

# Invasive *Streptococcus pneumoniae* Serotypes Associated with In-patient and Out-patient Isolates from the United States



#77.004

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## Revised Abstract

**Background:** *Streptococcus pneumoniae* (SPN) is a major cause of invasive diseases and upper respiratory tract infections. Introduction of the pneumococcal conjugate 7-valent vaccine (PV7) into the US childhood vaccine schedule in 2000 has significantly reduced invasive pneumococcal disease in children and adults, with concurrent reduction in the seven vaccine serotypes. Consistent monitoring of possible replacement serotypes is essential to determine possible antibiotic resistance patterns as certain serotypes are more closely associated with antibiotic resistance, as well as to determine targets for future vaccines. In this study we evaluate the serotypes of invasive SPN isolates from in-patients and out-patients from 2004. **Methods:** The capsular serotypes of 272 invasive SPN isolates collected in the US through the Tigecycline Evaluation Surveillance Trial (TEST) were determined using sequential multiplex PCR and confirmed using the Quellung reaction. Invasive isolates were defined as those from normally sterile sites, such as blood, CSF and other body fluid. **Results:**

	Total N	PV7 types (%)	Non-PV7 types
			Serotypes (% total non-PV7)
In-patient	162	37 (19)	19A(30), 7(6),33(6),3(6),22F(6),6A(5),12(5),others(26),nt <sup>*</sup> (10)
Out-patient	110	26 (22)	19A(17),3(10),22F(10),11(8),15(8),35(8), 7(7),12(6),others(4),nt <sup>*</sup> (2)

\*nt = non-typeable

**Conclusions:** While PV7 serotypes have declined since 2000, in 2004 approximately 20% of the invasive isolates from this study were from the seven vaccine types (4, 6B, 9V, 14, 18C, 19F, 23F). In-patients and out-patients were equally likely to carry PV7 serotypes. Serotype 19A was the most common serotype in both in- and out-patients, which is cause for concern as this serotype is often non-susceptible to penicillin and erythromycin. In-patients were more likely to carry non-typeable isolates, while equal percentages of isolates from both groups were non-encapsulated. Continued monitoring of post-vaccine serotype trends will be vital to the management of pneumococcal disease.

## Introduction

*Streptococcus pneumoniae* is an important cause of invasive disease and upper respiratory infections in children and the elderly. Contributing to the pathogenicity of this organism is a capsular polysaccharide (cps), the typing of which can differentiate isolates into more than 90 distinct serotypes, with about 15 of these serotypes causing the majority of invasive disease worldwide [1, 2]. The introduction of multi-valent pneumococcal conjugate vaccines has led to a decline in the serotypes covered by the vaccines, however the long-term impact on the prevalence of these serotypes and the emergence of non-vaccine serotypes is unknown[3-6]. The 7-valent conjugate vaccine (PV7) was introduced in the United States in 2000. This vaccine includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F [4]. Replacement of vaccine-serotypes by non-vaccine serotypes has already been reported [5, 6]. Post-vaccine surveillance studies of serotype prevalence patterns worldwide are necessary to monitor the changing seroepidemiology of this organism in order to evaluate the appropriate formulation for current and future vaccines. In addition monitoring of possible replacement serotypes is essential to determine possible antibiotic resistance patterns as certain serotypes are more closely associated with antibiotic resistance [9]. The established method for SPN serotyping is the Quellung reaction [7], which is costly and requires technical expertise. For this study, we utilized a combination of multiplex PCR, confirmed by the Quellung reaction, to determine the capsular serotypes of invasive SPN from in-patients and out-patients in the United States.

## References

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## Materials & Methods

272 SPN isolates from select sterile body sites from the TEST study were included in this analysis. All isolates were collected in 2004 in the United States (US). Sterile body sites include body fluids, cardiovascular system, including blood, central nervous system, lymph, ovary, lung, respiratory sinus and bone. Based on the seven- reaction sequential multiplex PCR described previously [8], we devised a PCR using only four reactions to identify the pneumococcal serotype, followed by confirmation via the conventional Quellung reaction [7]. This PCR-based approach is based on serotype-specific genes within the capsular polysaccharide synthesis genes (the cps locus) of SPN. While this scheme could not detect all serotypes without doing a traditional serotyping checkerboard, it served to streamline the project and reduce cost by reducing the number of Quellung reactions needed. Using primers targeted at different loci within the cps gene, multiplex PCR reactions were set up in a sequential fashion, including an internal cps control. When the PCR assigned isolates to a serotype subset, conventional serotyping was performed based on the subset type. All PCR serotype results were confirmed by Quellung reaction. Isolates that were negative by PCR were serotyped by Quellung reactions alone. All PCR reactions were run with positive and negative controls, including the internal cps locus. Pneumococcal antisera were purchased from Statens Serum Institute, Copenhagen, Denmark.

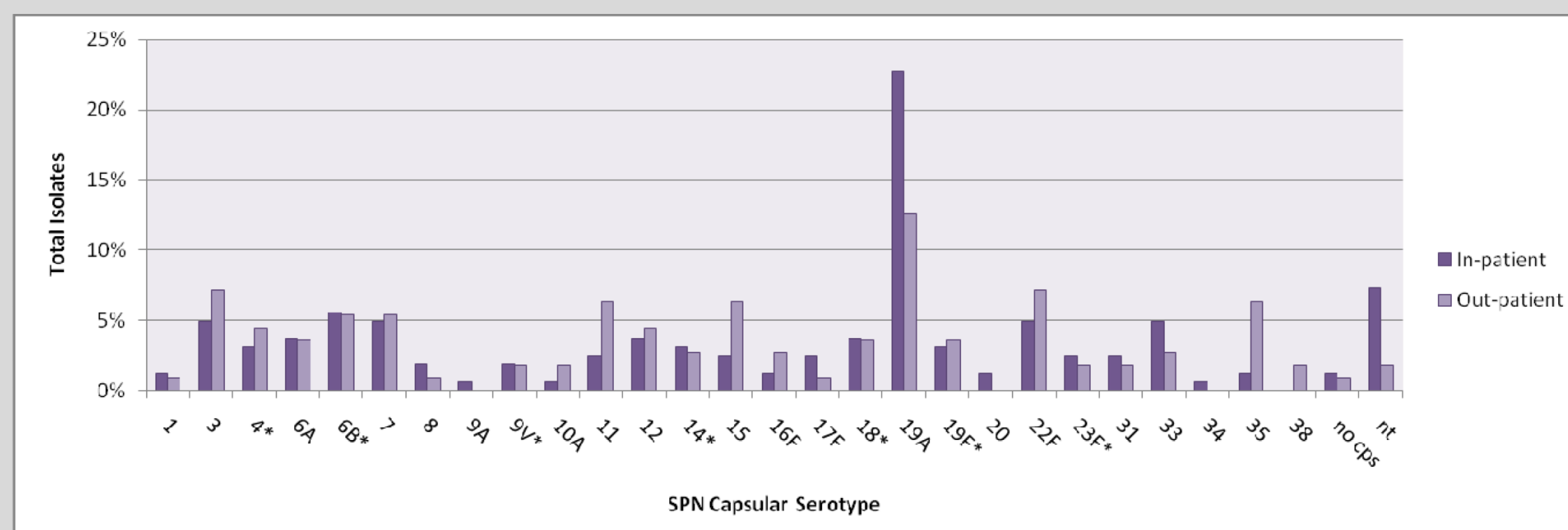
## Acknowledgements

This study was sponsored by Wyeth which was acquired by Pfizer Inc in October 2009.

## Results

Results are shown in the following figures and tables.

**Figure 1. Percentage of US SPN isolates in each capsular serotype.**



\* serotype included in PV7 vaccine

**Table 1. Distribution of SPN PV7 capsular serotypes.**

	Serotype						
	4	6B	9V	14	18	19F	23F
In-patient (n=37)	5	9	3	5	6	5	4
Out-patient (n=26)	5	6	2	3	4	4	2

**Table 2. Distribution of SPN non-vaccine capsular serotypes.**

	Serotype																			no CPS	nt <sup>*</sup>	
	1	3	6A	7	8	9A	10A	11	12	15	16F	17F	19A	20	22F	31	33	34	35			38
In-patient (n=125)	2	8	6	8	3	1	1	4	6	4	2	4	37	2	8	4	8	1	2	0	2	12
Out-patient (n=84)	1	8	4	6	1	0	2	7	5	7	3	1	14	0	8	2	3	0	7	2	1	2

\*nt = non-typeable

## Conclusions

- ❖ In this study approximately 20% of the invasive SPN isolate serotypes were from the seven vaccine types (4, 6B, 9V, 14, 18C, 19F, 23F). In-patients and out-patients were equally likely to carry PV7 serotypes.
- ❖ Serotype 19A, a common cause of respiratory infections, was the most common serotype in both in- and out-patients, which is cause for concern as this serotype is often non-susceptible to penicillin and erythromycin.
- ❖ Replacement disease caused by non-vaccine serotypes remains an important clinical issue. Continued monitoring of invasive pneumococcal disease is essential for future vaccine development.