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Identification of Newly Described *Streptococcus pneumoniae* Serotype 6D by Use of the Quellung Reaction and PCR▽

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We tested 121 pneumococcal serogroup 6 isolates (including 30 serotype 6C and 24 serotype 6D isolates) by serotype-specific PCR and the Quellung reaction, using "old" and "new" pool B, factor 6b, and new factor 6d antisera. In combination with group B and other factor antisera, factor 6d antiserum can reliably identify the newly described serotype 6D.

Serotyping of *Streptococcus pneumoniae* isolates is an important component of surveillance for pneumococcal disease and assessment of the impact of pneumococcal vaccines. The Quellung reaction is the accepted gold standard method and is used at the New South Wales (NSW) Pneumococcal Reference Laboratory for routine serotyping, supplemented by molecular methods (7, 8, 11). Recently, we developed serogroup 6 serotype-specific PCRs to identify the newly recognized serotype 6C (6, 10), which also allowed us to identify the first naturally occurring serotype 6D isolates (5).

In this study, we compared these PCRs with Quellung reactions, using a new factor, antiserum 6d, which reacts with serotype 6C (Statens Serum Institute, Copenhagen, Denmark). The use of this factor antiserum for identification of serotype 6C has recently been validated (4, 9). We also compared "old" (purchased before 2009) and "new" factor 6b and pool B antisera (purchased in 2009). We tested 64 *S. pneumoniae* isolates from nasopharyngeal swabs from Fijian children that had been serotyped in 2008 by the Quellung reaction, using old antisera (not including factor 6d), and by PCR (5). They included (i) 24 isolates initially identified as serotype 6B by Quellung reaction but subsequently identified as 6D by PCR (5), (ii) 30 serotype 6C isolates initially identified as 6A by the Quellung reaction but subsequently identified as 6C by PCR (6), and (iii) five isolates identified by the Quellung reaction as serotypes 6A and 6B and confirmed by PCR. In addition, 57 serogroup 6 isolates, referred to the NSW Pneumococcal Reference Laboratory in 2009, were retyped by both the Quellung reaction (using new reagents) and serogroup 6 PCRs (5, 6). To avoid any bias in interpretation, all the isolates were tested in a blind manner.

Previous PCR results for Fijian isolates belonging to serotypes 6A, 6B, 6C, and 6D were confirmed by Quellung reactions. Results are shown in Table 1. Serotype 6C isolates reacted with the new (absorbed), but not the old, pool B antiserum. Fourteen serotype 6D isolates did not react with the old pool B antiserum, and 10 showed weak or delayed (and easily missed) reactions; all 24 reacted with the new pool B antiserum, although reactions were very weak for three isolates.

Of the 57 NSW isolates, 32 had initially been identified as unusual 6A isolates on the basis of reactions with old group 6 and factor 6b antisera but not with old pool B antiserum. Subsequently, we identified these as serotype 6C by PCR. Consistent with this, the isolates reacted with the old 6b and 6d but not with the new (absorbed) 6b or 6c antiserum (Table 1). Reactions of the remaining NSW isolates (6A and 6B) were the same as with initial Quellung reactions and PCR. No serotype 6D isolates were identified among the NSW isolates tested.

Until recently, serogroup 6 comprised only serotypes 6A and 6B, which were identified by Quellung reactions on the basis of reactions with pool B and group 6 antisera and either factor 6b antiserum (for serotype 6A) or factor 6c antiserum (for 6B). We and others (3) had recognized that a proportion of apparently nontypeable isolates which react poorly or not at all with pool antisera reacted strongly with group 6 and factor 6b antisera. They were therefore generally classified as serotype 6A despite a negative reaction with pool B antiserum. Subsequently, we showed by PCR that these isolates belonged to serotype 6C (6), and this has now been confirmed using the new factor 6d antiserum. Hare et al., who recently reported similar atypical reactions with some serotype 6A isolates, referred to them as "dodgy 6As" and also showed that they were serotype 6C (3). Melnick et al. produced 6d antiserum specific for 6C and an absorbed 6b antiserum specific for 6A, which give the same results as ours for serotype 6C (9).

During development of our serogroup 6 serotype-specific PCRs, we found that some isolates, previously identified as 6B by the Quellung reaction (despite similar "dodgy" reactions with pool antiserum B), were positive in the serotype 6C-specific PCR (targeting *wciN* of serotype 6C). We showed that they belonged to serotype 6D, which had been hypothesized

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TABLE 1. Quellung reaction results for 121 *Streptococcus pneumoniae* serogroup 6 isolates using old and new antisera from the Statens Serum Institut

Serotype[a]	No. of isolates	Quellung reaction(s)[d] (no. of isolates) with Statens Serum Institut *S. pneumoniae* antisera of:					
		Pool B (old)[b]	Pool B (new)[c]	Factor 6b[b]	Factor 6b (new, absorbed)[c]	Factor 6c	Factor 6d
6A	16	+	+	+	+	−	−
6B	19	+	+	−	−	+	−
6C	62	−	+	+	−	−	+
6D	24	− (14), +/− (10)	+ (21), +/− (3)	−	−	+	+

[a] Confirmed by PCR.
[b] Old Statens Serum Institut antiserum (purchased before 2009).
[c] New Statens Serum Institut antiserum (purchased in 2009).
[d] +/−, weak and/or delayed reaction. All serotype 6D isolates reacted strongly with factor 6c and 6d antisera.

and produced experimentally (2) but has not previously been found among *S. pneumoniae* collections. More recently, two serotype 6D isolates were identified among a collection of serogroup 6 isolates from Korea (1).

This study has confirmed that serotypes 6C and 6D can reliably be identified by Quellung reactions on the basis of reactions with new pool antiserum B (albeit sometimes with weak or delayed reactions for 6D) and strong reactions with group 6 and factor 6d antisera (for 6C) or new factor 6c and 6d antisera (for 6D). Thus, cross-reaction between serotypes 6B and 6D remain with the new factor 6c.

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