How Many Factor Antisera Can Be Found for Serogroup 6 *Streptococcus pneumoniae*?†

We read the article of Jacobs et al. (3) with interest. The authors reported they could identify serotype 6C *Streptococcus pneumoniae* using factor 6d, which was developed by Statens Serum Institut, Copenhagen, Denmark.

We immunized a rabbit with one serotype 6C isolate identified with a monoclonal antibody (MAb6C) in the U.S. lab in which the new type was found. Based on absorption with serotypes 6A and 6B and the Quellung test (6), we got the specific antisera for MAb6C after absorption with 6A and 6B. The antisera identified 10 serotype MAb6C isolates among 91 traditional serotype 6A isolates, just like the multibead assay. We concluded that serotype 6C could be set up in the Denmark typing system because it could have a new factor antigen, 6X1 (5). More importantly, it must have a new antigenic formula, 6a, 6b, 6X1, which showed it must be a new serotype (5, 2). But we found the antisera was not specific for serotype 6C in further study. The antisera could be used to distinguish new type 6D from traditional 6B isolates. Six such isolates were identified among 111 isolates typed as 6B before (unpublished data). Type 6D was then confirmed using a PCR method that was described previously (4). The antisera should be specific for serotypes 6C and 6D, and it was reported that the two serotypes have replacements similar to those in the capsular genes of serotype 6A and 6B (1). It was not unexpected that the two new types have the same antigen.

We named the new factor antisera 6X1 instead of 6d in our publication (5) because we did not determine the antiserum with all known serotype antigens (7). Meanwhile, we found that factor 6b, after absorption completely by serotype 6C, was still against serotype 6aA, which means factor 6b might be divided into two factors. The specific antigen for 6A had already been shown by the multibead assay, which showed that serotype 6A bound to both monoclonal antibodies 6aA and 6AB whereas 6C bound to only one monoclonal antibody, 6AB (8).

The multibead assay based on monoclonal antibodies is an effective method to identify the serotypes of *S. pneumoniae*, but it could cause discrepancy because the monoclonal antibodies were developed from mice, whereas the traditional diagnostic antisera were developed from rabbits. We recommend, therefore, that the names of serotypes based on monoclonal antibodies contain special added markers, for example, MAb6A, MAb6B or m6A, m6B, etc., instead of monoclonal antibodies.

Authors’ Reply

We appreciate the interest shown by Yao et al. and Bratcher and Nahm (2) in our recent publication on the serologic identification of serotype 6C *Streptococcus pneumoniae* (4). As discussed in our paper, the same change that transformed serotype 6A into serotype 6C would transform serotype 6B into the new putative serotype 6D, which has now been documented experimentally and in naturally occurring isolates in Fiji and Korea (1, 3, 5).

Yao et al. found 10 serotype 6C isolates among 91 isolates previously characterized as serotype 6A and 6 putative serotype 6D strains among 111 isolates previously characterized as serotype 6B, extending the geographic distribution of these serotypes. This addition to our knowledge of the distribution of these new serotypes in China is very valuable.

As we did not detect any serotype 6D strains in our limited serotype 6B collection, we are unable to comment further on the question from Yao et al. regarding the reactivity of the 6d factor antisera with serotype 6D. However, the monoclonal antibody described by Yao et al. appears to have the same antigenicity as the 6d factor antisera. Bratcher and Nahm have evaluated the reactivity of the new 6d factor serum against serogroup 6 strains, including two of serotype 6D (2).

Using one lot of each factor serum, they obtained positive reactions by Quellung test, agglutination, and flow cytometry for factors 6b, 6c, and 6d with serotypes 6A, 6B, and 6C, respectively, while factor 6c and 6d sera both reacted with serotype 6D. These findings differ from our findings with serotype 6C, where we found factors 6b and 6d to be positive with serotype 6C strains. The reason for this difference is likely related to the lot of factor 6b antisera used by Bratcher and Nahm, as Statens Serum Institut has recently modified factor 6b antisera by absorbing out cross-reactivity with serotype 6C (L. M. Lambertsan, personal communication, 2010).

The Danish pneumococcal typing and nomenclature system...
has served us well over the past 70 years, evolving to accommodate new serotypes as they are discovered and characterized (6). Currently, the four serogroup 6 strains can be differentiated both serologically and genetically. However, we are not able to comment on the issues raised by Yao et al. on the terminology used to characterize serotypes, polyclonal antibodies, or monoclonal antibodies and leave these issues to others for guidance.

REFERENCES

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