)	*ptx*S1-A	*ptx*P-3	*prn*-2–631^632STOP:T>-	*fim*2-1	*fim*3-1	(*15*)
Profile C	20 (16.2)	*ptx*S1-A	*ptx*P-3	*prn*-2	*fim*2-1	*fim*3-2	(*13,14*)
Profile D	8 (6.50)	*ptx*S1-A	*ptx*P-3	*prn*-2–303+1326DEL	*fim*2-1	*fim*3-1	(*16*)
Profile E	3† (2.44)	*ptx*S1-A	*ptx*P-3	*prn*-2-IS481–1613rev	*fim*2-1	*fim*3-2	(*13,14*)
Profile F	2 (1.62)	*ptx*S1-A	*ptx*P-3	*prn*-2-IS481–1613rev	*fim*2-1	*fim*3-1	(*14*)
Profile G	1 (0.8)	*ptx*S1-A	*ptx*P-3	*prn*-2- DEL1742–1839‡	*fim*2-1	*fim*3-2	This study
Profile H	1 (0.8)	*ptx*S1-A	*ptx*P-3	*prn*-2-STOP-T223C	*fim*2-1	*fim*3-1	(*14*)
Profile I	1 (0.8)	*ptx*S1-A	*ptx*P-3	*prn*-2-C1273T	*fim*2-1	*fim*3-1	(*13,14*)

*NA, not applicable.
†One isolate was sequence type 83.
‡This isolate showed an insertion longer than 200 bp in the *prn* gene combined with a partial deletion at positions nt 1742–1839.

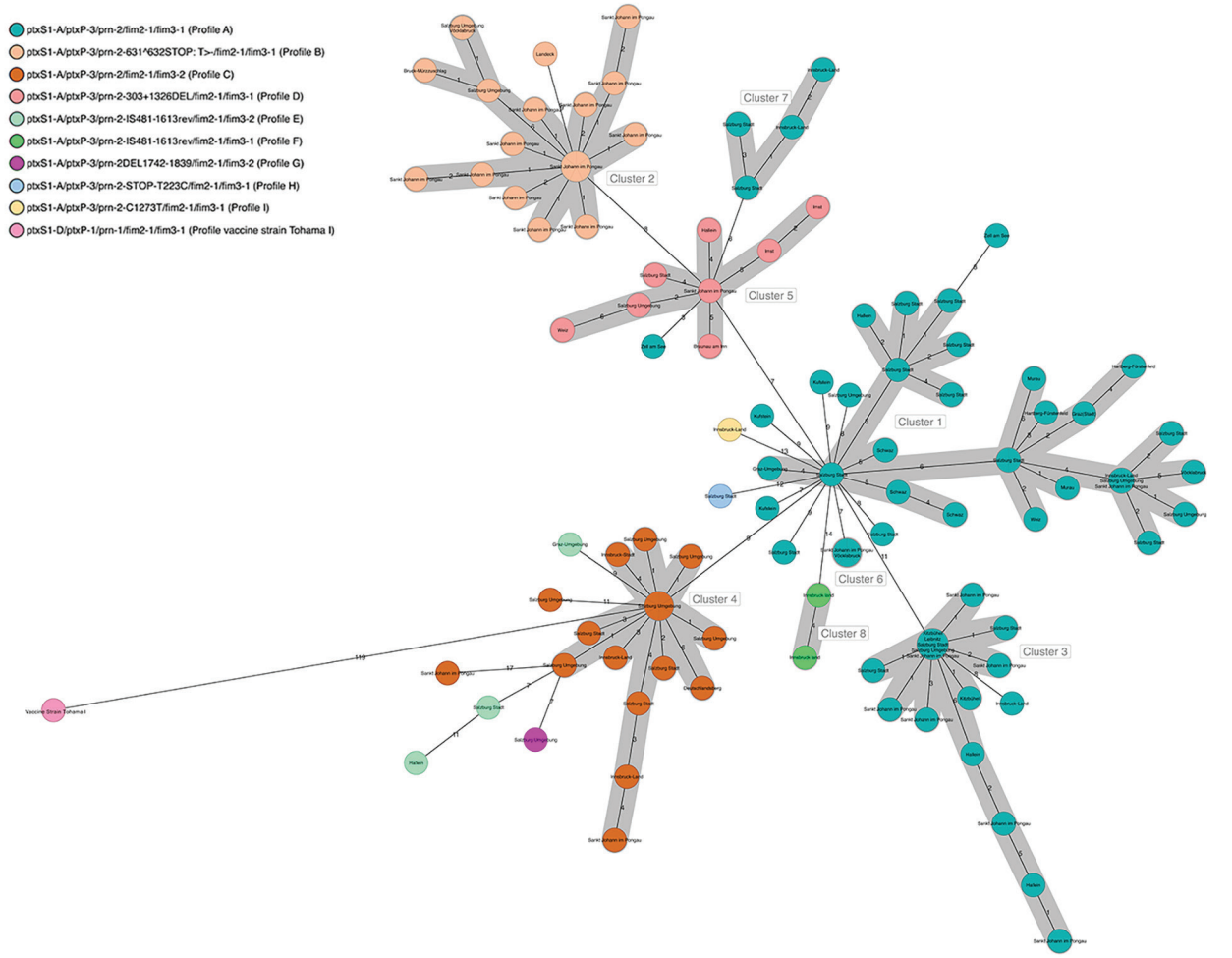

Figure 3. Minimum spanning tree of 123 *Bordetella pertussis* isolates and their clusters by genetic profile based on core-genome multilocus sequence typing in a *B. pertussis* isolate–based surveillance study, Austria, May 2018–May 2020. Numbers on connection lines represent the number of allelic differences among the isolates.

1). Cluster 1 integrated only profile A isolates (n = 26) from all states. Isolates in cluster 2 (n = 22) were of profile B. Most of the isolates in this cluster (n = 20) originated from case-patients living in Salzburg state. Eighteen of them resided in the district of St. Johann in Pongau. Of the 2 case-patients who did not live in Salzburg state, 1 had a confirmed epidemiologic link with a pertussis-positive relative in Salzburg. A trend compatible with a local outbreak of genetic profile B *B. pertussis* was distinguished (Appendix 1 Figure 1) for this cluster when we compared the number of profile B cases with the total number of reported cases (culture positive and nonculture positive) in the same period for St. Johann in Pongau (n = 160). In addition, a peak of cases in November 2018 corresponded to a small peak of cases with genetic profile B isolates. Cluster 3 had 19 isolates of profile A that were obtained from case-patients living in Salzburg, Tyrol, and Styria. Cluster 4 had 18 isolates of profile C from Salzburg, Tyrol, and Styria. Cluster 5 consisted of 9 profile D isolates from Salzburg, Styria, Tyrol, and Upper Austria. Cluster 6 included 5 profile A isolates from Salzburg and Upper Austria, and cluster 7 combined 4 profile A isolates from Salzburg and Tyrol. Cluster 8 had only 2 isolates, both of profile F, which originated from case-patients from Tyrol. Isolates from profiles E, G, H, and I did not cluster with any other isolate. The ST83 isolate did not cluster with any other isolate and differed from isolates in cluster 4 by ≥9 alleles.

In 6 households (A–E and G) (Appendix 2 Table 8), we confirmed the transmission of the same *B. pertussis* strain between 2 or 3 household members with a maximum of 4 allelic differences and an identical

genetic profile. In 1 household (F), transmission of *B. pertussis* was ruled out when cgMLST revealed 18 allelic differences between 2 isolates obtained from 2 case-patients living together, each of them with a *B. pertussis* strain of a different genetic profile (A and I).

We developed a comparative analysis between our 123 *B. pertussis* isolates and 106 *B. pertussis* genomes from NCBI, including mostly isolates from the United States and United Kingdom (Appendix 2 Table 4) from the 2010 and 2012 epidemics (*13*,*14*,*16*) (Figure 4). All *fim*3-1 isolates were clearly separated from *fim*3-2 isolates, but we could also differentiate isolates from the same country in different branches. On the basis of the *prn* type, which comprised the *prn* wild type alleles or mutations in this gene (i.e., insertions, deletions, and truncations), we distinguished isolates grouping closely to each other, sometimes originating from different countries. Genetic profiles A, C, H, and I were also represented in strains from outside Austria.

We compared target content between the Pasteur Institute's cgMLST scheme and ours (Appendix 2 Table 9). A total of 1,749 genes were common to both schemes; 1,239 genes were only present in our scheme, and 294 genes were only present in Pasteur Institute's scheme. The SNP analysis revealed isolates grouping in clusters in a similar way to that resulting from the cgMLST analysis (Appendix 1 Figure 2).

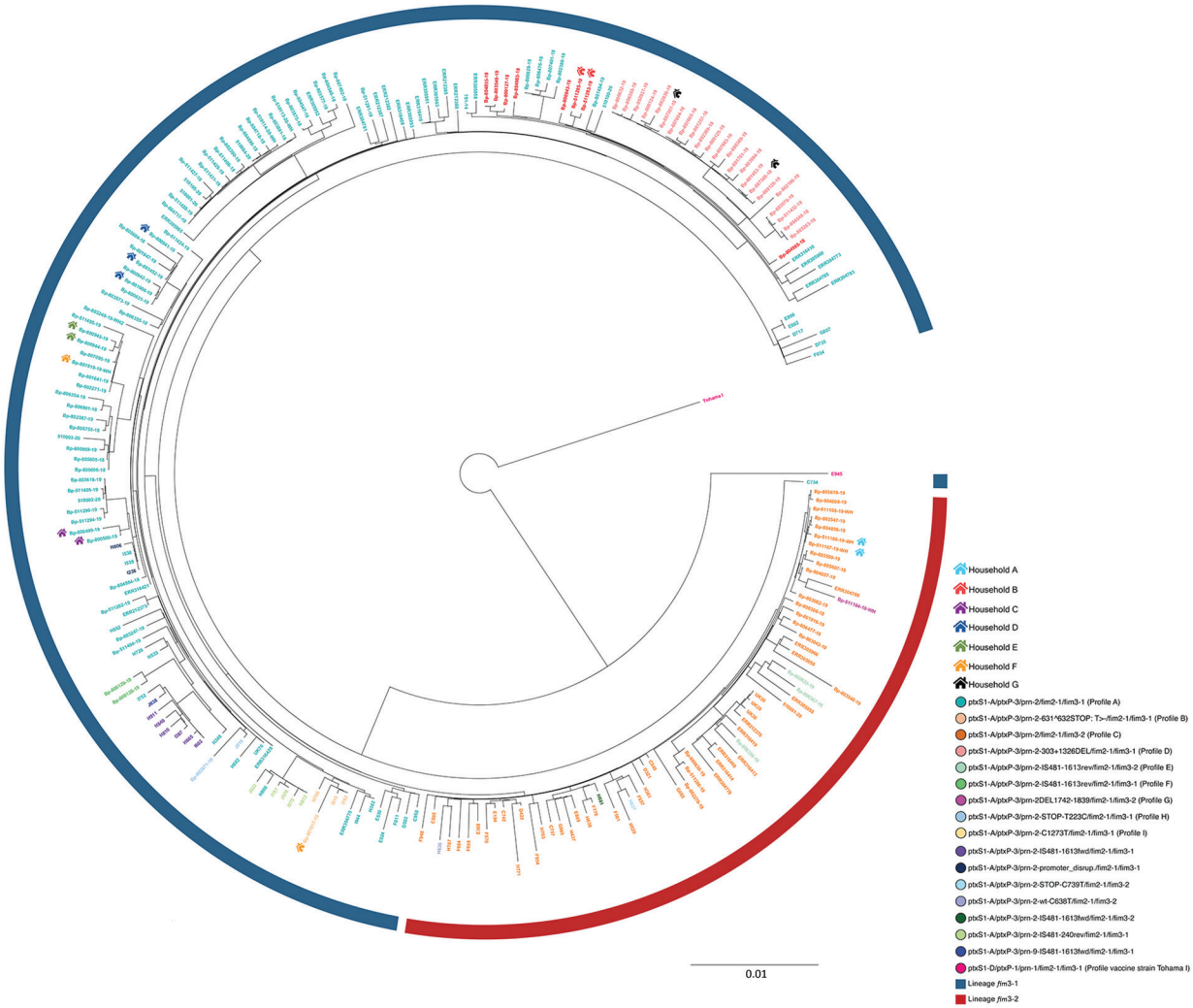

Figure 4. Maximum-likelihood phylogenetic tree generated using core-genome multilocus sequence typing data from 106 outbreak genome sequences from the United States and the United Kingdom and the 123 *Bordetella pertussis* isolates identified in an isolate-based surveillance study, Austria, May 2018–May 2020. Isolate identifiers are colored by genetic profile. These genetic profiles include profiles A–I, defined in this study, and other genetic profiles described outside of Austria. The circular blue line represents isolates of *fim*3-1 lineage; the circular red line represents *fim*3-1 isolates. A color-coded house-like symbol indicates isolates obtained from case-patients living in the same household. Scale bar indicates nucleotide substitutions per site.

Discussion

Our newly implemented *B. pertussis* isolate–based surveillance system has contributed to a better understanding of the molecular epidemiology of pertussis in Austria. No relationship existed between the pertussis incidence in the 3 states in Austria and the number of isolates collected, which depended mainly on the expertise of the respective laboratories in obtaining *B. pertussis* cultures. The estimated proportion (≈20%) of culture-positive pertussis cases found in relation to the total number of pertussis cases reported in Salzburg state was consistent with previous reports (*17*), whereas other authors reported up to 30% (*18*).

Results retrieved from the typing of the 123 *B. pertussis* isolates revealed the presence of the *ptxP*-3 allele in all isolates tested, consistent with other studies (*19*–*21*), thereby clearly indicating a divergence from the vaccine strain in Austria and a substitution among currently circulating *B. pertussis* strains of the *ptx*P-1 allele by the *ptxP*-3 allele. In comparison, during 2002–2008, a previous study in Austria still detected the *ptxP*-1 allele in 7% of the samples (*5*). Previous data also showed most of *B. pertussis* isolates (≈80%) grouping in the *fim*3-1 clade, which is more ancestral than *fim*3-2 (*22*). Therefore, not surprisingly, the genetic profile A (*fim3*-1 clade) was one of the most frequently detected genetic profiles globally (*22*–*24*), consistent with the findings of our study.

As for the proportion of pertactin-deficient isolates detected (31.7%), this finding was similar to the frequency reported during 2012–2015 in Norway, where ACVs were also introduced in 1998 and booster doses recommended after 2001 (*15*). In contrast, up to 98% of pertactin-deficient isolates were reported outside the European Union (*25*). In general, the proportion of *B. pertussis* isolates with pertactin deficiency seemed to be vary among countries depending on the vaccination schedule and vaccine type used (*26*). Those countries still using cellular pertussis vaccines have never or rarely reported pertactin-deficient isolates (*27*,*28*), whereas countries using ACVs have seen a direct association between the year of introduction of ACVs in the country and the appearance of pertactin-deficient isolates (*15*). Moreover, ACV-vaccinated persons seem more susceptible to pertactin-deficient strains than to pertactin-producing strains, given that pertactin-deficient strains are better able to colonize the respiratory tract (*29*). According to some authors, immunization with 2-component ACVs (instead of an ACV with 4 or 5 components) might affect immunogenicity (*30*–*32*). However, more time is needed to evaluate whether the lack of the pertactin component of 1 of the ACVs affects the incidence of pertussis in Austria in the coming years. Nevertheless, the higher likelihood of profile B strains found in the St. Johann in Pongau district seems not to have been influenced by vaccination.

Regarding the mechanisms causing pertactin deficiency, we reported a mutation at the 632 nt of the *prn* gene for our profile B isolates, previously described for isolates collected in Italy, Sweden, and Denmark during 2012–2015 (*15*). Likewise, this deletion was reported in Ireland in 2016 in an isolate (GenBank accession no. KX462969.1) differing from the isolates of cluster 2 in Austria by only 8 alleles. On the contrary, the mutation T223C in profile H had been previously reported during 2012–2015 in Australia, the Netherlands, Norway, Sweden, the United Kingdom, and the United States (*33*–*35*). The mutation in the *prn* gene at nt 1326 was reported in the United Kingdom (*16*) in 2012. Similar mutations at nt 1325 and 1340 of the *prn* gene were also detected in isolates from Australia (*35*) and the United States (*36*). Likewise, the mutation at nt 1273 had been previously found in Canada (*37*) and the United States as well (*13*,*14*). Last, the insertion of the *IS*481 at nt 1613 in reverse direction was also reported in Canada and the United States (*13*,*14*).

The preliminary cluster threshold proposed in this study has served to delineate clusters and can be adjusted when more epidemiologic data derived from contact tracing are available. Establishing a fixed cluster threshold for *B. pertussis* is challenging also because of its homogeneous core genome. Also, because the bacterium undergoes large genomic rearrangements that are only detectable with advanced bioinformatics (*14*), this diversity might not be captured by cgMLST alone. A possibility to increase the typing resolution obtained with cgMLST for detecting pertussis outbreaks might be to investigate the distribution of *IS*481 within the *B. pertussis* genome, as proposed elsewhere (*14*). In either case, cgMLST allowed the identification of a cluster (cluster 2) of pertactin-deficient isolates from case-patients living in St. Johann in Pongau, possibly indicating the presence of a local pertussis outbreak. We could not determine whether all cases occurring within the period of detection of the genetic profile B belonged to that profile or to another genetic profile because we did not receive *B. pertussis* cultures for each pertussis case. Except for cluster 2, we could neither confirm nor refute that all clusters identified in our study represented single outbreaks. However, we demonstrated the direct transmission of the same pertussis strain by cgMLST among members of the same household. We hypothesize that the 2 case-patients in the household where 2 different genetic

profiles and cgMLST (18 alleles of difference) were detected might have acquired pertussis from different sources.

Our results of the comparative genomic analysis using global strains were concordant with other studies, in which diverse *B. pertussis* genetic profiles are shown to be distributed across countries (*12*–*14*,*16*). The pertactin-deficient strains seemed not to belong to the same clone and the mutations observed in each country might consist of independent mutations, as previously described (*38*). In the absence of more sequences to compare with our isolates in Austria, profile B isolates seemed to be found only in Austria, although the mutation *prn*-2–631^632STOP:T>- had already been reported (*15*). In addition, the number of allelic differences between isolates not geographically nor temporally related to the isolates in Austria was sometimes as low as 2, matching recently reported findings (*12*).

The small differences observed between the SNP-based analysis and the cgMLST-based analysis might be attributable to the slightly higher discriminatory power of SNP-based analysis (*39*). Conversely, the differences in gene content between the Pasteur Institute's cgMLST scheme and ours indicate that our cgMLST was more discriminatory and therefore more suitable for cluster detection in Austria. The uneven number of loci composing each cgMLST might be partially attributable to the different algorithms used by Seqsphere and BIGSdb (*40*) but also to the fact that only targets that were present in all query genomes were included as targets of our cgMLST scheme. In contrast, the Pasteur Institute's scheme included targets present in ≥95% of all query genomes. Bouchez et al. (*12*) did not define a threshold for their cgMLST scheme, and hence both schemes might not be comparable in terms of isolate clustering.

The main limitation of our study was the incomplete information on the vaccination status of the case-patients and other epidemiologic data, which prevented better assessment of the effects of these genetic shifts on pertussis incidence. Because of the reduced sample size, whether detecting pertactin-deficient strains is linked to an increase in pertussis incidence is unclear; therefore, expanding the isolate-based surveillance system at the national level is advisable.

In summary, we found that *B. pertussis* strains in Austria differ genetically from the vaccine strain, both in their core genomes and their vaccine antigen genes. Furthermore, our cgMLST method has proven to be stable enough to be applied beyond our set of 123 *B. pertussis* isolates and proven useful to confirm transmission chains among household members and to detect 8 clusters, 1 of which indicated a possible local outbreak. To detect pertussis outbreaks and target pertussis-control strategies, we recommend performing genomic surveillance of *B. pertussis* using the proposed cgMLST scheme with a preliminary cluster threshold of ≤6 alleles, typing data on the vaccine antigen genes, and completing epidemiologic information on pertussis cases.

About the Author

Dr. Cabal Rosel is a postdoctoral researcher working as a public health microbiologist at the Austrian Agency for Health and Food Safety in Vienna, Austria. Her research interests include microbial genomics, One Health, antimicrobial resistance, and epidemiology.

References

1. Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ. Pertussis: microbiology, disease, treatment, and prevention. Clin Microbiol Rev. 2016;29:449–86. https://doi.org/10.1128/CMR.00083-15
2. Paisley RD, Blaylock J, Hartzell JD. Whooping cough in adults: an update on a reemerging infection. Am J Med. 2012;125:141–3. https://doi.org/10.1016/j.amjmed.2011.05.008
3. Lee AD, Cassiday PK, Pawloski LC, Tatti KM, Martin MD, Briere EC, et al.; Clinical Validation Study Group. Clinical evaluation and validation of laboratory methods for the diagnosis of *Bordetella pertussis* infection: culture, polymerase chain reaction (PCR) and anti-pertussis toxin IgG serology (IgG-PT). PLoS One. 2018;13:e0195979. https://doi.org/10.1371/journal.pone.0195979
4. Mir-Cros A, Moreno-Mingorance A, Martín-Gómez MT, Codina G, Cornejo-Sánchez T, Rajadell M, et al. Population dynamics and antigenic drift of *Bordetella pertussis* following whole cell vaccine replacement, Barcelona, Spain, 1986–2015. Emerg Microbes Infect. 2019;8:1711–20. https://doi.org/10.1080/22221751.2019.1694395
5. Wagner B, Melzer H, Freymüller G, Stumvoll S, Rendi-Wagner P, Paulke-Korinek M, et al. Genetic variation of *Bordetella pertussis* in Austria. PLoS One. 2015;10:e013262.
6. Latasa P, García-Comas L, Gil de Miguel A, Barranco MD, Rodero I, Sanz JC, et al. Effectiveness of acellular pertussis vaccine and evolution of pertussis incidence in the community of Madrid from 1998 to 2015. Vaccine. 2018;36:1643–9. https://doi.org/10.1016/j.vaccine.2018.01.070
7. Esposito S, Stefanelli P, Fry NK, Fedele G, He Q, Paterson P, et al.; World Association of Infectious Diseases and Immunological Disorders (WAidid) and the Vaccine Study Group of the European Society of Clinical Microbiology and Infectious Diseases (EVASG). Pertussis prevention: reasons for resurgence, and differences in the current acellular pertussis vaccines. Front Immunol. 2019;10:1344. https://doi.org/10.3389/fimmu.2019.01344
8. Burdin N, Handy LK, Plotkin SA. What is wrong with pertussis vaccine immunity? The problem of waning effectiveness of pertussis vaccines. Cold Spring Harb Perspect Biol. 2017;9:a029454. https://doi.org/10.1101/cshperspect.a029454

9. Chiappini E, Stival A, Galli L, de Martino M. Pertussis re-emergence in the post-vaccination era. BMC Infect Dis. 2013;13:151. https://doi.org/10.1186/1471-2334-13-151
10. Mooi FR, van Oirschot H, Heuvelman K, van der Heide HGJ, Gaastra W, Willems RJL. Polymorphism in the *Bordetella pertussis* virulence factors P.69/pertactin and pertussis toxin in the Netherlands: temporal trends and evidence for vaccine-driven evolution. Infect Immun. 1998;66:670–5. https://doi.org/10.1128/IAI.66.2.670-675.1998
11. Diavatopoulos DA, Cummings CA, Schouls LM, Brinig MM, Relman DA, Mooi FR. *Bordetella pertussis*, the causative agent of whooping cough, evolved from a distinct, human-associated lineage of *B. bronchiseptica*. PLoS Pathog. 2005;1:e45. https://doi.org/10.1371/journal.ppat.0010045
12. Bouchez V, Guglielmini J, Dazas M, Landier A, Toubiana J, Guillot S, et al. Genomic sequencing of *Bordetella pertussis* for epidemiology and global surveillance of whooping cough. Emerg Infect Dis. 2018;24:988–94. https://doi.org/10.3201/eid2406.171464
13. Bowden KE, Weigand MR, Peng Y, Cassiday PK, Sammons S, Knipe K, et al. Genome structural diversity among 31 *Bordetella pertussis* isolates from two recent U.S. whooping cough statewide epidemics. MSphere. 2016; 1:e00036–16. https://doi.org/10.1128/mSphere.00036-16
14. Weigand MR, Peng Y, Loparev V, Batra D, Bowden KE, Burroughs M, et al. The history of *Bordetella pertussis* genome evolution includes structural rearrangement. J Bacteriol. 2017;199:199. https://doi.org/10.1128/JB.00806-16
15. Barkoff A-M, Mertsola J, Pierard D, Dalby T, Hoegh SV, Guillot S, et al. Pertactin-deficient *Bordetella pertussis* isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill. 2019;24:1700832. https://doi.org/10.2807/1560-7917.ES.2019.24.7.1700832
16. Sealey KL, Harris SR, Fry NK, Hurst LD, Gorringe AR, Parkhill J, et al. Genomic analysis of isolates from the United Kingdom 2012 pertussis outbreak reveals that vaccine antigen genes are unusually fast evolving. J Infect Dis. 2015;212:294–301. https://doi.org/10.1093/infdis/jiu665
17. Vestrheim DF, Steinbakk M, Bjørnstad ML, Moghaddam A, Reinton N, Dahl ML, et al. Recovery of *Bordetella pertussis* from PCR-positive nasopharyngeal samples is dependent on bacterial load. J Clin Microbiol. 2012;50:4114–5. https://doi.org/10.1128/JCM.01553-12
18. Martini H, Rodeghiero C, VAN DEN Poel C, Vincent M, Pierard D, Huygen K. Pertussis diagnosis in Belgium: results of the National Reference Centre for *Bordetella anno* 2015. Epidemiol Infect. 2017;145:2366–73. https://doi.org/10.1017/S0950268817001108
19. Lam C, Octavia S, Bahrame Z, Sintchenko V, Gilbert GL, Lan R. Selection and emergence of pertussis toxin promoter *ptxP3* allele in the evolution of *Bordetella pertussis*. Infect Genet Evol. 2012;12:492–5. https://doi.org/10.1016/j.meegid.2012.01.001
20. Bowden KE, Williams MM, Cassiday PK, Milton A, Pawloski L, Harrison M, et al. Molecular epidemiology of the pertussis epidemic in Washington State in 2012. J Clin Microbiol. 2014;52:3549–57. https://doi.org/10.1128/JCM.01189-14
21. van Gent M, Heuvelman CJ, van der Heide HG, Hallander HO, Advani A, Guiso N, et al. Analysis of *Bordetella pertussis* clinical isolates circulating in European countries during the period 1998–2012. Eur J Clin Microbiol Infect Dis. 2015;34:821–30. https://doi.org/10.1007/s10096-014-2297-2
22. Bart MJ, Harris SR, Advani A, Arakawa Y, Bottero D, Bouchez V, et al. Global population structure and evolution of *Bordetella pertussis* and their relationship with vaccination. MBio. 2014;5:e01074–14. https://doi.org/10.1128/mBio.01074-14
23. Moriuchi T, Vichit O, Vutthikol Y, Hossain MS, Samnang C, Toda K, et al. Molecular epidemiology of *Bordetella pertussis* in Cambodia determined by direct genotyping of clinical specimens. Int J Infect Dis. 2017;62:56–8. https://doi.org/10.1016/j.ijid.2017.07.015
24. Miyaji Y, Otsuka N, Toyoizumi-Ajisaka H, Shibayama K, Kamachi K. Genetic analysis of *Bordetella pertussis* isolates from the 2008–2010 pertussis epidemic in Japan. PLoS One. 2013;8:e77165. https://doi.org/10.1371/journal.pone.0077165
25. Breakwell L, Kelso P, Finley C, Schoenfeld S, Goode B, Misegades LK, et al. Pertussis vaccine effectiveness in the setting of pertactin-deficient pertussis. Pediatrics. 2016;137:e20153973. https://doi.org/10.1542/peds.2015-3973
26. Etskovitz H, Anastasio N, Green E, May M. Role of evolutionary selection scting on vaccine antigens in the re-emergence of *Bordetella pertussis*. Diseases. 2019;7:35. https://doi.org/10.3390/diseases7020035
27. Safarchi A, Octavia S, Nikbin VS, Lotfi MN, Zahraei SM, Tay CY, et al. Genomic epidemiology of Iranian *Bordetella pertussis*: 50 years after the implementation of whole cell vaccine. Emerg Microbes Infect. 2019;8:1416–27. https://doi.org/10.1080/22221751.2019.1665479
28. Carriquiriborde F, Regidor V, Aispuro PM, Magali G, Bartel E, Bottero D, et al. Rare detection of *Bordetella pertussis* pertactin-deficient strains in Argentina. Emerg Infect Dis. 2019;25:2048–54. https://doi.org/10.3201/eid2511.190329
29. Safarchi A, Octavia S, Luu LD, Tay CY, Sintchenko V, Wood N, et al. Pertactin negative *Bordetella pertussis* demonstrates higher fitness under vaccine selection pressure in a mixed infection model. Vaccine. 2015;33:6277–81. https://doi.org/10.1016/j.vaccine.2015.09.064
30. Carvalho CFA, Andrews N, Dabrera G, Ribeiro S, Stowe J, Ramsay M, et al. National Outbreak of Pertussis in England, 2011–2012: a case-control study comparing 3-component and 5-component acellular vaccines with whole-cell pertussis vaccines. Clin Infect Dis. 2020;70:200–7. https://doi.org/10.1093/cid/ciz199
31. van Twillert I, Bonačić Marinović AA, Kuipers B, van Gaans-van den Brink JAM, Sanders EAM, van Els CACM. Impact of age and vaccination history on long-term serological responses after symptomatic *B. pertussis* infection, a high dimensional data analysis. Sci Rep. 2017;7:40328. https://doi.org/10.1038/srep40328
32. Olin P, Rasmussen F, Gustafsson L, Hallander HO, Heijbel H; Ad Hoc Group for the Study of Pertussis Vaccines. Randomised controlled trial of two-component, three-component, and five-component acellular pertussis vaccines compared with whole-cell pertussis vaccine. Lancet. 1997;350:1569–77. https://doi.org/10.1016/S0140-6736(97)06508-2
33. Zeddeman A, van Gent M, Heuvelman CJ, van der Heide HG, Bart MJ, Advani A, et al. Investigations into the emergence of pertactin-deficient *Bordetella pertussis* isolates in six European countries, 1996 to 2012. Euro Surveill. 2014;19:20881. https://doi.org/10.2807/1560-7917.ES2014.19.33.20881
34. Weigand MR, Williams MM, Peng Y, Kania D, Pawloski LC, Tondella ML; CDC Pertussis Working Group. Genomic survey of *Bordetella pertussis* diversity, United States, 2000–2013. Emerg Infect Dis. 2019;25:780–3. https://doi.org/10.3201/eid2504.180812

35. Xu Z, Octavia S, Luu LDW, Payne M, Timms V, Tay CY, et al. Pertactin-negative and filamentous hemagglutinin-negative *Bordetella pertussis*, Australia, 2013–2017. Emerg Infect Dis. 2019;25:1196–9. PubMed https://doi.org/10.3201/eid2506.180240
36. Weigand MR, Peng Y, Cassiday PK, Loparev VN, Johnson T, Juieng P, et al. Complete genome sequences of *Bordetella pertussis* isolates with novel pertactin-deficient deletions. Genome Announc. 2017;5:e00973–17. https://doi.org/10.1128/genomeA.00973-17
37. Tsang RS, Shuel M, Jamieson FB, Drews S, Hoang L, Horsman G, et al. Pertactin-negative *Bordetella pertussis* strains in Canada: characterization of a dozen isolates based on a survey of 224 samples collected in different parts of the country over the last 20 years. Int J Infect Dis. 2014;28:65–9. https://doi.org/10.1016/j.ijid.2014.08.002
38. Lam C, Octavia S, Ricafort L, Sintchenko V, Gilbert GL, Wood N, et al. Rapid increase in pertactin-deficient *Bordetella pertussis* isolates, Australia. Emerg Infect Dis. 2014;20:626–33. https://doi.org/10.3201/eid2004.131478
39. Uelze L, Grützke J, Borowiak M, Hammerl JA, Juraschek K, Deneke C, et al. Typing methods based on whole genome sequencing data. One Health Outlook. 2020;2:3. https://doi.org/10.1186/s42522-020-0010-1
40. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018;3:124. https://doi.org/10.12688/wellcomeopenres.14826.1

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Isolate-based surveillance of *Bordetella pertussis*, Austria, 2018–2020

Appendix 1

MLST Scheme

We obtained the seven *B. pertussis* MLST loci sequences and their allelic variants from an existing *Bordetella sp.* scheme (*1*) to type our Austrian isolates.

Definition of the cgMLST Scheme and Validation

We generated with Ridom SeqSphere+ software version 4.1.9., a stable cgMLST scheme for ad hoc usage containing all genes within the reference genome but the non-homologous, those with internal stop codons and those that overlapped other genes. Briefly, the reference genome (now called representative genome) Tohama I (NC_002929.2) was selected as a seed genome for the cgMLST using the *target definer* function of SeqSphere and applying the following filters:

1. A minimum length filter, which required a minimum of 50 bases.
2. A start codon filter, which required the presence of a start codon at beginning of each gene.
3. A stop codon filter, which required the presence of a single stop codon at end of gene. Two targets were discarded: BP1104, BP2738.
4. A homologous gene filter, which required the presence of no more than one gene copy with a BLAST (*2*) overlap of ≥100bp and an identity of ≥90.0%. 261 targets were discarded: BP0007, BP0023, BP0031, BP0041, BP0049, BP0058, BP0071, BP0080, BP0110, BP0118, BP0124, BP0137, BP0166, BP0175, BP0192, BP0202, BP0203, BP0210, BP0211, BP0228, BP0256, BP0268, BP0281, BP0295, BP0297, BP0327, BP0355, BP0365, BP0392, BP0401, BP0408,

BP0424, BP0439, BP0443, BP0473, BP0481, BP0496, BP0514, BP0517, BP0537, BP0540, BP0565, BP0579, BP0581, BP0582, BP0596, BP0597, BP0611, BP0645, BP0646, BP0676, BP0688, BP0704, BP0716, BP0729, BP0733, BP0739, BP0785, BP0786, BP0797, BP0812, BP0830, BP0838, BP0839, BP0867, BP0871, BP0891, BP0897, BP0910, BP0938, BP0957, BP0994, BP0995, BP0997, BP1020, BP1030, BP1035, BP1044, BP1048, BP1053, BP1064, BP1067, BP1080, BP1086, BP1093, BP1118, BP1130, BP1134, BP1142, BP1157, BP1177, BP1199, BP1201, BP1224, BP1268, BP1278, BP1287, BP1308, BP1332, BP1337, BP1361, BP1365, BP1384, BP1385, BP1388, BP1397, BP1439, BP1450, BP1459, BP1488, BP1489, BP1491, BP1493, BP1511, BP1544, BP1552, BP1557, BP1572, BP1594, BP1602, BP1629, BP1630, BP1633, BP1647, BP1653, BP1656, BP1678, BP1689, BP1697, BP1711, BP1717, BP1735, BP1748, BP1757, BP1792, BP1807, BP1809, BP1810, BP1844, BP1866, BP1879, BP1911, BP1914, BP1947, BP1955, BP1957, BP1958, BP1959, BP2018, BP2029, BP2048, BP2054, BP2087, BP2104, BP2105, BP2121, BP2135, BP2137, BP2166, BP2181, BP2207, BP2214, BP2221, BP2266, BP2272, BP2297, BP2316, BP2355, BP2390, BP2415, BP2453, BP2477, BP2485, BP2492, BP2524, BP2568, BP2577, BP2579, BP2582, BP2587, BP2608, BP2630, BP2666, BP2667, BP2672, BP2673, BP2679, BP2704, BP2721, BP2724, BP2733, BP2748, BP2763, BP2776, BP2781, BP2812, BP2819, BP2821, BP2845, BP2848, BP2852, BP2861, BP2884, BP2912, BP2947, BP2955, BP2976, BP3005, BP3046, BP3049, BP3055, BP3065, BP3091, BP3103, BP3111, BP3114, BP3149, BP3150, BP3164, BP3185, BP3186, BP3203, BP3210, BP3216, BP3220, BP3230, BP3243, BP3257, BP3272, BP3279, BP3294, BP3311, BP3312, BP3313, BP3323, BP3336, BP3386, BP3392, BP3406, BP3408, BP3423, BP3436, BP3451, BP3456, BP3478, BP3505, BP3510, BP3519, BP3520, BP3548, BP3593, BP3603, BP3607, BP3611, BP3698, BP3726, BP3806, BP3810, BP3811, BP3839, BP3851.

5. A gene overlap filter, which required no overlap between a core gene with other genes by more than 4 bases. Ninety-seven targets were filtered and moved to the

accessory genome scheme: BP0036, BP0044, BP0144, BP0169, BP0305, BP0320, BP0417, BP0437, BP0504, BP0619, BP0656, BP0684A, BP0799, BP0836, BP0946, BP1091, BP1106, BP1108, BP1136, BP1159, BP1182, BP1190, BP1344, BP1356, BP1393, BP1399, BP1401, BP1402, BP1433, BP1442, BP1591, BP1639, BP1650, BP1655, BP1665, BP1733, BP1754, BP1789, BP1825, BP1837, BP1883, BP1993, BP2061, BP2080, BP2107, BP2130, BP2150, BP2161, BP2192, BP2236, BP2246, BP2247, BP2264, BP2311, BP2320, BP2328, BP2330, BP2350, BP2381, BP2429, BP2475, BP2603, BP2612, BP2652, BP2658, BP2686, BP2710, BP2729, BP2878, BP2880, BP2902, BP2965, BP3042, BP3155, BP3188, BP3190, BP3277, BP3325, BP3349, BP3357, BP3363, BP3385, BP3389, BP3433, BP3517, BP3556, BP3770, BP3785, BP3792, BP3794, BP3796, BP3799, BP3801, BP3808, BP3824, BP3864, BP3865.

Afterwards we selected 15 publicly accessible *B. pertussis* genomes as query genomes as of 2nd of February 2018 (Appendix 2 Table 1). The selection was based on the center where the genome was sequenced and release date, so that the scheme contained *B. pertussis* strains obtained in different countries and years, avoiding over representing certain countries with more sequences available in NCBI. First, a query genome BLAST (version 2.2.12) search was performed. This required a BLAST hit with an overlap of 100% and an identity of ≥90.0% in every query genome. The following BLAST options were set: Mismatch penalty = −1, match reward = 1, gap open costs = 5, gap extension costs = 2. This query genome BLAST search filtered out 81 targets and all were moved to the accessory genome scheme: BP0184, BP0200, BP0422, BP0515, BP0593, BP0594, BP0635, BP0711, BP0911, BP0913, BP0914, BP0915, BP0918, BP0919, BP0920, BP0921, BP0922, BP0923, BP0924, BP0925, BP0926, BP0927, BP0928, BP0929, BP0930, BP0931, BP0932, BP0934, BP1014, BP1015, BP1016, BP1017, BP1018, BP1019, BP1054, BP1137, BP1138, BP1139, BP1141, BP1174, BP1323, BP1592, BP1948, BP1949, BP1950, BP1951, BP1953, BP1954, BP1961, BP1962, BP1965, BP1987, BP2075, BP2138, BP2139, BP2268, BP2369, BP2451, BP2452, BP2455, BP2820, BP2946, BP2990, BP3105, BP3106, BP3107, BP3108, BP3109, BP3110, BP3115, BP3160, BP3314, BP3315, BP3316, BP3317, BP3319, BP3320, BP3321, BP3322, BP3663, BP3764.

In addition, a stop codon percentage filter was applied to the query genomes (n = 15). This filter required a single stop codon at end of a gene in >80% of the query genomes. One target was filtered and moved to the accessory genome scheme: BP1123.

In summary, 2,983 targets (Appendix 2 Table 2) were defined for cgMLST with a total of 2,932,632 bases, 179 targets were used as accessory targets (Appendix 2 Table 3) with 159,468 bases and 263 targets were discarded. Of the reference genome NC_002929.2 71.8% bases were covered by cgMLST targets. Between 70.9% and 71.45% of query genomes bases were covered by cgMLST targets. Only targets present in all query genomes were included as targets of the final cgMLST scheme ("hard core" cgMLST scheme).

Further evaluation of the scheme included the addition of all available assembled *B. pertussis* genomes from NCBI (n = 537) as of 2nd February 2018. We manually discarded genomes with low WGS quality data. Finally, a collection of 359 *B. pertussis* genomes with a diverse genetic background (Appendix 2 Table 4) remained. All but two genomes presented more than 95% good targets. Afterwards, we also included two non-pertussis strains and one *B. pertussis* strain from an old Austrian isolate collection (Appendix 2 Table 5) and 32 genomes using Illumina reads from SRA (Appendix 2 Table 4).

Sequencing and de novo Assembly of the Austrian *B. pertussis* Isolates

DNA isolation from *B. pertussis* cultures was performed with MagAttract HMW DNA kit (Qiagen, Hilden, Germany) and quantified with Qubit version 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using the double-stranded DNA broad-range (dsDNA BR) assay kit (Thermo Fisher Scientific). Library preparation was performed using Nextera XT Kit (Illumina, San Diego, USA) followed by 300bp paired-end sequencing on an Illumina Miseq platform. Raw reads were de novo assembled with SPAdes version 3.11.1 (*3*). using default parameters. QUAST v5.0.2 (*4*) was used to evaluate the quality of the genome assemblies by extracting information on the N50, the number of contigs and the assembly length for each genome. Additionally, when pangenome assemblies were detected we used CheckM v1.1.2 (*5*) to assess the completeness and contamination level of the sequences (Appendix 2 Table 6).

Typing of the Austrian *B. pertussis* Isolates

Isolates were typed with our newly implemented cgMLST and accessory genome schemes using SeqSphere+, which uses BLAST version 2.2.12 and ignores *contigs* shorter than 200 bases. A target quality control (QC) procedure was included in all typing schemes. This QC required: 1) the length of the consensus being the same as the ref.-seq. area(s) length +-3 triplets, 2) no ambiguities (R,Y,K,M,S,W,B,D,H,V,N) in the consensus area(s) 3) no frame shift in the translatable consensus area(s). Afterwards, the software scanned with BLASTN the sequences of each scheme with the following parameters: mismatch = −1, match = 1, gap open = 5, gap extension = 2 and threshold for each hit of ≥90% identity and 100% aligned to the reference sequence. Isolates was re-sequenced when the percentage of "good targets" referring to the cgMLST scheme was inferior to 95%. To construct phylogenetic trees we always ignored missing values pairwise.

The cluster threshold was preliminary set at 6 allelic differences. We took into consideration the number of allelic differences between the 123 Austrian isolates (Figure 3, https://wwwnc.cdc.gov/EID/article/27/3/20-2314-F3.htm), the available epidemiologic data for the Austrian cases, such as district of residence or cohabitation, and the combination of allelic variants and mutations detected in the vaccine antigen genes *ptx*S1, *ptx*P, *prn*, *fim*2 and *fim*3 (here called as "genetic profiles").

Other Typing Schemes

We extracted the sequences of the *B. pertussis* vaccine antigen genes from the WGS data. To do so, we downloaded from PubMLST all those FASTA sequences comprising all the available allelic variants for the vaccine antigen genes until 2nd February 2018 (https://pubmlst.org/bigsdb?db = pubmlst_bordetella_seqdef&page = downloadAlleles&tree = 1) . Afterwards we generated an allele library typing scheme including the vaccine antigen genes *ptx*S1, *ptx*P, *prn*, *fim*2 and *fim*3 and their variants. The typing schemes were configured to detect new alleles for each of the targets with at least 90% identity and 98% overlap to the reference alleles.

Detection of PRN-Deficient Isolates

When the *prn* gene was not found using Seqsphere+, we first made a BLAST using the assembly file and the *prn* gene sequence from Tohama I (NC_002929.2). If two different fragments of the pertactin gene were present in at least two different *contigs*, the *prn* gene was most probably truncated by an insertion sequence. In this case, we mapped the raw reads against the *B. pertussis* Tohama I reference strain using the Burrows–Wheeler Aligner (BWA-MEM) version 0.7.16a-r1181 (*6*) to check whether these truncations in the *prn* gene were an artifact from the assembly process or a real genetic modification. Afterwards, we used PRODIGAL v2.6.3 (*7*) and BLAST 2.10.0+ to obtain the annotations and the predicted genes with tags on them. Lastly, the resultant BAM files were visualized using Tablet v1.19.09.03 (*8*) to distinguish possible areas near or within the *prn* gene with an absence of mapped reads. If no read pairs spanned the insertion sites, an insertion of >200 nt was suspected.

When the pertactin gene sequence differed from the alleles in the *prn* typing scheme, Seqsphere marked the target as “failed” or “new,” indicating the presence of a genetic modification (e.g.: insertion, deletion and/or base change, with or without a stop codon).

SNP Analysis

The cgMLST-based analysis on the 123 Austrian *B. pertussis* assembled genomes was compared with a SNP-based approach (Appendix 1 Figure 2) using a custom script for variant calling. First, the paired-end reads were trimmed with Trimmomatic (*9*) version 0.39 using the default parameters and then mapped against the reference genome Tohama I (NC_002929.2) using BWA-MEM. SNPs were called using the *mpileup* and *call* commands from bcftools version 1.6–14- geed5371 with default parameters (*10*). The SNPs were then filtered with the vcfutils.pl varFilter script available in SAMtools version 1.11 and indels were excluded with VCFtools 0.1.16 (*11*,*12*). Afterwards the SNPs in all genomes were concatenated in a single fasta alignment. We further inferred the phylogeny with VCF-toolkit version 0.1.6 (*13*). The resultant neighbor-joining tree was compared to the one obtained using the core genome with the Tanglegram option in Dendroscope version 3.7.2 (*14*).

References

1. Diavatopoulos DA, Cummings CA, Schouls LM, Brinig MM, Relman DA, Mooi FR. *Bordetella pertussis*, the causative agent of whooping cough, evolved from a distinct, human-associated lineage of *B. bronchiseptica*. PLoS Pathog. 2005;1:e45. PubMed https://doi.org/10.1371/journal.ppat.0010045

2. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421. PubMed https://doi.org/10.1186/1471-2105-10-421

3. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77. PubMed https://doi.org/10.1089/cmb.2012.0021

4. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5. PubMed https://doi.org/10.1093/bioinformatics/btt086

5. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 2015;25:1043–55. PubMed https://doi.org/10.1101/gr.186072.114

6. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60. PubMed https://doi.org/10.1093/bioinformatics/btp324

7. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119. PubMed https://doi.org/10.1186/1471-2105-11-119

8. Milne I, Bayer M, Cardle L, Shaw P, Stephen G, Wright F, et al. Tablet—next generation sequence assembly visualization. Bioinformatics. 2010;26:401–2. PubMed https://doi.org/10.1093/bioinformatics/btp666

9. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. PubMed https://doi.org/10.1093/bioinformatics/btu170

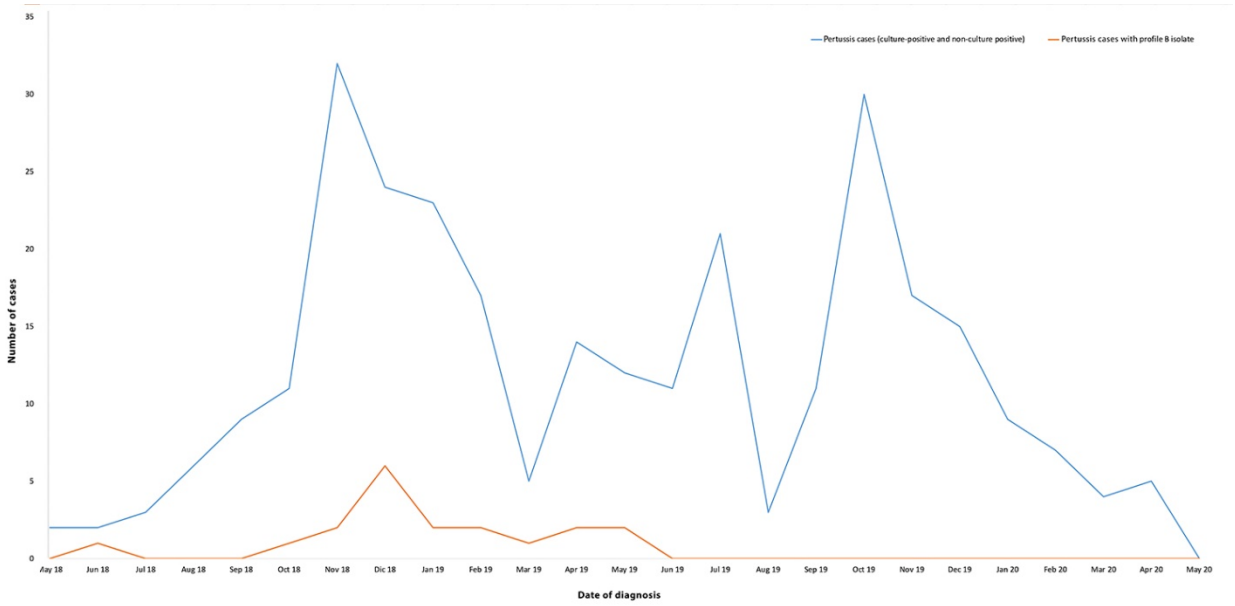
10. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011;27:2987–93. PubMed https://doi.org/10.1093/bioinformatics/btr509

11. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. PubMed https://doi.org/10.1093/bioinformatics/btp352

12. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al.; 1000 Genomes Project Analysis Group. The variant call format and VCFtools. Bioinformatics. 2011;27:2156–8. PubMed https://doi.org/10.1093/bioinformatics/btr330

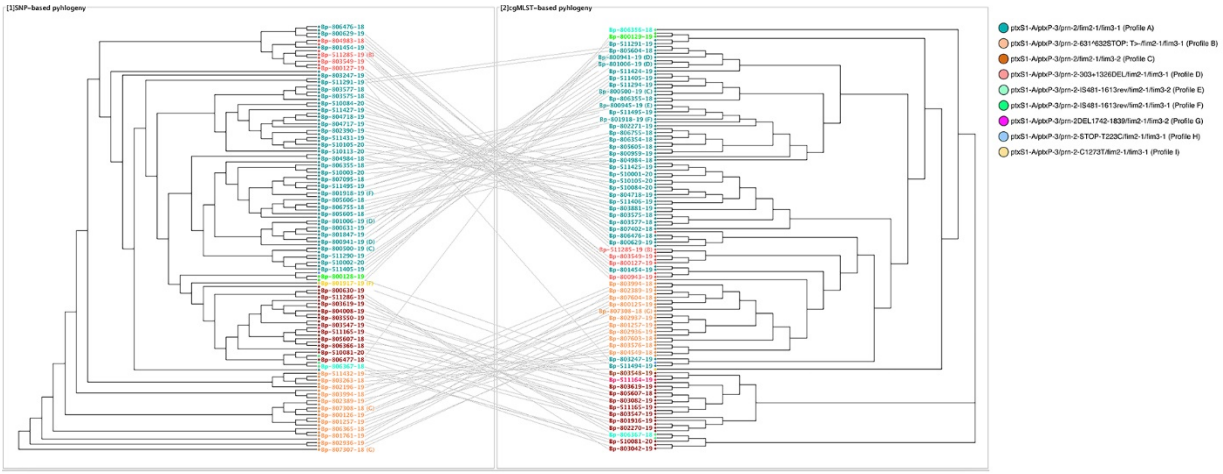
13. Cook DE, Andersen EC. VCF-kit: assorted utilities for the variant call format. Bioinformatics. 2017;33:1581–2. PubMed https://doi.org/10.1093/bioinformatics/btx011

14. Huson DH, Richter DC, Rausch C, Dezulian T, Franz M, Rupp R. Dendroscope: an interactive viewer for large phylogenetic trees. BMC Bioinformatics. 2007;8:460. PubMed https://doi.org/10.1186/1471-2105-8-460



Appendix Figure 1. Cases with a genetic Profile B isolate (orange) vs. total pertussis cases (blue) detected in Sankt Johann im Pongau between May 2018 and May 2020.
The total number of pertussis cases included both non-culture positive pertussis cases, for which no isolate was recovered and culture-positive cases of all genetic profiles, including Profile B.

Appendix 1 Figure 1. Cases with a genetic profile B isolate (orange) vs. total pertussis cases (blue) detected in Sankt Johann im Pongau between May 2018 and May 2020. The total number of pertussis cases included both culture-positive and non-culture positive pertussis cases (no isolate recovered) of any genetic profile, including profile B.



Appendix 1 Figure 2. Comparison of SNP (left) and cgMLST-based phylogenies (right) of the 123 Austrian *B. pertussis* isolates. Genetic profiles (A-I) are depicted in different colors. Letters (A-G) between parenthesis indicate households in which more than one pertussis case was detected. Due to limitations of the software, only a reduced number of isolates is displayed.