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Technical note

A rapid ELISA-based method for screening *Bordetella pertussis* strain production of antigens included in current acellular pertussis vaccines

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ABSTRACT

Introduction: Despite extensive vaccinations, there have been pertussis epidemics in many countries including the Netherlands, the UK, Australia and the USA. During these epidemics *Bordetella pertussis* strains not producing the vaccine antigen pertactin (Prn) are emerging and increasing in numbers. However, methods for confirming PRN production of *B. pertussis* isolates are combined PCR or PCR-based sequencing tests and western blotting. Furthermore, data about production of pertussis toxin (PT) and filamentous hemagglutinin (FHA) of these isolates are scarce. Fimbriae (Fim) production is usually determined by agglutination and reported as serotype. In this study we developed an easy, accurate and rapid method for screening PT and FHA production. Methods for Prn and Fim production have been published earlier.
Methods: We analyzed altogether 109 *B. pertussis* strains, including 103 Finnish *B. pertussis* strains collected during 2006–2013, international strain Tohama I, French strains FR3496 (PT-negative), FR3693 (Prn-negative) and FR4624 (FHA-negative) and Fim-serotype reference strains S1 (producing only Fim2) and S3 (producing only Fim3). An indirect ELISA with whole bacterial cells as coating antigen was developed and used for rapid screening of the *B. pertussis* strains. Production of different antigens (PT, FHA, Prn, Fim2 and Fim3) was detected with specific monoclonal antibodies (mAbs).
Results: From the 103 Finnish *B. pertussis* strains tested, all were positive for PT, FHA and Fim. Four were found negative for Prn, and they were isolated during 2011–2013.
Conclusions: The newly developed method proved to be useful and simple for rapid screening of different antigen production of *B. pertussis* isolates.

1. Introduction

Vaccination against pertussis is effective, but still the disease has been circulating among immunized populations in the 21st century with a yearly burden of 20–40 million cases (Celentano et al., 2005). Vaccination strategies have changed during the past years and booster doses have been implemented in national vaccination programs to further protect children,

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young adults and to prevent transmission of the disease to infants. In addition to this, different vaccine strategies to prevent pertussis have been tested during the past years and cocooning strategy and maternal vaccination for pregnant women have been recommended (van der Maas et al., 2013). In the beginning of the 21st century, Celentano et al. described the increasing trend of pertussis in Europe and in other countries including the USA, suggesting that pertussis cases in adolescents and adults will increase (Celentano et al., 2005). In the past few years, there have been major pertussis outbreaks in Europe, Australia and the USA (van der Maas et al., 2013; Kmietowicz, 2012; Winter et al., 2012; Campbell et al., 2012; Lam et al., 2014). Moreover, some seroprevalence studies have also shown that pertussis is circulating more and more among "healthy" people (de Greeff et al., 2010). The reasons behind the latest outbreaks are unclear, however several suggestions to explain the high circulation of the disease have been proposed. These suggestions include better awareness of the disease, waning immunity, vaccine failure or decrease of vaccine efficiency and pathogen adaptation, which includes many factors contributing to the survival of *Bordetella pertussis* (Cherry, 2013). One important factor of pathogen adaptation is the production of vaccine antigens. Recently several publications have described circulating strains mainly not producing pertactin (Prn), an important protein included in acellular pertussis vaccines (Barkoff et al., 2012; Bouchez et al., 2009; Miyaji et al., 2013; Pawloski et al., 2014). During the pertussis epidemic in Washington, USA, in 2012, more than 60% of *B. pertussis* isolates did not produce Prn (Pawloski et al., 2014). Isolates that did not produce PT, FHA and Fim were reported in France and Japan (Miyaji et al., 2013). Guiso et al. hypothesized that the change from whole-cell vaccine (WCV) to acellular vaccine (ACV) has increased the pressure for *B. pertussis* circulating strains not to produce antigens included in the ACVs (Bouchez et al., 2009; Guiso, 2009). The emergence and increase in number of isolates not producing antigens of ACVs are alarming.

In Finland, pertussis vaccination started in 1952 and WCV was replaced by ACV in 2005 (Barkoff et al., 2012). Although, there is increasing awareness of antigen negative *B. pertussis* strains, it seems that an easy, rapid and cheap method, for screening the production of different antigens by *B. pertussis*, is lacking. Western blotting, which is referred to as the gold standard for detecting protein production, is still used in many studies (Bouchez et al., 2009; Pawloski et al., 2014), although methods based on immunoblotting are laborious.

In this study we aimed to develop an ELISA based method for rapid screening of different antigens produced by *B. pertussis*. The targeted antigens were PT and FHA. For Prn and Fim, similar screening methods have already been published earlier (Barkoff et al., 2012; Heikkinen et al., 2008; Tsang et al., 2005). Furthermore, we screened the production of PT, FHA, Prn [part of the Prn data have already been published, (Barkoff et al., 2012)] and Fim of all our clinical *B. pertussis* strains collected during 2006–2013.

2. Materials and methods

2.1. Reference strains and clinical isolates

For method development and strain screening, we included altogether 109 *B. pertussis* strains and two *Bordetella parapertussis* strains. *B. pertussis* strains included 103 Finnish clinical *B. pertussis* isolates collected from different cities in Finland during 2006–2013. All isolates collected in the Pertussis reference laboratory, National Institute for Health and Welfare, Turku, Finland were included (Fig. 1), international strain Tohama I and three French *B. pertussis* strains FR3496 (negative for PT), FR3693 (negative for Prn), and FR4624 (negative for FHA) (Bouchez et al., 2009) were used as negative controls in this study. In this study, we used purified PT, FHA and Prn, kindly provided by GlaxoSmithKline Biologicals, Rixensart, Belgium as positive controls to show the binding capacity of the mAbs used. For *B. parapertussis*, international reference strain ATCC15311 and one clinical isolate (PRCB661) were included.

2.2. Optimization of the ELISA assays

For testing inter-assay variation and the stability of the bacterial suspensions used, seven strains were included: Tohama I, FR3469, FR3693, FR4624 and three Finnish clinical isolates: PRCB723, PRCB728 and PRCB742 (isolated in 2012–2013). Inter-assay variation (three paired replicates) and stability of bacterial suspension during the storage for PT, FHA and Prn were measured.

We prepared three independent cultures (charcoal agar) from the selected strains and made bacterial suspensions in 1 μM 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride [(AEBSF), A8456, Sigma-Aldrich, Helsinki, Finland] –PBS. According to Sigma-Aldrich, AEBSF is known to improve the stability of proteins in aqueous solutions. Optical density (OD) values at three different time points from the same tube were measured: 0 h, 72 h and 8 days after the preparation of AEBSF-PBS tubes. In addition, inter-plate difference for these cultures at the same time points was tested. We also tested, whether AEBSF could have interfered to ELISA results by comparing PT in AEBSF-PBS to PT in PBS simultaneously at the same plate.

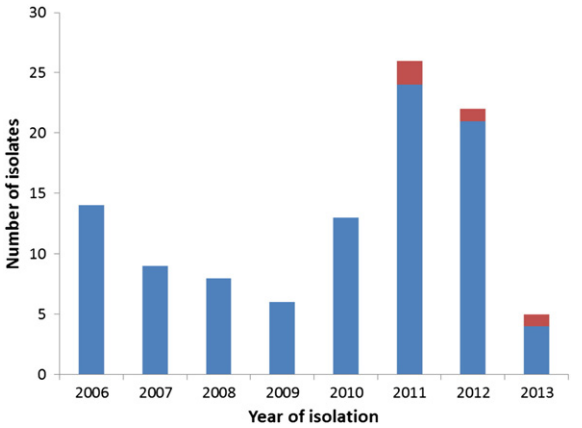

Fig. 1. Yearly distribution of Finnish *B. pertussis* isolates and appearance of Prn negative isolates. No PT, FHA or Fim negative isolates were found during 2006–2013. The number of Prn negative isolates is indicated in red. Part of the result of Prn and Fim was published previously (Barkoff et al., 2012; Heikkinen et al., 2008).