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## Research note

***Bordetella pertussis* isolates in Finland after acellular vaccination: serotype change and biofilm formation**Vili Niinikoski<sup>1,2</sup>, Alex-Mikael Barkoff<sup>1,2</sup>, Jussi Mertsola<sup>1,2</sup>, Qiushui He<sup>1,2,\*</sup><sup>1</sup> Finnish Reference Laboratory for Pertussis and Diphtheria, Institute of Biomedicine, University of Turku, Turku, Finland<sup>2</sup> InFLAMES Research Flagship Center, University of Turku, Turku, Finland

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## ABSTRACT

**Objectives:** In Finland, whole cell pertussis vaccine (wP) was introduced in 1952 and was replaced by acellular pertussis vaccine (aP) without fimbrial (FIM) antigen in 2005. We aimed to analyse the changes in serotypes of circulating *Bordetella pertussis* before and after acellular vaccination and to explore the relationship between biofilm formation and serotype diversity after the introduction of aP vaccine.

**Methods:** Serotyping of 1399 *B. pertussis* isolates collected at the Finnish National Reference Laboratory for Pertussis and Diphtheria in Turku, Finland, from 1974 to 2023 was performed by slide agglutination or indirect ELISA. Of 278 isolates collected after 2005, 53 were selected, genotyped for *fim3* and *fim2* alleles, and tested for biofilm formation. The selection criteria included maintaining a relatively equal distribution of isolates per time interval, ensuring approximately a 50:50 ratio of FIM2 ( $N = 26$ ) and FIM3 ( $N = 27$ ) serotypes. The reference strain Tohama I was used as a control.

**Results:** During the wP era, the majority of circulating *B. pertussis* exhibited the FIM2 serotype. However, FIM3 strains have appeared since 1999 and become prevalent. After the implementation of aP vaccines, the distribution of serotypes has exhibited substantial variability. FIM3 isolates displayed an enhanced biofilm formation compared to FIM2 isolates (Geometric mean value (95% CI): 0.90 (0.79–1.03) vs. 0.75 (0.65–0.85);  $p < 0.05$ ). Of the 27 FIM3 isolates, 8 harboured *fim3-1* and 19 *fim3-2* alleles. FIM3 isolates with *fim3-2* allele were significantly associated with increased biofilm formation when compared to those with *fim3-1* (1.07 (0.96–1.19) vs. 0.61 (0.52–0.72);  $p < 0.0001$ ).

**Conclusion:** Following the implementation of aP vaccines, the distribution of serotypes in Finland has exhibited substantial variability. FIM3 isolates with the *fim3-2* allele displayed an enhanced biofilm formation capability compared to FIM2 isolates. **Vili Niinikoski, Clin Microbiol Infect 2024;•:1**

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## Introduction

With the introduction of acellular pertussis vaccines (aP), significant changes have been observed among circulating *Bordetella pertussis* [1–4]. These changes include an increasing prevalence of vaccine antigen pertactin (PRN) deficient isolates and shifts in fimbrial (FIM) serotype distribution.

Studies have shown that recently isolated *B. pertussis* strains have a higher capability for biofilm formation than old ones [5]. Lately, the FIM serotype and the ability of the strains to form

biofilms have been linked [6]. However, the previously published data were derived from a limited number of test isolates, and a systemic comparison was not conducted. Therefore, understanding the relation of biofilm formation with FIM serotypes in *B. pertussis* is crucial for developing strategies to combat pertussis infections.

In 1952, whole cell pertussis vaccine (wP) was introduced in Finland. The vaccine was produced at the National Public Health Institute, Helsinki, Finland. Strain 18530 (FIM3) has been used since 1962. In 1976, strain 1772 (FIM2/3) was added to the vaccine, and the vaccine composition did not change since then. In 2005, wP was replaced by aP vaccine. The aP vaccine containing pertussis toxin and filamentous hemagglutinin was used for primary immunization from 2005 to 2008, and since 2009, a three-component aP, including PRN, has been in use. Subsequently, the first PRN-deficient isolates were detected in 2011 [7], and dynamic changes

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between serotypes FIM2 and FIM3 have been observed. During the era of wP, the vast majority of circulating *B. pertussis* strains were FIM2 serotypes [8]. However, since 1999, FIM3 strains have emerged, and a notable reversal in serotype distribution has occurred following the implementation of aP vaccine without FIM antigen in 2005 (FIM not included in any aP in Finland).

This study aimed to analyse the changes in serotypes of circulating *B. pertussis* in Finland before and after acellular vaccination and to explore the relationship between biofilm formation and serotype diversity after the introduction of aP vaccine.

## Methods

Serotyping of 1399 *B. pertussis* isolates collected at the Finnish National Reference Laboratory for Pertussis and Diphtheria in Turku, Finland, from 1974 to 2023 was performed by slide agglutination or indirect ELISA. In the ELISA, two international reference strains (S1 and S3) and monoclonal antibodies (NIBSC 06/124 and 06/128) to FIM2 and FIM3 were used to detect the expression of FIM serotype. The serotyping result of the isolates collected before 2006 was previously published [8]. Of 278 isolates collected after 2005, 53 were tested for biofilm formation. These isolates were selected to represent different time periods and geographic regions within Finland. The selection criteria also included maintaining a relatively equal distribution of isolates per time interval, ensuring approximately a 50:50 ratio of FIM2 ( $N = 26$ ) and FIM3 ( $N = 27$ ) serotypes. The reference strain Tohama I, isolated in Japan in 1952, expressing FIM2 and carrying the *fim2-1* allele, was used as a control.

For biofilm quantification, *B. pertussis* isolates were initially cultured on Bordet-Gengou agar for 72 hours at 35°C. Colonies were transferred into 150 µL Stainer-Scholte medium in microtiter plates to reach an OD = 0.1 and incubated at 35°C in place for an additional 72 hours. Fresh medium (volume = 100 µL) was added every 24 hours to maintain optimal growth conditions. Biofilm formation was quantified as previously described by O'Toole et al. [9]. The plate was inverted and rinsed. This step was repeated to minimize background staining. The staining was performed using 200 µL of a 0.1% crystal violet solution in water, which was incubated at room temperature for 10–15 minutes. The plate was then rinsed 3–4 times with water. Once dried, 200 µL of 30% acetic acid solution in water was added to each well to solubilize the crystal violet. The plate was then further incubated at room temperature for 10–15 minutes on a plate shaker. Finally, the quantification of biofilm was achieved using a plate reader at an OD of 550 nm, with a 30% acetic acid solution in water serving as the blank. The biofilm formation results were then corrected with bacterial growth OD-values (from 0.15 to 0.2) to have comparable results.

Both serotyping and genotyping of *fim3* and *fim2* alleles were previously described [1,10].

## Results

As shown in Fig. 1, the FIM2 serotype held dominance in the wP vaccine era until 1999, when a significant shift to FIM3 occurred. This shift coincided with a nationwide epidemic of pertussis [11]. However, following the introduction of aP vaccine, dynamic fluctuations were observed, ultimately leading to a resurgence of FIM2 isolates as the dominant serotype in Finland.

All 53 isolates had the FIM2 and FIM3 genes, and their expression is controlled by variation in the promoter. Of the 53 isolates, 51 harboured the *ptxP3* allele, and two harboured *ptxP1*. Of 27 FIM3 isolates, 8 harboured *fim3-1* allele, while 19 exhibited *fim3-2* allele. Of 26 FIM2 isolates, all except one harboured *fim3-1* allele.

Variation in biofilm formation was observed among isolates with the same serotypes (Fig. 2). However, the variation of the

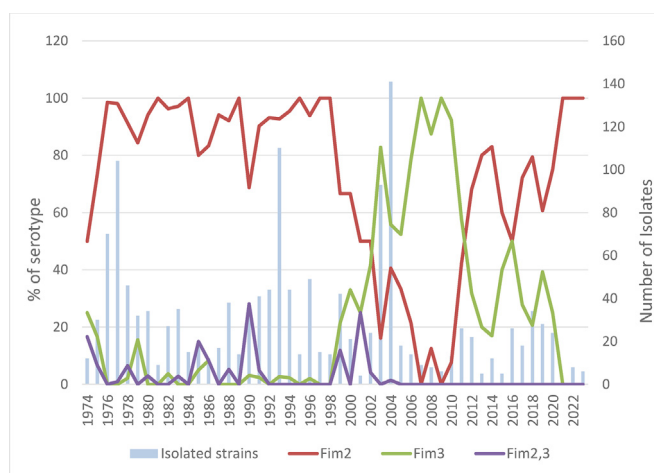


Fig. 1. Distribution (%) of fimbrial serotypes of *Bordetella pertussis* isolates ( $N = 1399$ ) in Finland, from 1974 to 2023. The number of isolates collected each year varied from 4 to 141.

reference strain Tohama I was marginal. Particularly noteworthy was the observation that starting from the year 2010. Although FIM3 isolates displayed a higher capacity for biofilm formation compared to FIM2 counterparts (Geometric mean value (95% CI): 0.90 (0.79–1.03) vs. 0.75 (0.65–0.85);  $p < 0.05$ ), isolates carrying the *fim3-2* allele exhibited a markedly enhanced ability to form biofilms when compared to those with *fim3-1* allele (1.07 (0.96–1.19) vs. 0.61 (0.52–0.72);  $p < 0.0001$ ).

## Discussion

It became evident that following the implementation of aP vaccine, Finland has experienced substantial fluctuations in the

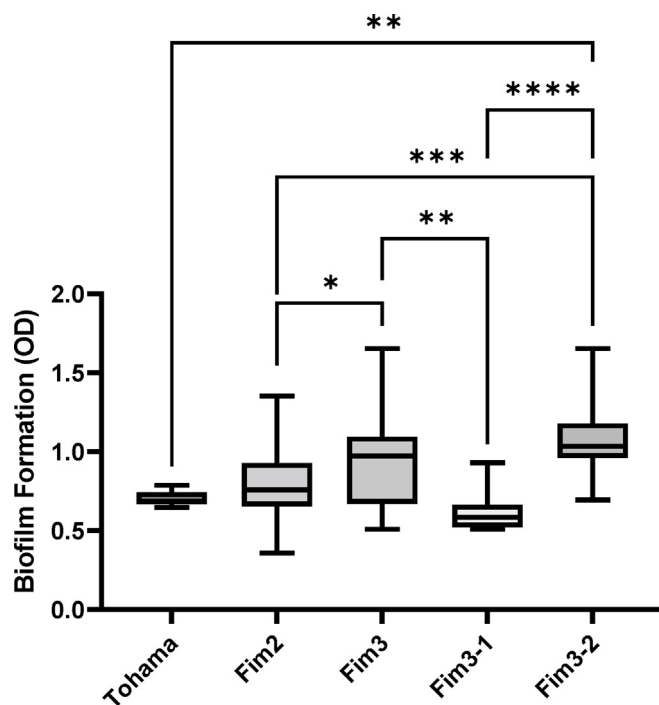


Fig. 2. Biofilm formation capacity between serotype and genotype of *Bordetella pertussis* clinical isolates tested. Min/Max-values, 25 and 75 quartiles, and median values are shown in the graph. \*indicates  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  and \*\*\*\* $p < 0.0001$ .

distribution of *B. pertussis* serotypes. In Finland, the aP vaccines used for primary and booster vaccinations do not contain FIM. Therefore, the dynamic changes in frequency from FIM3 to FIM2 are most likely caused by infections or the combination of infection and vaccinations. It has been shown that FIM2 strains could exhibit stronger colonization than FIM3 strains. Furthermore, based on a previous study, it seems that individuals infected with FIM2 strains carry antibodies to both FIM2 and FIM3, indicating that FIM2 strains could express both FIM2 and FIM3 during the course of the disease [8]. Therefore, the change in frequency from FIM3 to FIM2 may indicate additional benefits for *B. pertussis* colonization.

On the other hand, this study demonstrates that FIM3 isolates with the *fim3-2* allele exhibit a greater capacity for biofilm formation than FIM2 isolates. Strains currently used for aP vaccine harbour *fim2-1* and/or *fim3-1* alleles. The difference between *fim3-1* and *fim3-2* alleles is a point mutation of C260A causing an amino acid change (Ala87Glu). In pre-vaccination isolates, only the *fim3-1* allele has been identified. The *fim3-2* allele probably arose after the introduction of vaccination [12]. In this study, almost all FIM2 isolates harbour *fim2-1* allele. Both *fim2-1* and *fim2-2* alleles are observed in isolates from the pre-vaccine era, and the difference between the two alleles is a point mutation of G521A, causing an amino acid change (Arg174Lys). The enhanced biofilm-forming capabilities observed among FIM3 isolates carrying the *fim3-2* allele underscore the importance of fimbrial serotyping and genotyping.

While our study demonstrates a significant association between FIM3 isolates and enhanced biofilm formation capacity among Finnish *B. pertussis* strains, it is important to note that regional variations within serotypes and genotypes may exist. A recent study from France suggested that FIM2 strains exhibit a propensity to produce more biofilm [6]. This regional disparity underscores the complexity of *B. pertussis* epidemiology and the potential influence of local factors on strain behaviour. It should be kept in mind that the number of isolates tested for biofilm formation in the above study is limited, and only *fim3-1* carrying isolates were included.

In Finland, aP vaccine without PRN was started in 2005, and aP with PRN in 2009. The PRN-deficient isolates have been detected since 2011. While comparing isolates with PRN deficiency, we found that they not only include both serotypes but also both Fim3-1 and Fim3-2 genotypes, suggesting that introducing PRN in the vaccine component may not have directly impacted the serotype change observed. Moreover, we did not observe the difference in biofilm formation between PRN-deficient and PRN-positive isolates. Whole genome sequencing may reveal possible differences between isolates with *fim3-1* and *fim3-2* alleles.

In conclusion, after the implementation of aP vaccines, the distribution of serotypes in Finland has exhibited substantial variability. FIM3 isolates with *fim3-2* allele displayed an enhanced biofilm formation capability compared to FIM2 isolates. These findings underscore the necessity for additional research into the impact of fimbrial genotypes on biofilm formation. Our study also stresses the importance of continuously monitoring emerging strains of *B. pertussis* and the need to obtain a better understanding of the relationship of the evolution of *B. pertussis* in the vaccinated population.

## Author contributions

QH and AMB conceptualized the project, and all authors designed the study. VN performed experiments. VN, AMB, and QH were responsible for data collection, and all authors analysed and interpreted the data. VN drafted the manuscript, and all authors were involved in critically revising the manuscript for important intellectual content. All authors approved the final manuscript and accepted final responsibility for the decision to submit for publication.

## Transparency declaration

The authors declare that they have no conflicts of interest.

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## References

- [1] Barkoff AM, 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>.
- [2] Martin SW, Pawloski L, Williams M, Weening K, DeBolt C, Qin X, et al. Pertactin-negative *Bordetella pertussis* strains: evidence for a possible selective advantage. *Clin Infect Dis* 2015;60:223–7. <https://doi.org/10.1093/cid/ciu788>.
- [3] 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>.
- [4] Barkoff AM, Mertsola J, Pierard D, Dalby T, Hoegh SV, Guillot S, et al. Surveillance of circulating *Bordetella pertussis* strains in Europe during 1998 to 2015. *J Clin Microbiol* 2018;56. <https://doi.org/10.1128/JCM.01998-17>.
- [5] Cattelan N, Jennings-Gee J, Dubey P, Yantorno OM, Deora R. Hyperbiofilm formation by *Bordetella pertussis* strains correlates with enhanced virulence traits. *Infect Immun* 2017;85:003733–e417. <https://doi.org/10.1128/IAI.00373-17>.
- [6] Matczak S, Bouchez V, Leroux P, Douché T, Collinet N, Landier A, et al. Biological differences between FIM2 and FIM3 fimbriae of *Bordetella pertussis*: not just the serotype. *Microbe*. *Infect* 2023;25:105152. <https://doi.org/10.1016/j.micinf.2023.105152>.
- [7] Barkoff AM, Mertsola J, Guillot S, Guiso N, Berbers G, He Q. Appearance of *Bordetella pertussis* strains not expressing the vaccine antigen pertactin in Finland. *Clin Vaccine Immunol* 2012;19:1703–4. <https://doi.org/10.1128/CVI.00367-12>.
- [8] Heikkinen E, Xing DK, Olander RM, Hytönen J, Viljanen MK, Mertsola J, et al. *Bordetella pertussis* isolates in Finland: serotype and fimbrial expression. *BMC Microbiol* 2008;8:162. <https://doi.org/10.1186/1471-2180-8-162>.
- [9] O'Toole GA. Microtiter dish biofilm formation assay. *J Vis Exp* 2011;47:2437. <https://doi.org/10.3791/2437>.
- [10] Barkoff AM, Guiso N, Guillot S, Xing D, Markey K, Berbers G, et al. A rapid ELISA-based method for screening *Bordetella pertussis* strain production of antigens included in current acellular pertussis vaccines. *J Immunol Methods* 2014;408:142–8. <https://doi.org/10.1016/j.jim.2014.06.001>.
- [11] Elomaa A, Advani A, Donnelly D, Antila M, Mertsola J, Hallander H, et al. Strain variation among *Bordetella pertussis* isolates in Finland, where the whole-cell pertussis vaccine has been used for 50 years. *J Clin Microbiol* 2005;43:3681–7. <https://doi.org/10.1128/JCM.43.8.3681-3687.2005>.
- [12] van Gent M, Bart MJ, van der Heide HG, Heuvelman KJ, Mooi FR. Small mutations in *Bordetella pertussis* are associated with selective sweeps. *PLoS One* 2012;7:e46407. <https://doi.org/10.1371/journal.pone.0046407>.