

A report of *Streptococcus pneumoniae* serotype 6D in Europe

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Serotype 6D of *Streptococcus pneumoniae* has been reported in Asia and the Fijian islands among nasopharyngeal carriage isolates. We now report a 6D isolate from a Finnish adult with invasive pneumococcal disease. Interestingly, the Finnish isolate and Asian isolate capsule gene loci are almost identical.

INTRODUCTION

Recently, serotype 6D was predicted to be a new member of serogroup 6 of *Streptococcus pneumoniae* (pneumococcus) (Bratcher *et al.*, 2009), but, until recently, extensive searches by one of us (M.H.N) and investigators at the Centers for Disease Control and Prevention in the USA failed to show its presence in North America, South America, Europe and Africa (Bratcher *et al.*, 2009; Da Gloria Carvalho *et al.*, 2009; Hermans *et al.*, 2008). Finally, serotype 6D was found in Asia (Bratcher *et al.*, 2010) and the Fiji islands (Jin *et al.*, 2009). However, since its presence has not been detected in large parts of the world, the clinical significance of serotype 6D has been unclear. We now report an invasive European 6D isolate with a capsule gene locus (*cps*) that is highly related to an Asian isolate.

METHODS

Quellung reaction. Pneumococci were serotyped using various factor sera from Statens Serum Institute (SSI) as previously described (Bratcher *et al.*, 2009).

Flow cytometry. To investigate the epitopes being expressed, pneumococci in suspension were stained first with one of several hybridoma culture supernatants and then with fluorescein-conjugated goat anti-mouse immunoglobulin antibody as previously described (Bratcher *et al.*, 2010).

PCR and DNA sequencing. Bacterial genomic DNA was extracted and the *cps* region was PCR amplified with primers as described previously (Bratcher *et al.*, 2010). The resulting amplicon was sequenced

Abbreviations: SSI, Statens Serum Institute; ST, sequence type.

The GenBank/EMBL/DDBJ accession number for the sequence of the 15.9 kb *cps* region of the MNZ920 isolate reported in this paper is HM448897.

at the Genomics Core at the University of Alabama at Birmingham (Birmingham, AL, USA) as previously described (Bratcher *et al.*, 2010).

RESULTS

A pneumococcal isolate (MNZ920) was obtained in 2009 from a blood culture of an elderly female in Finland. The isolate was identified as pneumococcus (*S. pneumoniae*), based on colony morphology, typical Gram stain appearance, bile solubility and optochin sensitivity. It was initially typed as 6B by counter-immunoelectrophoresis and the quellung reaction, although serotype 6B is known to be reactive with factor serum 6c, but not 6d (Table 1). Later, along with a validation process for setting up new serotyping methods, the isolate was found to be reactive with new factor serum 6d and to harbour *wciN_β* (Table 1), a gene associated with serotypes 6C and 6D, by PCR and sequencing (Bratcher *et al.*, 2009). The isolate was then sent to the SSI in Denmark and the University of Alabama at Birmingham in the USA for additional studies.

In Denmark, serological investigation was performed with the factor sera available at that time. The Danish study confirmed that the isolate was positive with both factor sera 6c and 6d but negative with factor serum 6b (Table 1). This distinct serological pattern has recently been shown to be indicative of serotype 6D, whereas serotype 6C is negative to factor serum 6c but positive to factor serum 6d (Table 1) (Bratcher & Nahm, 2010). In the USA, the isolate was examined with mAb and flow cytometry, and was found to be reactive with Hyp6BM8 but not with Hyp6AG1, Hyp6AM3 and Hyp6BM7. This reaction pattern was previously found to be the serological pattern characteristic of serotype 6D (Bratcher *et al.*, 2009, 2010). Additional DNA sequencing studies showed that the isolate

Table 1. Comparison of the serological properties of pneumococcal isolate MNZ920 and the established serological properties for the four serotypes in serogroup 6

	Finland		SSI Denmark	Reaction patterns with known serotypes*			
	First typing†	Second typing‡		6A	6B	6C	6D
Factor sera							
6b	–	–	–	+	–	–	–
6c	+	+	+	–	+	–	+
6d	+	+	+	–	–	+	+
wciN _β	ND	+	ND	–	–	+	+

ND, Not done.

*Bratcher & Nahm (2010).

†The lot numbers of the SSI factor sera used for the first typing were: 6b (H6b11A1); 6c (H6c11C1); and 6d (L6d11C1).

‡The lot numbers of the SSI factor sera used for the second typing were: 6b (L6b22A1); 6c (H6c12A1); and 6d (L6d11C1).

had 6B/6D *wciP* (but not 6A/6C *wciP*) (GenBank accession no. HM448897) and a novel sequence type (ST), ST5163.

To investigate the evolutionary origin of the Finnish 6D *cps*, the DNA sequence of the 15.9 kb in the *cps* region of MNZ920 was determined as described previously (Park *et al.*, 2007) and deposited in GenBank (GenBank accession no. HM448897). The 15.9 kb region includes all 14 genes from *cpsA* to *rmlD* that are necessary for producing serotype 6D polysaccharide. Comparison of the sequence with the Korean 6D *cps* (GenBank accession no. HM171374) showed the entire 15.9 kb sequence to be highly homologous (99.2% identity) and the great majority of the differences are found in *rmlA*, *rmlB*, *rmlC* and *rmlD* genes. When the 5 kb central regions with low G+C content (from the *wciN_β* to the *wzx* region) were compared, the homology between the Korean and Finnish isolates was even greater (99.9% identity). It is interesting to note that the 6D *cps* regions of these two isolates from very distant parts of the world are almost identical, even though the ST of the Korean 6D is quite different from the ST of the Finnish 6D (ST282 vs ST5163). Further studies with additional serotype 6D isolates are needed to investigate how serotype 6D originated in different parts of the world.

DISCUSSION

Until the Finnish isolate was found, all of the 6D isolates described had been obtained from the nasopharynges of healthy children (Bratcher *et al.*, 2010; Jin *et al.*, 2009). However, the Finnish isolate was obtained from an elderly adult suffering from invasive pneumococcal disease. Thus, disease profiles of serotype 6D infections may be as heterogeneous as those associated with other pneumococci expressing different serotypes, although further studies are required to confirm this.

As this case illustrates, serotype 6D may be confused with either 6B or 6C. DNA testing may be difficult because the

genetic distinction between 6C and 6D may be just 1 bp in the *wciP* gene (Mavroidi *et al.*, 2004). However, currently available factor sera can be used to easily distinguish 6D from the other three members of serogroup 6, as shown in Table 1. Since serotype 6D *cps* appears to have spread to two distant locations (Korea and Finland) after its first appearance, serotype 6D *cps* might also have spread to many other locations in the world. We therefore believe that serotype 6D will eventually be identified worldwide. Awareness of the serological pattern of 6D and vigilance during serotype screening should facilitate the recognition of additional 6D isolates. In fact, since our manuscript was submitted for review, a group have reported finding 6D isolates in Poland, which were identified among invasive pneumococcal disease isolates with PCR tests alone (Kuch *et al.*, 2010).

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