ORIGINAL ARTICLE 10.1111/j.1469-0691.2009.02938.x

Changes in serotypes causing invasive pneumococcal disease (2005–2007 vs. 1997–1999) in children under 2 years of age in a population with intermediate coverage of the 7-valent pneumococcal conjugated vaccine

L. Salleras[1,2,3], A. Domínguez[1,2], P. Ciruela[4], C. Izquierdo[4], E. Navas[4], N. Torner[2,4] and E. Borras[2,4]

1) *Department of Public Health, School of Medicine, University of Barcelona,* 2) *CIBER Epidemiologia y Salud Pública (CIBERESP),* 3) *Preventive Medicine Unit, Hospital Clinic Barcelona and* 4) *Department of Health, Autonomous Government of Catalonia (Spain), Barcelona, Spain*

Abstract

Serotypes causing invasive pneumococcal disease (IPD) in children aged <2 years in Catalonia (Spain) before and after licensing of the 7-valent pneumococcal conjugated vaccine (7vPCV) were assessed, using samples taken during 1997–1999 and 2005–2007 respectively. The distribution of serotypes causing IPD within these groups was obtained by serotyping strains sent by 22 Catalan hospitals to the Carlos III Health Institute, Madrid. Between 1997–99 and 2005–2007, the proportion of vaccine serotypes causing IPD in Catalonia fell from 70.54% to 31.67% ($p < 0.0001$). The proportion of vaccine-related serotypes, mainly serotype 19A, increased from 9.82% to 32.50% ($p < 0.0001$). The proportion of non-vaccine, non-related serotypes (serotypes not related to vaccine serotypes) rose from 19.64% to 35.83% ($p < 0.05$). Within this group, the proportions of serotype 24F increased significantly. There has been a change in the distribution of serotypes isolated from cases of IPD in children <2 years old in Catalonia, comprising a reduction in the proportion of 7-valent vaccine serotypes, a rise in vaccine-related serotypes, especially 19A, and a smaller rise in non-vaccine, non-related serotypes, especially serotype 24F. A new 13-valent vaccine will cover 77.91% of the serotypes causing IPD in children <2 years old in Catalonia from 2005 to 2007.

Keywords: Invasive pneumococcal disease, pneumococcus, serotype, 7-valent pneumococcal conjugated vaccine
Original Submission: 10 December 2008; **Revised Submission:** 30 March 2009; **Accepted:** 21 May 2009
Editor: J.-L. Mainardi
Article published online: 18 August 2009
Clin Microbiol Infect 2009; **15:** 997–1001

Corresponding author and reprint requests: L. Salleras, Cr Casanova 143, 08036 Barcelona, Spain
E-mail: salleras@ub.edu

Introduction

The 7-valent pneumococcal conjugated vaccine (7vPCV) was licensed in Spain in 2001 [1] and, although not yet incorporated into the routine vaccination schedule, except in the Community of Madrid, its use has been recommended by the Spanish Association of Paediatrics [2] and by many private paediatric offices. In Catalonia in 2003, a telephone survey investigating vaccination coverage in children aged 2 years, born in October 2001, revealed a 7vPCV vaccination coverage of approximately 35% [3].

The 7vPCVserotypes (4, 6B, 9V, 14, 18C, 19F and 23F) caused 82.2% of the cases of invasive pneumococcal disease (IPD) in children in the USA in the 1990s [4]. In Spain, before introduction of the 7vPCV, the proportion of vaccine serotypes causing IPD in children aged <2 years was 68.2% [5].

The objective of this study was to analyse the evolution of the distribution of serotypes causing IPD in children aged <2 years in Catalonia before and after licensing of the 7vPCV, between 1997–1999 and 2005–2007, respectively.

Materials and Methods

All serotypes isolated from children aged <2 years in the periods 1997–1999 and 2005–2007 at 22 hospitals of Catalonia and sent for serotyping to the Pneumococcal Reference Laboratory, Carlos III Health Institute, Madrid, Spain, were included in the study.

Serotyping was carried out using the Quellung reaction calibrated according to international standards. Serotypes were classified in accordance with the Danish nomenclature

[5], and divided into three groups: serotypes included in the vaccine (vaccine serotypes); serotypes belonging to the same serogroup as vaccine serotypes (vaccine-related serotypes); and serotypes not related to vaccine serotypes (non-vaccine, non-related serotypes).

The χ^2 test was used to determine differences between proportions, with a value of $p < 0.05$ considered as statistically significant [6]. The SPSS v12 program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

During 1997–1999, 112 pneumococcal strains were sent to the Carlos III Health Institute, of which the disease-causing serotype was identified in 111 (99.11%). The most frequent serotypes were 6B (16.96%), 14 (16.07%), 19F (15.18%), 18C (7.14%) and 4 (6.25%), all of which are vaccine serotypes.

During 2005–2007, 240 strains were sent and the serotype was identified in 238 (99.17%). The most frequent serotypes were vaccine-related serotype19A (22.08%), vaccine serotype 14 (14.58%), vaccine-related serotype 6A (7.08%), vaccine serotype 19F (6.25%) and non-vaccine, non-related serotypes 7F (5.83%) and 5 (5.0%) (Table 1).

Between 1997–1999 and 2005–2007, the proportion of vaccine serotypes in children aged <2 years fell from 70.54% to 31.67% ($p < 0.05$) and the proportion of vaccine-related serotypes increased from 9.82% to 32.50% ($p < 0.0001$). Non-vaccine, non-related serotypes increased from 19.64% to 35.83% ($p < 0.05$) (Table 1).

Serotypes whose proportion increased significantly were vaccine-related 19A and non-vaccine, non-related 24F (Table 1). The proportion of vaccine serotypes 4, 6B, 18C and 19F fell significantly (Table 1).

The serotypes included in the future 10-valent vaccine (the seven serotypes included in the 7vPCV plus serotypes 1, 5 and 7F) represented 81.25% and 46.67% of serotypes causing IPD in children aged <2 years in Catalonia in 1997–1999 and 2005–2007, respectively (Table 2). Serotypes included in the future 13-valent vaccine (the above serotypes plus serotypes 3, 6A and 19A) represented 90.18% and 77.92%, respectively (Table 2).

TABLE 1. Serotypes causing invasive pneumococcal disease in children aged <2 years in Catalonia (Spain) before and after licensing of the 7-valent pneumococcal conjugate vaccine (1997–1999 and 2005–2007, respectively)

	1997–1999		2005–2007		
	Absolute	%	Absolute	%	p
Vaccine serotypes					
4	7	6.25	3	1.25	<0.05
6B	19	16.96	9	3.75	0.0002
9V	5	4.46	4	1.67	NS
14	18	16.07	35	14.58	NS
18C	8	7.14	5	2.08	<0.05
19F	17	15.18	15	6.25	<0.05
23F	5	4.46	5	2.08	NS
Total	79	70.54	76	31.67	<0.05
Vaccine-related serotypes					
6A*	5	4.46	17	7.08	NS
9N	1	0.89	2	0.83	NS
19A	4	3.57	53	22.08	<0.0001
23B	1	0.89	6	2.50	NS
Total	11	9.82	78	32.50	<0.0001
Non-vaccine, non-related serotypes					
1	4	3.57	10	4.17	NS
3	1	0.89	5	2.08	NS
5	5	4.46	12	5.8	NS
7F	3	2.68	14	5.83	NS
8	0	0	1	0.42	NS
10A	1	0.89	6	2.50	NS
12F	0	0	7	2.92	NS
15A	2	1.79	1	0.42	NS
15BC	1	0.89	5	2.08	NS
16F	0	0	1	0.42	NS
22F	2	1.79	1	0.42	NS
24F	0	0	11	4.58	<0.05
28F, 28A	0	0	1	0.42	NS
32F,32 A	1	0.89	0	0	NS
33 F	0	0	5	2.08	NS
35F	1	0.89	0	0	NS
35B	0	0	2	0.83	NS
38	0	0	2	0.83	NS
Not serotyped	1	0.89	2	0.83	NS
Total	22	19.64	86	35.83	<0.05
All serotypes	112	100	240	100	

NS, not significant.
*May be serotype 6C, at least in part; the classic methods of serotyping (Quellung reaction) cannot distinguish between serotype A and serotype C.

Discussion

Since the licensing of the 7vPCV, there has been expectation that the ecological niche left by vaccine serotypes could be occupied by non-vaccine serotypes, leading to the emergence of serotypes not included in the vaccine [7].

Routine administration of the 7vPCV in the USA in children aged <2 years has provided substantial health benefits, both direct (reduced incidence of IPD caused by vaccine serotypes, and a reduced proportion of antibiotic-resistant serotypes) [8–11] and indirect (reduced prevalence of vaccine serotype carriers, and reduced incidence of IPD in unvaccinated children and elderly people, all due to the effect of herd immunity) [12–17]. The possible negative effects of vaccination (such as the emergence of non-vaccine serotypes in both carriers and cases) have also been widely studied in the USA [18–23] and the latest data indicate that, 5 years after the introduction of routine vaccination, the magnitude of emergence is very small compared with the benefits of vaccination [21,24,25]. The incidence of IPD cases caused by vaccine serotypes has fallen by 97%, whereas cases due to non-vaccine, non-related serotypes have increased by only

TABLE 2. Serotypes causing invasive pneumococcal disease in children aged <2 years included in the 7-valent, 10-valent and 13-valent vaccines. (1997–1999 and 2005–2007), Catalonia (Spain)

		% Coverage			
		1997–1999		2005–2007	
Vaccine	Serotypes	Δ	Accumulated	Δ	Accumulated
7-valent	4, 6B, 9V, 14, 18C, 19F, 23F	–	70.54%	–	31.67%
10-valent	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	+10.71%	81.25%	+15.0%	46.67%
13-valent	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	+8.93%	90.18%	+31.25%	77.92%

20% [24,25]. Therefore, in the USA, current expert consensus is that the direct and indirect benefits of routine paediatric administration of the 7vPCV have greatly exceeded the possible negative effects [26–28]. Vaccination coverage in the USA has been very high and the distribution of vaccination quite uniform, with only small differences according to social class [10,12,27]. Therefore, if vaccination coverage is high, the benefits of vaccination are only slightly eroded by the possible negative impact of the increase in non-vaccine serotypes [27,29,30].

However, the effects of individual 7vPCV administration in private paediatric offices, which results in a less uniform demographic distribution of vaccination, with better coverage in higher social classes, as applies in Catalonia, have been little studied.

Our study shows a reduction in the proportion of vaccine serotypes and an increase in the proportion of vaccine-related and non-vaccine, non-related serotypes in Catalonia in children aged <2 years with IPD after introduction of the 7vPCV. The increase in vaccine-related serotype 6A may be due, at least in part, to the increase in the newly discovered serotype 6C [31]. The classic Quellung reaction cannot distinguish between serotypes 6A and 6C [31]. Most, but not all, studies have found that the 7vPCV provides cross-protection against serotype 6A but not against 6C [32]. In the USA, introduction of the 7vPCV has led to a reduction in serotype 6A but not in serotype 6C [32].

Our results are similar to those observed in the whole of Spain by the Pneumococcal Reference Centre, Carlos III Health Institute, which serotypes all pneumococcal strains sent by Spanish hospitals [33]. A similar pattern has been noted in studies of different Spanish regions [34–38]. In other reports, however, no relevant changes in serotype distribution after the introduction of the 7vPCV [39,40] have been documented. A substantial increase in the incidence of paediatric empyemata has also been observed, a high proportion of which is caused by non-vaccine serogroup 1 [35,37,38].

In some of these studies incidence rates have been calculated, but in these studies data were drawn either from small autonomous communities [34,39] or from poorly defined populations within an autonomous community [35,37,38]. The lack of data on the national incidence of IPD according to serotype before and after licensing of the 7vPCV makes it difficult to analyse the replacement phenomenon in Spain.

The emergence and increase of some non-vaccine serotypes, especially 19A, has coincided with the introduction of the 7vPCV, leading to some reports suggesting that they are related phenomena. However, the increase in new non-vaccine serotypes can occur without introduction of the 7vPCV [27,29,30,41]. An example is South Korea, where there was a substantial increase in the frequency of serotype 19A isolated from children aged <5 years before licensing of the 7vPCV in 2004 (from 0% of isolates of serotypes of *Streptococcus pneumoniae* during 1991–1994 to 8–10% during 1995–2000) [41]. Likewise, a recent study in Israeli Bedouins found an increase of 68% in the proportion of cases of otitis media caused by serotype 19A in the absence of 7vPCV administration [42].

Two recent studies on routine 7vPCV vaccination in Norway [43] and in Native-American reservations in New Mexico [44] both found a dramatic reduction in the incidence of cases caused by vaccine serotypes, with no replacement, i.e. no corresponding increase in non-vaccine serotypes. These results indicate that vaccination is not necessarily accompanied by replacement of serotypes.

When vaccination already has a substantial impact on vaccine serotypes, the reasons for the replacement of vaccine serotypes by some non-vaccine serotypes are not completely clear [27,29,30].

Moore and colleagues [30,45] recently suggested that, although the emergence of serotype 19A in the USA occurred after the introduction of the 7vPCV, there is not sufficient evidence to implicate vaccination as the only causal factor, or even to consider it as the most important factor. They suggest that serotype 19A was far better positioned to occupy the ecological niche left by vaccine serotypes than other non-vaccine serotypes. Unlike most non-vaccine serotypes, 19A can cause the three forms of interaction between the pneumococcus and the human host: nasopharyngeal colonization, otitis media and IPD. Before licensing of the 7vPCV, 19A was the ninth most important

serotype causing IPD in the USA in children aged <5 years, only exceeded by the seven vaccine serotypes and by serotype 6A. At that time, it was the main serotype causing carrier state in the USA. In addition, *c.* 60% of cases of serotype 19A isolated in IPD and 25–75% of carriers identified were resistant to penicillin. These results suggest that serotype 19A is more aggressive than other non-vaccine serotypes. In addition, 7vPCV vaccination has been shown not to be effective against serotype 19A according to the studies carried out before introduction of the vaccine, which showed no cross-immunity between vaccine serotype 19F and serotype 19A [11,46,47].

Black has recently suggested that serotype replacement is not due to vaccination, or at least that vaccination is not the most important factor [48]. He suggests that the determining factor is the uncontrolled use of antibiotics, especially macrolides, without specific indications for the treatment of paediatric respiratory infections. In Native-American reservations in Arizona, where azithromycin is approved only for the treatment of sexually transmitted diseases and children with documented allergy to penicillin, replacement has not been observed after vaccination [44]. By contrast, among indigenous Alaskans, substantial replacement of serotypes was observed after vaccination [22], coinciding with widespread use of azithromycin to treat pneumonia and otitis media. Similarly, among Israeli Bedouins, the emergence of serotype 19A coincided with mass use of azithromycin to treat acute paediatric respiratory infections in the absence of 7vPCV vaccination [42]. Black also suggested that the emergence of vaccine serotypes in Barcelona observed by Muñoz-Almagro *et al.* [37] would have occurred with relatively low vaccination coverage (only 36% of children had received one dose in 2005 and 47% in 2007), and that this could have been due, at least in part, to mass, uncontrolled use of antibiotics, especially macrolides, to treat mild paediatric infections [48].

Like all epidemiological studies, our results may have been subject to bias. One factor could be changes in the criteria for sending strains of pneumococcus to the Carlos III Health Institute for serotyping. For example, some hospitals did not begin to send isolates in cases of empyemata until 2004, which could have led to underestimation of the most frequent serotypes in empyemata (serotype 1, for example) during 1997–1999. However, the increase in the number of strains sent (240 during 2005–2007 vs. 112 during 1997–1999), probably due to increased interest in the disease by paediatricians after licensing of the vaccine, does not seem to have caused any bias.

Currently, a new 13-valent vaccine that includes serotypes 6A and 19A is under preparation [49]. Our results show that this vaccine would cover 77.92% of current serotypes causing IPD in children aged <2 years in Catalonia and therefore would be a welcome addition.

Acknowledgements

The authors would like to thank the reporting physicians and technicians of the Microbiological Notification System of Catalonia (SNMC) for their collaboration, and express their gratitude for the expert comments of A. Fenoll (National Centre of Microbiology, Carlos III Health Institute, Madrid).

Transparency Declaration

This work was partially funded by CIBER Epidemiología y Salud Pública (CIBERESP), Spain of the Institute of Health Carlos III, Madrid, Spain. The authors declare no conflict of interests.

References

1. Salleras L, Dominguez A, Navarro JA. Vacuna antineumocócica conjugada. In: Salleras L, ed. *Vacunaciones preventivas*, 2nd edn. Barcelona: Masson, 2003: 399–420.
2. Bernaola E, Gimenez F, Baca M *et al.* [Vaccination schedule of the Spanish Association of Pediatrics: recommendations 2006]. *An Pediatr (Barc)* 2006; 64: 74–77.
3. Borras E, Dominguez A, Batalla J *et al.* Vaccination coverage in indigenous and immigrant children under 3 years of age in Catalonia (Spain). *Vaccine* 2007; 25: 3240–3243.
4. Butler JC, Breiman RF, Lipman HB *et al.* Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. *J Infect Dis* 1995; 171: 885–889.
5. Fenoll A, Jado I, Vicioso D, Perez A, Casal J. Evolution of *Streptococcus pneumoniae* serotypes and antibiotic resistance in Spain: update (1990 to 1996). *J Clin Microbiol* 1998; 36: 3447–3454.
6. Norusis MJ. *Advanced statistics*. Chicago: SPSS [computer program], 1998.
7. Salleras L. [The emergence of nonvaccine serotypes in invasive pneumococcal disease: a probably multifactorial origin]. *Vacunas* 2008; 9 (suppl 1): 3–11.
8. CDC. Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction—eight states, 1998–2005. *MMWR Morb Mortal Wkly Rep* 2008;57:144–148.
9. Kyaw MH, Lynfield R, Schaffner W *et al.* Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006; 354: 1455–1463.
10. Whitney CG. Impact of conjugate pneumococcal vaccines. *Pediatr Infect Dis J* 2005; 24: 729–730.
11. Whitney CG, Farley MM, Hadler J *et al.* Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med* 2003; 348: 1737–1746.

12. CDC. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease – United States, 1998–2003. *MMWR Morb Mortal Wkly Rep* 2005;54:893–897.
13. Hammitt LL, Bruden DL, Butler JC *et al.* Indirect effect of conjugate vaccine on adult carriage of *Streptococcus pneumoniae*: an explanation of trends in invasive pneumococcal disease. *J Infect Dis* 2006; 193: 1487–1494.
14. Lexau CA, Lynfield R, Danila R *et al.* Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *JAMA* 2005; 294: 2043–2051.
15. McBean AM, Park YT, Caldwell D, Yu X. Declining invasive pneumococcal disease in the U.S. elderly. *Vaccine* 2005; 23: 5641–5645.
16. Metlay JP, Fishman NO, Joffe M, Edelstein PH. Impact of pediatric vaccination with pneumococcal conjugate vaccine on the risk of bacteremic pneumococcal pneumonia in adults. *Vaccine* 2006; 24: 468–475.
17. Musher DM. Pneumococcal vaccine—direct and indirect ("herd") effects. *N Engl J Med* 2006; 354: 1522–1524.
18. Byington CL, Samore MH, Stoddard GJ *et al.* Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clin Infect Dis* 2005; 41: 21–29.
19. Klugman KP, McGee L. Resurgence of the multiresistant pneumococcus in the United States: a commentary. *Pediatr Infect Dis J* 2007; 26: 473–474.
20. Pai R, Moore MR, Pilishvili T, Gertz RE, Whitney CG, Beall B. Post-vaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis* 2005; 192: 1988–1995.
21. Pelton SI, Huot H, Finkelstein JA *et al.* Emergence of 19A as virulent and multidrug resistant Pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2007; 26: 468–472.
22. Singleton RJ, Hennessy TW, Bulkow LR *et al.* Invasive pneumococcal disease caused by nonvaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA* 2007; 297: 1784–1792.
23. Steenhoff AP, Shah SS, Ratner AJ, Patil SM, McGowan KL. Emergence of vaccine-related pneumococcal serotypes as a cause of bacteremia. *Clin Infect Dis* 2006; 42: 907–914.
24. Hanage WP. Serotype replacement in invasive pneumococcal disease: where do we go from here? *J Infect Dis* 2007; 196: 1282–1284.
25. Hicks LA, Harrison LH, Flannery B *et al.* Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J Infect Dis* 2007; 196: 1346–1354.
26. Bernatoniene J, Finn A. Advances in pneumococcal vaccines: advantages for infants and children. *Drugs* 2005; 65: 229–255.
27. Grijalva CG, Griffin MR. Population-based impact of routine infant immunization with pneumococcal conjugate vaccine in the USA. *Expert Rev Vaccines* 2008; 7: 83–95.
28. Lockhart SP, Hackell JG, Fritzell B. Pneumococcal conjugate vaccines: emerging clinical information and its implications. *Expert Rev Vaccines* 2006; 5: 553–564.
29. Beall B. Vaccination with the pneumococcal 7-valent conjugate: a successful experiment but the species is adapting. *Expert Rev Vaccines* 2007; 6: 297–300.
30. Moore MR, Gertz RE Jr, Woodbury RL *et al.* Population snapshot of emergent *Streptococcus pneumoniae* serotype 19A in the United States, 2005. *J Infect Dis* 2008; 197: 1016–1027.
31. Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MC, Nahm MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. *J Clin Microbiol* 2007; 45: 1225–1233.
32. Park IH, Moore MR, Treanor MJ *et al.* Differential effects of pneumococcal vaccines against serotypes 6A and 6C. *J Infect Dis* 2008; 198: 1818–1822.
33. Fenoll A, Tarrago D, Casal J. Cambios epidemiológicos en España desde la introducción de la vacuna neumocócica heptavalente. In: Campins M, Moraga FA, eds. *Vacunas 2007*. Madrid: GlaxoSmithKline, 2008: 137–149.
34. Barricarte A, Gil-Setas A, Torroba L *et al.* [Invasive pneumococcal disease in children younger than 5 years in Navarra, Spain (2000–2005). Impact of the conjugate vaccine]. *Med Clin (Barc)* 2007; 129: 41–45.
35. Calbo E, Diaz A, Canadell E *et al.* Invasive pneumococcal disease among children in a health district of Barcelona: early impact of pneumococcal conjugate vaccine. *Clin Microbiol Infect* 2006; 12: 867–872.
36. Cercenado F, Arenas C, Fenoll A, Bouza E. Evidence for the emergence of nonvaccine types causing invasive disease in Spain. *Clin Microbiol Infect* 2005; 11(suppl 2): 436. Abstract.
37. Muñoz-Almagro C, Jordan I, Gene A, Latorre C, Garcia-Garcia JJ, Pallares R. Emergence of invasive pneumococcal disease caused by nonvaccine serotypes in the era of 7-valent conjugate vaccine. *Clin Infect Dis* 2008; 46: 174–182.
38. Obando I, Arroyo LA, Sanchez-Tatay D *et al.* Molecular epidemiology of paediatric invasive pneumococcal disease in southern Spain after the introduction of heptavalent pneumococcal conjugate vaccine. *Clin Microbiol Infect* 2007; 13: 347–348.
39. Aristegui J, Bernaola E, Pocheville I *et al.* Reduction in pediatric invasive pneumococcal disease in the Basque Country and Navarre, Spain, after introduction of the heptavalent pneumococcal conjugate vaccine. *Eur J Clin Microbiol Infect Dis* 2007; 26: 303–310.
40. Benito-Fernandez J, Raso SM, Pocheville-Gurutzeta I, SanchezEtxaniz J, Azcunaga-Santibanez B, Capape-Zache S. Pneumococcal bacteremia among infants with fever without known source before and after introduction of pneumococcal conjugate vaccine in the Basque Country of Spain. *Pediatr Infect Dis J* 2007; 26: 667–671.
41. Hwa CE, Hee KS, Wook EB *et al. Streptococcus pneumoniae* serotype 19A in children, South Korea. *Emerg Infect Dis* 2008; 14: 275–281.
42. Dagan R, Givon-Lavi N, Leibovitz E, Greenberg D, Porat N. Introduction and proliferation of multidrug-resistant *Streptococcus pneumoniae* serotype 19A clones that cause acute otitis media in an unvaccinated population. *J Infect Dis* 2009; 199: 776–785.
43. Vestrheim DF, Lovoll O, Aaberge IS *et al.* Effectiveness of a 2 + 1 dose schedule pneumococcal conjugate vaccination programme on invasive pneumococcal disease among children in Norway. *Vaccine* 2008; 26: 3277–3281.
44. Lacapa R, Bliss SJ, Larzelere-Hinton F *et al.* Changing epidemiology of invasive pneumococcal disease among White Mountain Apache persons in the era of the pneumococcal conjugate vaccine. *Clin Infect Dis* 2008; 47: 476–484.
45. Moore MR. Rethinking replacement and resistance. *J Infect Dis* 2009; 199: 771–773.
46. Penn RL, Lewin EB, Douglas RG Jr, Schiffman G, Lee CJ, Robbins JB. Antibody responses in adult volunteers to pneumococcal polysaccharide types 19F and 19A administered singly and in combination. *Infect Immun* 1982; 36: 1261–1262.
47. Yu X, Gray B, Chang S, Ward JI, Edwards KM, Nahm MH. Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. *J Infect Dis* 1999; 180: 1569–1576.
48. Black S. Changing epidemiology of invasive pneumococcal disease: a complicated story. *Clin Infect Dis* 2008; 47: 485–486.
49. Scott DA, Komjathy SF, Hu BT *et al.* Phase I trial of a 13-valent pneumococcal conjugate vaccine in healthy adults. *Vaccine* 2007; 25: 6164–6166.