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Review

Current methods for capsular typing of *Streptococcus pneumoniae*

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ABSTRACT

Streptococcus pneumoniae is a major respiratory tract pathogen causing pneumococcal disease mainly in children aged less than five years and in the elderly. Ninety-eight different capsular types (serotypes) of pneumococci have been reported, but pneumococcal conjugate vaccines (PCV) include polysaccharide antigens against only 7, 10 or 13 serotypes. It is therefore important to track the emergence of serotypes due to the clonal expansion of non-vaccine serotypes. Increased numbers of carried and disease-causing pneumococci are now being analysed as part of the post-PCV implementation surveillance studies and hence rapid, accurate and cost-effective typing methods are important.

Here we describe serotyping methods published prior to 10th November 2014 for pneumococcal capsule typing. Sixteen methods were identified; six were based on serological tests using immunological properties of the capsular epitopes, eight were semi-automated molecular tests, and one describes the identification of capsular type directly from whole genome data, which also allows for further intra and inter-genome analyses. There was no single method that could be recommended for all pneumococcal capsular typing applications. Although the Quellung reaction is still considered to be the gold-standard, laboratories should take into account the number of pneumococcal isolates and the type of samples to be used for testing, the time frame for the results and the resources available in order to select the most appropriate method. Most likely, a combination of phenotypic and genotypic methods would be optimal to monitor and evaluate the impact of pneumococcal conjugate vaccines and to provide information for future vaccine formulations.

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1. Introduction

Streptococcus pneumoniae (the pneumococcus) causes ~500,000 deaths of children under the age of five years annually, primarily in developing countries (WHO, 2012). There are ninety-eight reported pneumococcal serotypes classified on biochemical and genetic differences in the structure of the capsular polysaccharide (CPS), including the novel serotypes 6D (Jin et al., 2009), 6F and 6G (Oliver et al., 2013), 11E (Calix and Nahm, 2010), 20A and 20B (Calix et al., 2012) and putative serotype 6E (Ko et al., 2013), which are grouped into 46 serogroups based on their antigenic similarities (Henrichsen, 1995). The specific distribution of pneumococcal serotypes and serogroups has been associated with age, site of infection, pre-existing medical conditions, social status and geographic region (Hausdorff et al., 2000a,b, 2005). Reports suggest that the biochemical properties of the CPS are predictors for serotype specific carriage prevalence, growth and invasive pneumococcal disease (IPD) potential (Brueggemann et al., 2003; Hathaway et al., 2012; Sjostrom et al., 2006; Weinberger et al., 2009). The majority of IPD in adults and children as well as acute otitis media in children under five years of age can be attributed to only 11 serogroups (Hausdorff et al., 2000b; Johnson et al., 2010).

The accurate identification of pneumococcal serotypes is paramount for disease surveillance and pre- and post-pneumococcal vaccine evaluation. In the UK and other countries, the emergence of non-vaccine serotypes after PCV7 and PCV13 implementations has been reported in carriage and disease. Increases in carriage of serotypes 6C, 33F and 22F were reported in the paediatric population (Tocheva, 2011; Tocheva et al., 2013; van Hoek et al., 2014). Changes in disease-causing serotypes after pneumococcal vaccine implementations included the decrease of 19A, but increase in serotypes 15A and 15B, 35B, 23B and 6C (Jefferies et al., 2010; Hanage et al., 2011; Miller et al., 2011; Rosen et al., 2011; Richter et al., 2014; Regev-Yochay et al., 2015).

While pneumococcal capsular typing uses antisera to detect serogroup and serotype-specific pneumococcal capsule epitopes, molecular techniques identify the pneumococcal serotypes based on the nucleotide sequence of the capsule gene. Here, we review studies published on pneumococcal capsular typing methods identified using the PubMed database before 10th November 2014. Search terms used to identify relevant articles were: [*S. pneumoniae*], [pneumococcus], [capsule type identification], [serotype identification] and [serotyping methods]. Further studies were identified from the reference lists of primary studies. Only studies published in English and with available full text articles were used in this review. To provide a convenient summary of strengths and limitations of the various methods available, we used the following criteria: sensitivity, specificity, number of serotypes that can be identified by the method, approximate costs, time required to prepare the samples and perform a test, level of training required to carry out the method, shelf life of reagents, possibility of using the method for batch processing, detection of multiple serotypes within the same sample and direct pneumococcal serotype detection from clinical samples (Table 1).

2. Phenotypic methods

2.1. Quellung reaction

The Quellung reaction (Neufeld, 1902; Sorensen, 1993) is still considered the gold-standard for pneumococcal serotyping (Austrian, 1976). It is reported to be highly sensitive and specific and remains the method against which all other methods are compared (Satzke et al., 2013). It is an in-situ immunoprecipitation method whereby rabbit antiserum is added to the pneumococcal suspension. If the antibodies recognise a specific capsule epitope, they bind to the cell wall and produce a change in the refractile index of light passing through the capsule, which appears swollen under a microscope (Sorensen, 1993). One limitation of the Quellung reaction is that the test is unable to detect multiple serotypes within the same sample (Kaijalainen, 2006). It is also inconvenient for serotyping large number of isolates and is relatively laborious and costly as it requires a supply of pneumococcal antisera. To improve the latter, Habib and colleagues (Habib et al., 2014) developed an improved Quellung protocol that minimizes the amount of antisera used (Habib et al., 2014) (a video for the protocol is also available at http://www.jove.com/video/51208/). This is done by testing 1 μl of pneumococcal suspension against pools made up from the most common antisera and testing the sample against only those individual antisera from the pools that give a positive result (Habib et al., 2014).

2.2. Co-agglutination and latex agglutination

The development of agglutination tests reduced the time required to serotype a batch of pneumococcal isolates (Kirkman et al., 1970). A fresh culture of pneumococci is mixed with type specific serum raised against 1 of 46 pneumococcal serogroups in each well of a microtitre plate. The agglutination reaction that occurs between the pneumococcus and the type-specific antibodies in the serum results in visible clumping eliminating the requirement for microscopy (Kirkman et al., 1970). This method was improved by incorporating factor-specific antiserum and carried out on a microscope slide, i.e., slide agglutination test (Kronvall, 1973). Commercially available omni antiserum enables the detection of 91 of the 98 reported pneumococcal serotypes with this method (Christensen et al., 1973; Kronvall, 1973; Lund and Rasmussen, 1966; SSI, 2012).

In 1986, the co-agglutination method was improved for pneumococcal typing from culture and clinical samples by binding formalin fixed staphylococcus protein A to a serotype specific (factor) antibodies rather than serogroup specific antibodies as done previously, thus minimizing cross-reactivity (Smart, 1986; Smart and Henrichsen, 1986).

The chess-board system for typing pneumococci was introduced in 1993 and served as the basis for the Pneumotest-latex test developed by the Statens' Serum Institute. It identifies the 23 serotypes included in the 23-valent pneumococcal polysaccharide vaccine and additional 25 cross-reacting types (Table 2). This method was later improved by introducing the latex agglutination test for identification and capsular typing of pneumococci, which allowed pneumococcal identification

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