JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2010, p. 1456–1457 Vol. 48, No. 4
0095-1137/10/$12.00 doi:10.1128/JCM.01907-09

Validation of Factor 6d Antiserum for Serotyping *Streptococcus pneumoniae* Serotype 6C[▽]

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Received 26 September 2009/Returned for modification 21 December 2009/Accepted 4 February 2010

Factor 6d antiserum reacts with the new *Streptococcus pneumoniae* serotype 6C. Serogroup 6 isolates, consisting of 49 6A, 42 6B and 98 6C strains from the United States and Israel, serotyped in parallel by PCR and capsular swelling methods, were all identified correctly. The new factor 6d antiserum accurately identifies serotype 6C.

Until recently there were 90 recognized distinct pneumococcal serotypes, with serogroup 6 represented by two serotypes, 6A and 6B. The serogroup 6 capsular *wciP* gene loci differ from each other by only 1 nucleotide, and this difference produces 6A WciP with a serine at residue 195, while 6B WciP has an asparagine at the same residue (2). In 2007, however, a 91st serotype was described: serotype 6C (15, 16). Serotype 6C cross-reacts serologically with serotype 6A and was initially differentiated from serotype 6A by using monoclonal antibodies and shown to have a different capsular polysaccharide from that of serotype 6A (16). The same investigators then characterized the genetic basis of the new serotype as having a new *wciN* gene region of the capsular locus encoding glucosyltransferase, replacing the original galactosyltransferase (15). Furthermore, they found that this change was present in strains isolated up to 27 years ago and postulated that the 6C capsule type originated more than 27 years ago from a single recombination event in a 6A locus, in which 6A *wciN* was replaced by a gene of unknown origin. Serotype 6C has subsequently been recognized in several countries and is an important replacement serotype, following the introduction of a conjugate vaccine (3–10, 12–14). Recent U.S. surveillance showed a significant 164% increase in the prevalence of invasive disease due to serotype 6C, increasing from 0.22 cases per 100,000 in 1999 to 0.57 and 0.58 cases per 100,000 in 2006 and 2007, respectively, while rates of invasive disease due to serotypes 6A and 6B markedly decreased (3).

The investigators who described serotype 6C also postulated that the same change that transformed serotype 6A into serotype 6C would transform serotype 6B into a new serotype (2). Although they were initially unable to detect this change among 264 serotype 6B strains examined, a strain with this change was produced experimentally. Naturally occurring serogroup 6 strains with this change have recently been detected in 14 of 34 nasopharyngeal isolates collected from Fijian children between 2004 and 2007, as well as in 2 of 14 nasopharyngeal isolates collected from Korean children in 2008; these strains have been designated the new putative serotype 6D (1, 9). Initial findings with monoclonal antisera indicate that serotype 6D can be differentiated from the other serogroup 6 serotypes serologically (1, 2). Reactions of serotype 6D with commercially available polyvalent antisera have not yet been characterized.

The absence of commercially available serotyping reagents has limited the detection of serotypes 6C and 6D. Recently, however, a serotype 6C-specific antiserum, factor 6d, was developed by Statens Serum Institut, Copenhagen, Denmark. This report describes the validation of the new factor serum 6d in two laboratories. Isolates of serogroup 6 from various strain collections were recovered from frozen storage and tested by PCR, as described by Park et al. (15), and the standard serotyping method using the capsular swelling reaction (11).

Serotyping by the capsular swelling method was performed using the standard antisera for serogroup 6, consisting of group 6, factor 6b and factor 6c antisera, as well as the newly introduced factor 6d antiserum (Statens Serum Institut). All serogroup 6 isolates are identified by the group 6 antiserum, with serotype 6A being additionally positive with factor 6b, serotype 6B with factor 6c, and serotype 6C with factors 6b and 6d. Serotyping by PCR of DNA extracts was performed using two forward primers, 5101, which attaches to nucleotides 6949 to 6966 in the gene *wciN*, and 5106, which attaches to nucleotides 5897 to 5916 in gene *wchA*, and a common reverse primer, 3101, which attaches to nucleotides 7888 to 7905 in gene *wciO* (15). Primer pair 5101-3101 produces products of 958 or 1,267 bp with serotypes 6A and 6B, while no product is produced with serotypes 6C or 6D. Primer pair 5106-3101 produces *wciN* PCR products of 2.0 or 2.3 kb with serotypes 6A and 6B and 1.8 kb with serotypes 6C and 6D (2, 9). PCR was performed as a simplex reaction with the two primer pairs or as a multiplex reaction with all three primers.

One-hundred eighty-nine serogroup 6 isolates, originating from several surveillance collections in the United States and Israel, were tested (Table 1). Serotypes identified by capsular swelling reactions with serogroup 6 factor antisera were 49 serotype 6A, 42 serotype 6B, and 98 serotype 6C. PCR ampli-

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[▽] Published ahead of print on 17 February 2010.

TABLE 1. Results of PCR amplification and capsular swelling reactions with serogroup 6 factor antisera for 189 isolates of *S. pneumoniae*

PCR primer set (product size [bp])	No. of isolates with capsular swelling reaction with factor antisera[a]		
	6b only	6c only	6b and 6d
5101-3101 (958)	48	6	—
5101-3101 (1,267)	1	36	—
5106-3101 (1,800)	—	—	98

[a] All isolates reacted with serogroup 6 antiserum, while additional reaction to factor antisera indicate the following: 6b, serotype 6A; 6c, serotype 6B; 6b and 6d, serotype 6C. —, no isolates had these characteristics.

fication product sizes differentiated all serotype 6A and 6B strains from serotype 6C strains with the 5101-3101 primer pair set, with band sizes of 958 bp for 48 serotype 6A and 6 serotype 6B strains and 1,267 bp for one serotype 6A and 36 serotype 6B strains; no product was obtained with serotype 6C strains. Products of 1,800 bp in size were produced with the 5106-3101 primer pair set for all 98 serotype 6C strains, with no product for any serotype 6A or 6B strains. None of the strains with 1,800-bp products produced with the 5106-3101 primer pair set reacted with factor 6c antiserum, indicating that they were not serotype 6D.

Although serotype 6C was described only recently, it has now been identified in many countries and shown to be present in some pneumococcal collections for nearly 3 decades (15). Serotype 6C has also been shown to be an important, and often antimicrobial-resistant, replacement serotype in countries where the conjugate pneumococcal vaccine containing serotype 6B is used (3, 8). The increase in serotype 6C prevalence was recently postulated to be associated with the induction of small amounts of functional anti-6C antibody, compared with anti-6A and anti-6B antibodies, by the conjugate pneumococcal vaccine (12). The availability of commercially produced factor antiserum for identification of serotype 6C allows accurate identification of this serotype and allows scientists to avoid the need to differentiate this serotype from serotype 6A by PCR.

We thank Moon H. Nahm for assistance with PCR interpretations and for information on serotype 6D.

REFERENCES

1. **Bratcher, P. E., K.-H. Kim, J. H. Kang, J. Y. Hong, and M. H. Nahm.** 2010. Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical and serological characterization. Microbiology **156**(Pt. 2)**:**555–560.
2. **Bratcher, P. E., I. H. Park, S. K. Hollingshead, and M. H. Nahm.** 2009. Production of a unique pneumococcal capsule serotype belonging to serogroup 6. Microbiology **155:**576–583.
3. **Carvalho, M. D. G., F. C. Pimenta, R. E. Gertz, Jr., H. H. Joshi, A. A. Trujillo, L. E. Keys, J. Findley, I. S. Moura, I. H. Park, S. K. Hollingshead, T. Pilishvili, C. G. Whitney, M. H. Nahm, and B. W. Beall.** 2009. PCR-based quantitation and clonal diversity of the current prevalent invasive serogroup 6 pneumococcal serotype, 6C, in the United States in 1999 and 2006 to 2007. J. Clin. Microbiol. **47:**554–559.
4. **du Plessis, M., A. von Gottberg, S. A. Madhi, O. Hattingh, L. de Gouveia, and K. P. Klugman.** 2008. Serotype 6C is associated with penicillin-susceptible meningeal infections in human immunodeficiency virus (HIV)-infected adults among invasive pneumococcal isolates previously identified as serotype 6A in South Africa. Int. J. Antimicrob. Agents **32**(Suppl. 1)**:**S66–S70.
5. **Hare, K. M., H. Smith-Vaughan, M. Binks, I. H. Park, M. H. Nahm, and A. J. Leach.** 2009. "Dodgy 6As": differentiating pneumococcal serotype 6C from 6A by use of the Quellung reaction. J. Clin. Microbiol. **47:**1981–1982.
6. **Hermans, P. W., M. Blommaart, I. H. Park, M. H. Nahm, and D. Bogaert.** 2008. Low prevalence of recently discovered pneumococcal serotype 6C isolates among healthy Dutch children in the pre-vaccination era. Vaccine **26:**449–450.
7. **Jacobs, M. R., S. Bajaksouzian, R. A. Bonomo, C. E. Good, A. R. Windau, A. M. Hujer, C. Massire, R. Melton, L. B. Blyn, D. J. Ecker, and R. Sampath.** 2009. Occurrence, distribution, and origins of *Streptococcus pneumoniae* serotype 6C, a recently recognized serotype. J. Clin. Microbiol. **47:**64–72.
8. **Jacobs, M. R., C. E. Good, S. Bajaksouzian, and A. R. Windau.** 2008. Emergence of Streptococcus pneumoniae serotypes 19A, 6C, and 22F and serogroup 15 in Cleveland, Ohio, in relation to introduction of the protein-conjugated pneumococcal vaccine. Clin. Infect. Dis. **47:**1388–1395.
9. **Jin, P., F. Kong, M. Xiao, S. Oftadeh, F. Zhou, C. Liu, F. Russell, and G. L. Gilbert.** 2009. First report of putative Streptococcus pneumoniae serotype 6D among nasopharyngeal isolates from Fijian children. J. Infect. Dis. **200:** 1375–1380.
10. **Jin, P., M. Xiao, F. Kong, S. Oftadeh, F. Zhou, C. Liu, and G. L. Gilbert.** 2009. Simple, accurate, serotype-specific PCR assay to differentiate *Streptococcus pneumoniae* serotypes 6A, 6B, and 6C. J. Clin. Microbiol. **47:**2470–2474.
11. **Lund, E., and J. Henrichsen.** 1978. Laboratory diagnosis, serology and epidemiology of Streptococcus pneumoniae, p. 244–255. Methods in microbiology, vol. 12. Academic Press, London, United Kingdom.
12. **Nahm, M. H., J. Lin, J. A. Finkelstein, and S. I. Pelton.** 2009. Increase in the prevalence of the newly discovered pneumococcal serotype 6C in the nasopharynx after introduction of pneumococcal conjugate vaccine. J. Infect. Dis. **199:**320–325.
13. **Nahm, M. H., I. H. Park, T. Camou, and M. Hortal.** 2008. Serotype 6C has been present in Uruguay, abstr. P1-063. Sixth International Symposium on Pneumococci and Pneumococcal Diseases, Reykjavik, Iceland.
14. **Nunes, S., C. Valente, R. Sa-Leao, and H. de Lencastre.** 2009. Temporal trends and molecular epidemiology of recently described serotype 6C of *Streptococcus pneumoniae*. J. Clin. Microbiol. **47:**472–474.
15. **Park, I. H., S. Park, S. K. Hollingshead, and M. H. Nahm.** 2007. Genetic basis for the new pneumococcal serotype, 6C. Infect. Immun. **75:**4482–4489.
16. **Park, I. H., D. G. Pritchard, R. Cartee, A. Brandao, M. C. Brandileone, and M. H. Nahm.** 2007. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. J. Clin. Microbiol. **45:**1225–1233.