Development and testing of *Streptococcus pneumoniae* conjugate vaccines

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INTRODUCTION

Pneumococci are the largest cause of death resulting from infectious disease in the elderly, and cause the majority of ear infections in young children [1,2]. This organism is also an important cause of meningitis in young children and the elderly [3–5]. Although ear infections in young children generally do not lead to meningitis or other serious pneumococcal diseases, they do result in costly clinic visits for the children and much lost work by their parents [6]. Because of its ability to infect the very young, the very old, and the immunodeficient, the pneumococcus has one of the largest public-health and economic impacts of any infectious disease. Globally, the pneumococcus remains a leading bacterial pathogen in adults and the foremost cause of morbidity and mortality in infants and children in developing countries. Patients recovering from viral infections such as measles or influenza and those already afflicted with chronic diseases such as HIV constitute especially susceptible hosts in whom morbidity from the co-infecting pneumococcus is high. Overall, pneumococcal disease is a universal problem and has demanded an even greater amount of attention in recent years due to the advent of drug-resistant organisms.

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Pneumococcal strains with resistance to many antibiotics now exist, and indiscriminate use by physicians and healthcare providers has contributed to their widespread occurrence. This frequently introduces elements of uncertainty into formerly satisfactory treatment practices, particularly in the case of highly resistant strains [7,8]. Laboratory confirmation of antibiotic susceptibility patterns may take several days to complete, and clinicians are often forced to initiate treatment with broad-spectrum antibiotics such as cephalosporins and quinolones, a situation that may only accelerate antimicrobial resistance [9,10]. Therefore, preventing these infections with safe and effective vaccines will not only slow down the development of antibiotic resistance, but is a cost-effective way to control pneumococcal disease. The present conventional vaccine contains a mixture of 23 different polysaccharides [11]. This vaccine is not immunogenic in young children, because of their inability to mount an adequate immune response to polysaccharide antigens. Additional data in groups that are considered to be at high risk for life-threatening pneumococcal infections, such as the elderly, the immunocompromised, the splenectomized, those with sickle cell disease, and HIV-positive patients, have also shown only moderate to little efficacy after immunization with the conventional pneumococcal vaccine [12,13]. Among the elderly population, vaccine efficacy of the licensed pneumococcal vaccine is estimated to be approximately 60%, but goes down appreciably with increasing age.

Much work has been done in developing pneumococcal conjugate vaccines as the next generation of promising vaccines against pneumococcal diseases. As with most T-independent antigens, the capsular pneumococcal polysaccharide vaccine induces an immune response that is short-lived and characterized by variable amounts of antibody that is disproportionately IgM [14]. It also fails to produce high antibody affinity and a booster response upon repeated immunization. Because of these problems, attention has

been given to the development of pneumococcal vaccines that more closely mimic protein vaccines currently used in infants and other high-risk populations. One approach that has been used to immunize infants against infections from encapsulated bacteria (e.g. *Haemophilus influenzae* type b vaccine) is to present the capsular polysaccharide antigens in a form that is more immunogenic, such as in a protein–polysaccharide conjugate vaccine [15]. The coupling of polysaccharides to protein carriers such as diphtheria and tetanus toxoids demonstrates that these polysaccharides can acquire new antigenic properties typical of 'T-dependent' protein antigens [16,17]. They can stimulate a T-cell response, as shown in experimental animals, by their ability to use T-cell help to generate stronger 'booster' responses on re-stimulation [18]. The goal of a pneumococcal conjugate vaccine is, therefore, to convert the normally T-independent saccharide into a T-dependent antigen that will be immunogenic and efficacious in non-responsive populations [19].

The introduction of pneumococcal conjugate vaccines into the US vaccine armamentarium will address many of the major concerns about changing patterns of pneumococcal infection. It is anticipated that these new second-generation vaccines will help: (1) offset drug resistance and reduce antibiotic usage; (2) protect against the spread of uncontrolled invasive strains of pneumococci; (3) reduce the incidence of pneumococcal otitis media and tympanostomy; (4) reduce carriage and household/community transmission of pneumococci; and (5) promote a significant degree of herd immunity. A more effective vaccine for pneumococcal infection, therefore, represents a major public-health priority.

Four major companies are involved in the development and manufacture of pneumococcal conjugate vaccines. The products vary slightly in their chemical composition and construct (e.g. different carrier proteins), and each of the products is in a different stage of clinical development (Table 1). There is a select group of important serotypes that is now included in all conjugate pneumococcal vaccines, because of their relative importance in the pathogenicity of the disease. The seven-valent vaccines contain serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, while the nine-valent vaccines add serotypes 1 and 5 and the 11-valent vaccines add serotypes 3 and 7F. The protein carriers used to date are all functional entities and include tetanus toxoid, diphtheria toxoid, CRM 197 (a mutant protein of diphtheria toxoid), meningococcal outer-membrane complex, and non-typable *H. influenzae* outer-membrane protein (Table 1).

Widespread vaccination against a select group of common and important serotypes of *Streptococcus pneumoniae* could reduce infant mortality and protect against antibiotic resistance by inducing titers of mucosal antibodies sufficient to eliminate nasopharyngeal carriage. Based on the use of a seven-valent vaccine, a highly efficacious vaccine would have the potential to prevent up to 85% of invasive pneumococcal disease and 65% of pneumococcal otitis media in US children [20]. Little general clinical value will accrue for US children from the addition of serotypes 1, 3, 5 and 7F to a seven-valent conjugate vaccine. The nine- and 11-valent vaccines were designed to benefit individuals in countries outside the USA as well as special high-risk groups (e.g. Inuits and American Indians).

In the development of pneumococcal conjugates, the number of vaccine serotypes included in the construct is limited because of the need to conjugate each serotype as an individual entity. For now, the 11-valent vaccine appears to be the industry standard, although there has been some discussion on whether an additional two serotypes should be added to the mix. The FDA would probably favor a uniform approach among the different vaccine manufacturers with regard to the number of valences that are included in each of the multivalent pneumococcal conjugate vaccines, to avoid confusion within the medical community.

CLINICAL DATA THAT HAVE CONTRIBUTED TO THE CHARACTERIZATION OF CONJUGATE VACCINES

Some of the earliest and most significant clinical studies to date have been conducted in Finland. Table 2 shows the results of four separate clinical trials, all done in a common setting using four different conjugate pneumococcal vaccines administered at 2, 4 and 6 months of age [21]. Each trial used a similar protocol and incorporated common laboratory procedures that included a standardized ELISA assay. All four vaccines were considered safe, with no indications of any serious adverse events. All four vaccines also induced significant levels of antibody to each of the serotypes tested after three immunizations at 2, 4 and 6 months. Some serotypes were more immunogenic than others (e.g. 14 and 19F), while serotypes 6B and 23F were considerably less immunogenic. This appears to be the trend for all the pneumococcal conjugate vaccines, regardless of manufacturer. Unfortunately, it is not possible to make any true comparisons here, since the studies were not done head-to-head and the vaccines were not optimal end-stage formulas, but early prototype vaccines.

Another study demonstrated the ability to boost a primary response among infants immunized with a four-valent conjugate vaccine at 2, 4 and 6 months of

Table 1 Selected characteristics of glycoprotein conjugate pneumococcal vaccines

Manufacturer	Protein carrier	Linker	Saccharide length	Vaccine serotypes	Clinical studies
Pasteur/Merieux/ Connaught	Tetanus toxoid/ diphtheria toxoid	Short linker	—	8-valent: 3, 4, 6B, 9V, 14, 18C, 19F, 23F	Phase I/II Phase I/II
				11-valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F (D carrier—3, 6B, 14, 18C) (T carrier—1, 4, 5, 7F, 9V, 19F, 23F)	Phase I/II
Wyeth/Lederle Vaccines & Pediatrics	CRM 197	Reductive amination (amine)	Long	7-valent: 4, 6B, 9V, 14, 18C, 19F, 23F	Phase III
				9-valent: 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F	Phase III
				11-valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	Preclinical
Merck Research Laboratories	OMP meningococcus B	Bivalent linker (thioether)	Long	4, 6B, 9V, 14, 18C, 19F, 23F	Phase II/III
SmithKline Beecham	Non-typable *H. influenzae* OMP	—	Long	4-valent: 6B, 14, 19F, 23F	Phase I
				11-valent: 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	Phase I

CRM 197, CRM—a non-toxic variant of diphtheria toxin; D, diphtheria toxoid; T, tetanus toxoid; OMP, outer membrane protein complex.

Table 2 Immune response in infants to four different quadrivalent pneumococcal conjugate vaccines administered at 2, 4 and 6 months of age: antibody concentration (mg/L) at 7 months of age

Vaccine	6B	14	19F	23F
PncCRM	0.40	2.50	0.79	1.10
PncOMP	1.30	8.27	9.85	1.90
PnCD	0.88	2.30	5.29	0.88
PncT	0.77	3.06	3.20	0.67

PncCRM, Wyeth/Lederle; PncOMP, Merck Research Laboratories; PnCD, Pasteur/Merieux/Connaught; PncT, Pasteur/Merieux/Connaught.

age (Table 3) [22]. One group of children was primed and boosted with a conjugate vaccine at 14 months, while a second group was primed with conjugate vaccine, but boosted with plain polysaccharide, again at 14 months. The data indicate that antibody levels declined significantly (2–4-fold) by 14 months following the initial priming immunization. However, 1 month after a booster dose of vaccine, there was a 6–10-fold increase in antibody activity for each of the vaccine serotypes in both study groups. Furthermore, >90% of children in both groups had antibody concentrations >1.0 mg/L for all serotypes measured. The results indicate that pneumococcal conjugate vaccines confer properties of T-dependent antigens to polysac-charides and elicit immunologic memory and that it is possible to boost with a polysaccharide vaccine after priming infants first with a conjugate vaccine.

The capacity of pneumococcal conjugate vaccines to prime for an anamnestic response was also observed in a study conducted in The Gambia [23]. Children, who were all previously primed at infancy with either two or three doses of a five-valent pneumococcal conjugate vaccine or three doses of a control Hib conjugate vaccine, were boosted at 2 years of age with a 23-valent polysaccharide vaccine. The data reveal that three priming doses were better than two in producing consistently higher antibody levels (51 mg/L versus 28 mg/L respectively) (Table 4). Antibody concentrations were measured 2 years post-priming, but immediately before the boost had dropped precipitously (pre-3 levels). The rise in antibody concentrations 10 days following the polysaccharide boost was striking, regardless of whether two or three priming doses were used. It was surprising to see high antibody titers to serotype 6B in Gambian children, given its status as a weak immunogen. Again, these results reinforce the idea that pneumococcal conjugate vaccines are capable of priming the immune system to respond to subsequent exposures to capsular polysaccharide antigens associated with the pneumococcal organism.

Several studies have been done in healthy adults and the elderly to determine the impact of pneumococcal conjugate vaccines in these populations [24–27]. In one such study, healthy adults received either one of four conjugate vaccines or a 23-valent polysaccharide vaccine (Figure 1) [27]. The results are expressed as a ratio of conjugate vaccine/polysaccharide vaccine geometric mean concentrations (GMC), with ratios >1.0 indicative of a better response for the conjugate vaccine and ratios <1.0 indicative of a better response for the polysaccharide vaccine. In general, the levels of IgG antibodies to serotypes 6B and 23F were greater following immunization with a conjugate vaccine (with one exception), and the levels of antibodies to serotype 14 were greater following immunization with a polysaccharide vaccine. This equivocal pattern of response has prompted additional studies in the elderly with newer versions of conjugate vaccines to determine whether other vaccine schedules may provide for a more favorable outcome.

In addition to measuring antibody concentrations following vaccination, it is important to evaluate the qualitative characteristics of antibody production (i.e. avidity and opsonic antibody activity) that are important in assessing the effectiveness of pneumococcal conjugate vaccines [28]. To address this issue, a study was conducted in which infants were primed with a tetravalent conjugate vaccine at 2, 4 and 6 months of

Table 3 Infants primed with a four-valent pneumococcal conjugate vaccine at 2, 4 and 6 months and boosted at 14 months: GMC mg/L in infants at various months of age

	Vaccine			PS 6B			PS 23F		
Study group	Primary	Booster	*n*	7	14	15	7	14	15
1	PncD	PncD	36	0.81	0.39	2.62	0.75	0.21	1.17
2	PncD	PncPS	35	1.45	0.49	3.03	0.71	0.29	2.79

PncD, conjugate of PSs of serotypes 6B, 14, 19F and 23F to diphtheria toxoid; PncPS, 23-valent pneumococcal PS vaccine; GMC, geometric mean concentration; PS, polysaccharide.

Table 4 Antibody concentrations after a PncPS boost at two years of age in Gambian infants primed with either three doses of a Hib conjugate vaccine or with two or three doses of a five-valent pneumococcal conjugate vaccine [23]

	Antibody concentration (mg/L)			
	None	Pre-3	Post-2	Post-3
6B	0.37	0.89	27.62	50.90
14	0.73	1.98	15.28	30.52
18C	1.62	0.30	5.82	7.26
19	0.57	0.99	5.70	10.33
23F	0.28	0.63	4.17	6.64

None=immunized 17 months previously with a Hib conjugate vaccine.
Pre-3=antibody levels 2 years after priming.
Post-2=received two doses of conjugate vaccine at 2 and 4 months.
Post-3=received three doses of conjugate vaccine at 2, 3 and 4 months.
PncPS=pneumococcal polysaccharide.

age and boosted at 14 months with either the same conjugate vaccine or a 23-valent polysaccharide vaccine [29]. An increase in the avidity of antibody binding to the vaccine serotypes was observed only among children boosted with the conjugate vaccine, but not among the recipients of plain polysaccharide (Figure 2). Avidity was also shown to correlate with functional opsonic antibody activity. These results may have significant cost implications with regard to vaccine strategies. Although data indicate that boosting with a plain polysaccharide vaccine produces a significant increase in antibody activity against each of the serotypes contained in the priming vaccine, the functional activity of these antibodies compared to antibodies following a boost with a conjugate vaccine may be inferior and, therefore, provide for a lesser degree of protection. It is also possible that boosting with a conjugate vaccine may improve the duration of the protective antibody response [30].

There was concern over whether children less than 2 years of age with recurrent respiratory infections, who failed to respond to polysaccharide vaccine, would be able to respond to a pneumococcal conjugate vaccine. It is estimated that between 5% and 10% of children with recurrent respiratory infections are unresponsive to the conventional polysaccharide vaccine [31]. To examine the effectiveness of a conjugate vaccine in this population, children 2–13 years of age with no known immunodeficiencies were recruited into the study (Figure 3). All the children received a pneumococcal polysaccharide vaccine followed six months later by either a heptavalent conjugate vaccine or plain pneumococcal polysaccharide [32]. The results indicate that poor responders to the polysaccharide vaccine are able to respond significantly better, overall, to a single dose of conjugate vaccine, with increased antibody concentrations in response to all serotypes tested. Immunization with conjugate vaccines may thus represent a viable option for patients at high risk for

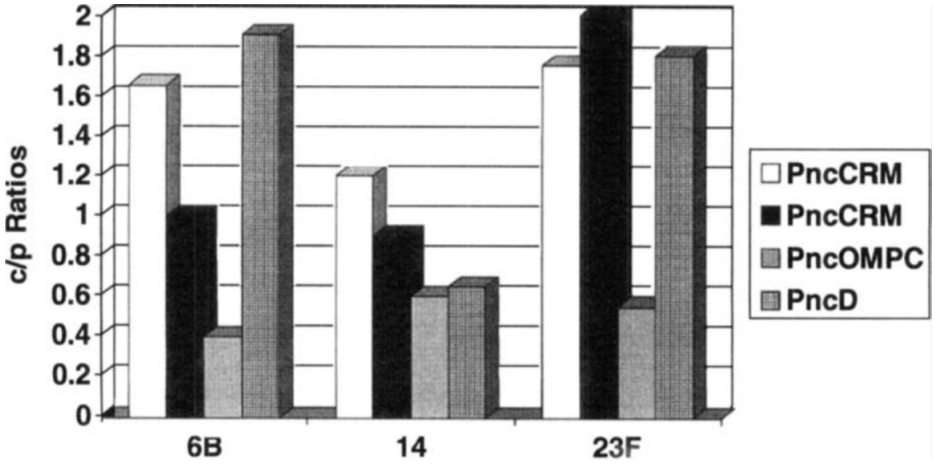

Figure 1 Ratio of GMCs (conjugate/PS) after pneumococcal vaccination in healthy adults: C/P, conjugate vaccine/polysaccharide vaccine ratio; GMC, geometric mean concentration; PS, polysaccharide; PncCRM, five-component pneumococcal oligosaccharide conjugate with CRM 197 protein carrier (Wyeth Lederle); PncOMPC, pneumococcal conjugate with outer-membrane protein complex carrier (Merck Research Laboratories); PnCD, pneumococcal conjugate with diphtheria carrier (Pasteur/Merieux/Connaught); PnCRM, seven-component pneumococcal conjugate with CRM 197 protein carrier (Wyeth-Lederle) [24,27].

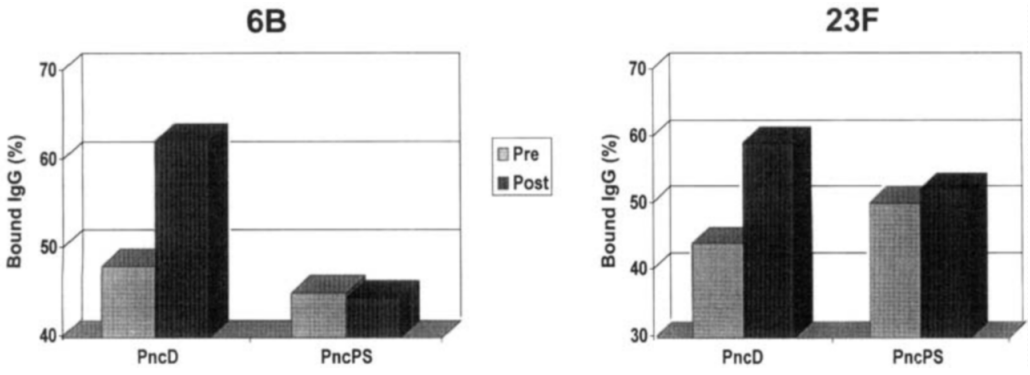

Figure 2 Increase in avidity of antibodies after booster vaccination: PncD, PMC pneumococcal conjugate vaccine; PncPS, 23-valent pneumococcal polysaccharide vaccine [29].

recurrent respiratory infections who fail to respond initially to the currently available 23-valent polysaccharide vaccine.

Pneumococcal conjugate vaccines have also demonstrated tremendous value in other high-risk groups, including Hodgkin's disease patients [33], and HIV-infected children [34]. In general, a significant increase was observed in the number of responders with antibody levels greater than 1.0 mg/L following one or more doses of conjugate vaccine. These results further suggest that priming with conjugate vaccines may represent a good strategy for high-risk populations.

An important consideration when administering pneumococcal conjugate vaccines to children is the potential impact they may have on colonization and transmission of the organism. A good example of the effect that a multivalent pneumococcal conjugate vaccine has on nasopharyngeal carriage was observed in Israeli toddlers. Day-care children received either a single dose of a nine-valent conjugate vaccine or a conjugate meningococcal C vaccine [35]. Figure 4 shows the nasopharyngeal carriage results 8 months following vaccination. Carriage rates were reduced for all vaccine serotypes as well as for penicillin-resistant, multi-resistant and non-resistant vaccine types, especially in children less than 24 months of age at the time of vaccination. These data indicate that conjugate vaccines are capable of reducing the colonization of vaccine-related serotypes significantly, as well as decreasing the burden of antibiotic-resistant strains.

Similar studies were conducted in Gambian and South African infant populations [36,37]. Again, a reduction was observed in the pharyngeal carriage rates of serotypes associated with the 7-valent pneumococcal conjugate vaccine following its vaccination into infants (Figure 5). Interestingly, the figure also shows an increase in non-vaccine serotypes among infants in both locations who received the pneumococcal conjugate vaccine compared to a placebo (i.e. conjugate meningococcal C vaccine). This latter data suggest that replacement and/or unmasking of pneumococcal serotypes occurs in the host following immunization with a pneumococcal conjugate vaccine due to successful competition by non-vaccine serotypes. Whether this is truly a replacement phenomenon (i.e. new serotypes occupying available niches) or the unmasking of serotypes present previously, but not expressed due to the prevalence of more dominant types, or both, is unclear at this time. The real question and concern is whether replacement carriage will translate into replacement pneumococcal disease.

RECENT VACCINE TRIALS THAT DEFINE EFFICACY

Currently, there are four efficacy trials in progress evaluating three different pneumococcal multivalent conjugate vaccines manufactured by two companies (Wyeth-Lederle Vaccines and Pediatrics and Merck Research Laboratories) (Table 5). The endpoints for these four trials vary considerably from acute otitis media to invasive disease. Recently, a large-scale phase III clinical trial involving approximately 38 000 infants

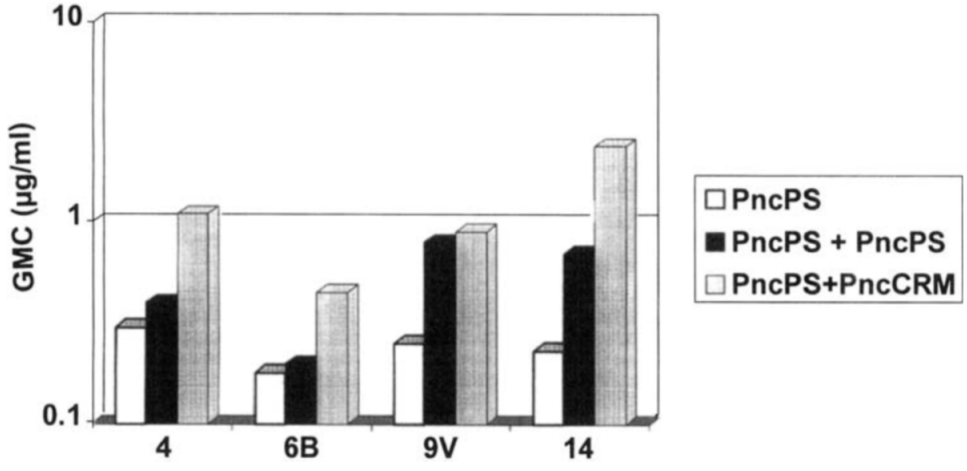

Figure 3 Response to 23-valent pneumococcal polysaccharide vaccine (PncPS) or Wyeth-Lederle pneumococcal conjugate vaccine (PncCRM) in children with recurrent infections [32].

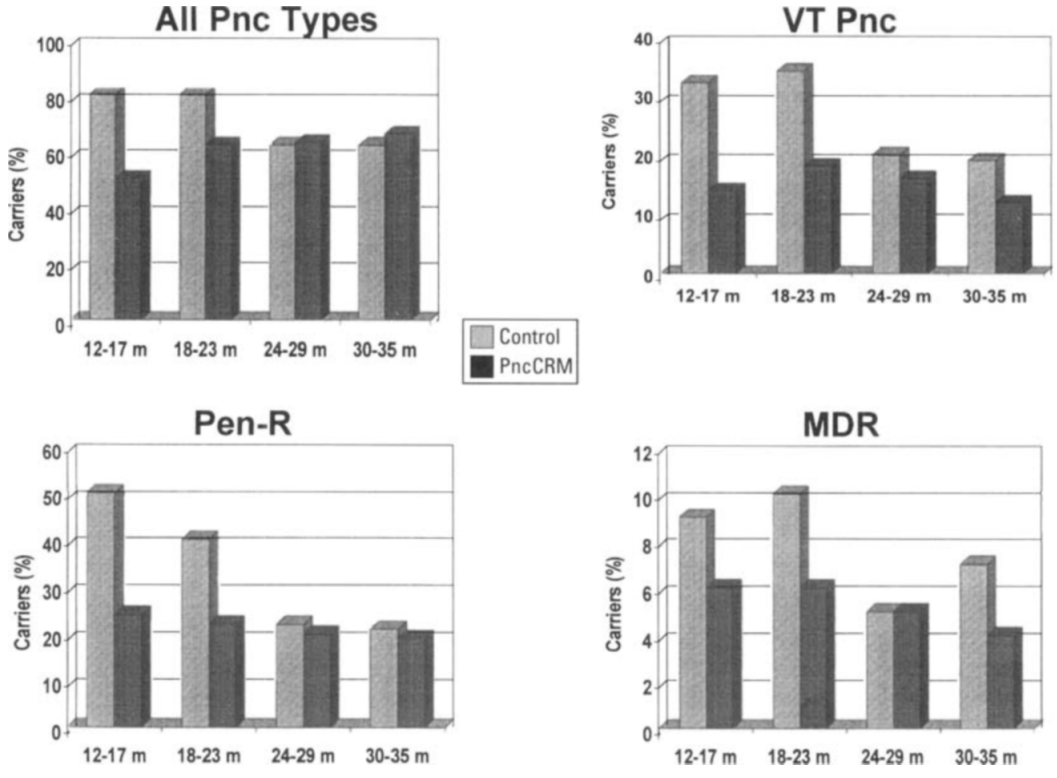

Figure 4 Pneumococcal carriage after PncCRM in Israeli toddlers: PncCRM, Wyeth-Lederle pneumococcal conjugate vaccine; Pnc, pneumococci; VT Pnc, vaccine-type pneumococci; Pen-R, penicillin-resistant types; MDR, multiresistant; Control, meningococcal C conjugate vaccine [35].

Table 5 Ongoing and planned efficacy trials with pneumococcal conjugate vaccines

Site	Vaccine	Starting year	Endpoint
USA (recently completed)	PncCRM, 7-valent	1995	Invasive disease
Finland	PncCRM, 7-valent PncOMPC, 7-valent	1995	Otitis media
USA (Navajo/Apache infants)	PncCRM, 7-valent	1998	Invasive disease/herd immunity
South Africa	PncCRM, 9-valent	1998	Invasive disease
Gambia (phase II ongoing)	PncCRM, 9-valent	1999	Mortality/morbidity
Israel	PncD+T, 11-valent	1999	Otitis media
Philippines	PncD+T, 11-valent	1999	Invasive disease
Chile	PncD+T, 11-valent	2000	Invasive disease

PnCRM, Wyeth-Lederle; PncOMPC, Merck Research Laboratories; PncD+T, Pasteur/Merieux/Connaught.

Table 6 Breakdown of cases reported in the Northern California Kaiser Permanente Efficacy Trial

	Number of cases		
Group	Vaccine group	Control group	Serotypes
Fully vaccinated	0	17	6B, 9V, 14, 18C 19F, 23F
Partially vaccinated	0	5[a]	6B, 14, 19F, 23F
Non-vaccine serotype	3[b]	5	3, 10F, 11A, 18B, 19A, 23A, 38

[a]Three with one dose, two with two doses.
[b]Two fully vaccinated and one partially vaccinated.

was unblinded and the results published [38]. The trial involved the use of a Wyeth-Lederle pneumococcal conjugate vaccine and was conducted at a Northern California Kaiser Permanente Health Maintenance Organization (HMO). The infants were randomized to receive either a pneumococcal conjugate vaccine or a meningococcal C conjugate vaccine, both produced by Wyeth-Lederle. The pneumococcal conjugate vaccine was formulated to protect against the seven most common strains of *S. pneumoniae* in the USA (polysaccharide serotypes 4, 6B, 9V, 14, 19F and 23F, and oligosaccharide serotype 18C) and included 20 μg of the carrier CRM197, a mutant form of diphtheria toxin. The infants were vaccinated at 2, 4, and 6 months of age, in addition to a conjugate booster at 12–15 months of age, and followed for disease. The trial was designed to have an initial look at the efficacy data once 17 fully vaccinated cases of invasive disease

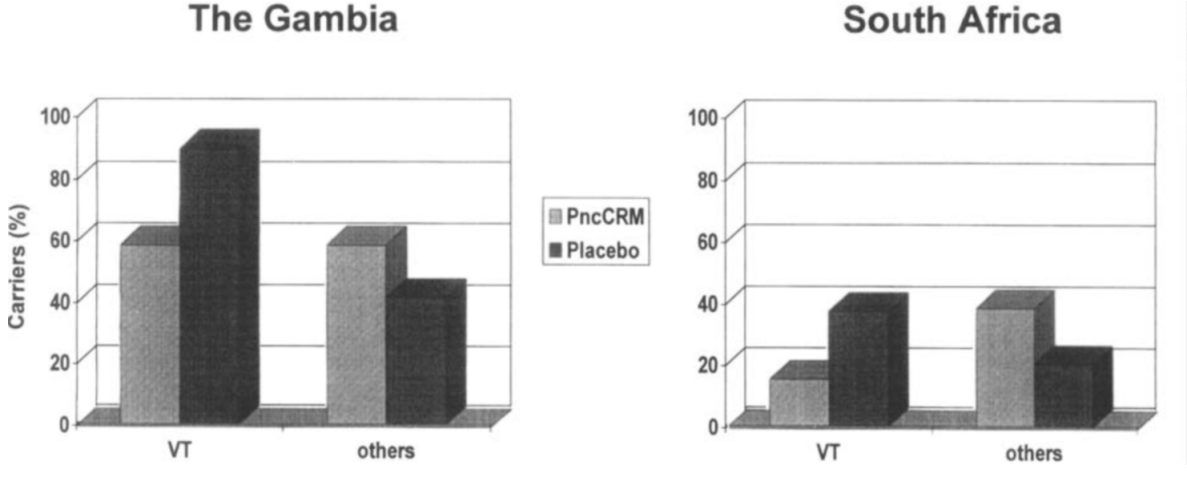

Figure 5 Reduction of pharyngeal carriage after PncCRM vaccination in infancy: PncCRM, Wyeth-Lederle pneumococcal conjugate vaccine; Placebo, meningococcal C conjugate vaccine; VT, vaccine types; Others, non-vaccine serotypes [36].

were documented. The results outlined in Table 6 are very encouraging and reveal 100% efficacy (95% CI = 75.7–100.0, $p<0.0001$) for the conjugate pneumococcal vaccine against invasive disease following either full vaccination or partial vaccination. Unfortunately, none of the cases in the vaccinated control group included meningitis, and none of the children in the study was considered to be at high risk.

A randomized, double-blind safety and immunogenicity study was run concurrently with the efficacy study. The trial enrolled 212 healthy 2-month-old infants at four clinical sites. The infants received identical vaccines to those used in the efficacy trial (i.e. half received the pneumococcal conjugate vaccine and the other half the meningococcal C conjugate vaccine). Each child was given doses of vaccine at 2, 4 and 6 months, and those who remained in the study received a booster at 12–15 months. Results showed that the conjugate vaccine was highly immunogenic, produced a good antibody response against all seven strains of *S. pneumoniae*, was well tolerated, with only minor reactions at the injection site, and caused only mild-to-moderate post-vaccination fever in some children [39].

During the next 12–18 months, several additional efficacy trials are expected to begin at four sites outside the USA (i.e. Israel, The Gambia, the Philippines, and Chile) (Table 5). Many important issues will be addressed in addition to the question of vaccine safety and the effect that these conjugate vaccines have on protective efficacy. These include: (1) determining laboratory correlates of protection; (2) examining interference with other childhood vaccines; and (3) evaluating the overall impact on ecology (i.e. herd immunity) and colonization. Many of the conjugate vaccines in these new trials will contain as many as 11 serotypes. In the near future, other phase II and phase III studies may be needed in special populations to adequately describe the ability of pneumococcal conjugate vaccines to serve as good immunogens as well as protect against disease. These populations will include several high-risk groups, such as sickle cell patients, the elderly, pregnant women in their third trimester, immunocompromised patients, and premature infants of low birth weight. The main objectives will be to determine whether conjugate vaccines offer any significant advantage over conventional 23-valent capsular polysaccharide vaccines with regard to safety and immunogenicity. Many of the existing data suggest that the pneumococcal conjugate vaccines will have their greatest impact in populations (e.g. infants and high-risk groups) that do not respond well to the 23-valent polysaccharide vaccine when used as a primary inoculum [40].

ALTERNATIVE PNEUMOCOCCAL VACCINE STRATEGIES

The use of polysaccharide–protein conjugate vaccines, while overcoming many of the liabilities of polysaccharide vaccines, still involves a number of problems. First, new studies indicate that immunity induced by pneumococcal conjugate vaccines may be short-lived, especially in infants. Such a limitation would necessitate repeat vaccinations through the first several years of life—an expensive procedure even in relatively wealthy nations, but an even greater and prohibitive expense for the developing world, where cost factors play a major role in deciding whether or not a vaccine gets used. Second, regional variations in the predominance of infecting pneumococcal serotypes necessitate the formulation of capsule-based vaccines that are appropriate to the local epidemiology. Such modifications are not only technologically difficult, but also exceedingly expensive. Third, because serotype coverage is severely restricted by the inclusion of only 11 serotypes, the capacity to promote wide protection against infections in developing countries is limited. At best, an 11-valent conjugate vaccine would cover only three-quarters of serotypes causing disease that may vary over time. Fourth, because the multivalent vaccines are made up of individual vaccines, a large total dose of carrier protein is required that may subsequently lead to carrier-induced suppression/overload, or even anti-carrier proteins [41, 42]. Last, the ability of pneumococci to change their capsular serotype as a result of uptake of heterologous DNA suggests that the protective effect of anti-capsular antibody may be all too temporary, as vaccine serotypes 'deliberately' modify their surface polysaccharide in response to mucosal antibodies and other selective pressures [43].

All of these considerations lead to the conclusion that new generations of pneumococcal vaccines will be needed to address many of these problems. Just as capsular polysaccharide vaccines have now given way to protein–polysaccharide conjugates, future pneumococcal surface proteins, which represent excellent virulence determinants that are immunogenic and conserved among global serotypes, will lead the way as third-generation vaccines. These new surface protein vaccines most probably will be employed either as the carrier component in conjugate vaccines or as an individual vaccine combined with an adjuvant or cytokine [44–49]. The use of such proteins as immunizing antigens might serve not only to prevent colonization in fully immunized hosts, but also to ameliorate the effects of breakthrough infections in incompletely protected populations such as infants. Work in international laboratories has shown that several different pneumococcal surface proteins such as

Table 7 Questions remaining after current efficacy trials

Persistence of antibodies and protection
Impact on the epidemiology of pneumococcal infections
Potential indications in other target groups, such as premature infants, pregnant women, elderly individuals, immunocompromised individuals, including HIV-positive infants, nephrotics, splenectomized subjects and bone marrow transplant recipients
Optimal use of the vaccine with regard to:
Schedule, combinations, immunization route
Possible interference with other childhood vaccines
Cost-effectiveness analysis
Benefit of adding adjuvants to improve immune response
Need for additional serotypes and improved formulations
Standardization of laboratory methodology for total and functional antibody
Role of cell-mediated immunity in protection
Search for surrogates of protection
Role of genetic transformation on conjugate vaccine effectiveness

Table 8 Problems associated with pneumococcal conjugate vaccines

Cost of producing vaccine and limited potential in developing countries
Need for multivalent products made up of individual vaccines
Need for different formulations, dosages and combinations of serotypes to accommodate different populations, age groups and geographic needs
Problem with carrier-induced suppression, or overload due to large total dose of carrier protein
Currently, vaccine covers, at best, only three-quarters of types causing disease that may vary over time
The majority of pneumococcal morbidity is associated with mucosal infections that conjugate vaccines do not address
Each conjugated antigen is a unique, separate vaccine with different immunologic properties—makes for a very complex product

pneumococcal surface protein A (PspA) [45,49], pneumococcal surface adhesin A (PsaA) [46], neuraminidase [44] and autolysin [48] are able to elicit active protection against pneumococcal infection in mice when challenged with lethal doses of pneumococci. More recent data strongly indicate that antibody to PspA in human serum can protect mice from fatal pneumococcal infection [50]. As a result of these studies, PspA is now being developed as a human vaccine by a major vaccine manufacturer, and is now in phase I trials. Because of the conserved nature of these surface proteins, it is possible that they will provide a broader vaccine application and greater overall protection against serotypes causing pneumococcal disease than do conjugate vaccines. In addition, surface protein vaccines may have the ability to stimulate a protective respiratory tract mucosal immune response when administered parenterally, something not observed generally with conjugate vaccines [51].

SUMMARY

Even after the completion of the phase III trials and the possible licensing of pneumococcal conjugate vaccines, numerous outstanding issues and questions, both basic and clinical, remain to be addressed, as outlined in Tables 7 and 8.

Pneumococcal conjugate vaccines have evolved considerably over the past several years and appear to offer a number of opportunities for various high-risk groups compared to the licensed polysaccharide vaccines. What we know and can say about pneumococcal conjugate vaccines at this time is summarized below:

- All pneumococcal conjugate vaccines are not created equally. Furthermore, each conjugated antigen is a unique, separate vaccine with different immunologic properties.
- Conjugate vaccines produce increased immunologic responses compared to the plain polysaccharide vaccine.
- All pneumococcal conjugate vaccines have been found to be relatively safe and well tolerated, with no reported serious adverse events.
- Repeated injections following a priming immunization with conjugate vaccine elicits both IgG and functional antibody.
- Conjugate vaccines appear to work best in infants and high-risk groups.
- Conjugate vaccines induce immunologic memory.
- Immunogenicity has been shown to vary significantly among serotypes in terms of magnitude and kinetics of response.
- Conjugate vaccines can decrease nasopharyngeal carriage rates and, thus, reduce the transmission of pneumococci in community and specialized settings.
- There are no antibody data that show what antibody concentrations are needed for protection.
- Pneumococcal conjugate vaccines may protect against the spread of uncontrolled invasive strains.
- The routine use of pneumococcal conjugate vaccines may represent the most successful approach to decreasing the burden of antibiotic-resistant strains of pneumococci.

References

1. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: Recommendations of the Advisory Committee on Immunization Practice (ACIP). MMWR 1997; 47(No. RR-8)

2. Mufson M. *Streptococcus pneumoniae*. In Mandell GL, Douglas RG, Bennett JE eds. Principles and practices of infectious disease. Churchill Livingston, 1990; 1539–50.
3. Leowski J. Mortality from acute respiratory infections in children under 5 years of age: global estimates. World Health Stat Q 1986; 39: 138–44.
4. WHO Technical Advisory Group on ARI. A program for controlling acute respiratory infections in children: memorandum from a WHO meeting. Bull WHO 1984; 64: 47–58.
5. Gray BM, Dillon HC Jr. Clinical and epidemiological studies of pneumococcal infection in children. Pediatr Infect Dis 1986; 5: 201–7.
6. Klein JO. The epidemiology of pneumococcal disease in infants and children. Rev Infect Dis 1981; 3: 246–53.
7. Hofmann J, Cetron MS, Farley MM, et al. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. N Engl J Med 1995; 333: 481–6.
8. Tomasz A. Antibiotic resistance in *Streptococcus pneumoniae*. Clin Infect Dis 1997; 24(suppl 1): S85–8.
9. Deka K, Ware JC, Chonmaitree T, et al. Penicillin-resistant *Streptococcus pneumoniae* in acute otitis media: prevalence, serotypes, and clinical significance. In Lim DJ, Bluestone CD, Casselbrant M, Klein JO, Ogra PL, eds. Proceedings of the Sixth International Symposium on Recent Advances in Otitis Media. Hamilton, Canada: BC Decker Inc. 1996: 303–5.
10. Sih T. Drug-resistant *Streptococcus pneumoniae*. In Lim DJ, Bluestone CD, Casselbrant M, Klein JO, Ogra PL, eds. Proceedings of the Sixth International Symposium on Recent Advances in Otitis Media. Hamilton, Canada: BC Decker Inc., 1996: 305–7.
11. Henrichsen J. Six newly recognized types of *Streptococcus pneumoniae*. J Clin Microbiol. 1995; 33: 2759-62.
12. Kayhty H, Eskola J. New vaccines for the prevention of pneumococcal infections. Emerg Infect Dis. 1996; 2: 289–95.
13. Siber GR. Pneumococcal disease: prospects for a new generation of vaccines. Science 1994; 265: 1385–7.
14. Stein KE. Network regulation of the immune response to bacterial polysaccharide antigens. Curr Top Microbiol Immunol. 1985; 119: 57–74.
15. Murphy TV. Vaccines for *Haemophilus influenzae* type b. Semin Pediatr Infect Dis 1991; 2: 120–34.
16. Beuvery ECF, van Rossum F, Nagle J. Comparison of the induction of immunoglobulin M and G antibodies in mice with purified pneumococcal type 3 and meningococcal group C polysaccharides and their protein conjugates. Infect Immun 1982; 37: 15–22.
17. Chu C, Schneerson R, Robbins JB, Rastogi SC. Further studies on the immunogenicity of *Haemophilus influenzae* type b and pneumococcal type 6A polysaccharide–protein conjugates. Infect Immun 1983; 40: 245–56.
18. Gordon LK. Characterization of a hapten-carrier conjugate vaccine: *H. influenzae*-diphtheria conjugate vaccine. In Chanock RM, Lerner RA (eds), Modern approaches to vaccines. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1984; 393–6.
19. Robbins JB, Schneerson R. Polysaccharide–protein conjugates: new generation vaccines. Rev Infect Dis 1990; 161: 821–32.
20. Butler JC, Dowell SF, Breiman RF. Epidemiology of emerging pneumococcal drug resistance: implications for treatment and prevention. Vaccine 1998; 16: 1693–7.
21. Käyhty H, Eskola J. New vaccines for the prevention of pneumococcal infections. Emerg Infect Dis 1996; 2: 289–98.
22. Åhman H, Käyhty H, Lehtonen H, et al. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to induce immunologic memory. Pediatr Infect Dis J 1998; 17: 211–16.
23. Obaro SK, Huo Z, Banya WAS, et al. A glycoprotein pneumococcal conjugate vaccine primes for antibody responses to a pneumococcal polysaccharide vaccine in Gambian children. Pediatr Infect Dis J 1997; 16: 1135–40.
24. Powers DC, Anderson EL, Lottenbach K, Mink CM. Reactogenicity and immunogenicity of a protein-conjugated pneumococcal oligosaccharide vaccine in older adults. J Infect Dis 1996; 173: 1014–18.
25. Shelly MA, Jacoby H, Riley GJ, et al. Comparison of pneumococcal polysaccharide and CRM197-conjugated pneumococcal oligosaccharide vaccines in young and elderly adults. Infect Immun 1997; 65: 242–7.
26. Storck J, Mendelman PM, Witherspoon RP, et al. IgG response to pneumococcal polysaccharide-protein conjugate appears similar to IgG response to polysaccharide in bone marrow transplant recipients and healthy adults. Clin Infect Dis 1997; 25: 1253–5.
27. Nieminen T, Kayhty H, Virolainen A, Eskola J. Circulating antibody secreting cell response to parenteral pneumococcal vaccines as an indicator of a salivary IgA antibody response. Vaccine 1997; 16: 313–19.
28. Granoff DM, Lucas AH. Laboratory correlates of protection against *Haemophilus influenzae* type b disease. Importance of assessment of antibody avidity and immunological memory. Ann NY Acad Sci 1995; 745: 278–88.
29. Anttila M, Eskola J, Åhman H, Käyhty H, et al. Avidity of IgG for *Streptococcus pneumoniae* type 6B and 23F polysaccharides in infants primed with pneumococcal conjugates and boosted with polysaccharide or conjugate vaccines. J Infect Dis 1998; 177: 1614–21.
30. Schlesinger Y, Granoff DM. Vaccine Study Group. Avidity and bactericidal activity of antibody elicited by different *Haemophilus influenzae* type b conjugate vaccines. JAMA 1992; 267: 1489–94.
31. Hidalgo H, Moore C, Leiva L, Sorensen RU. Pre- and post-pneumococcal antibody titers in children with recurrent infections. Ann Allergy Asthma Immunol 1996; 76: 341–6.
32. Sorensen RU, Leiva LE, Giangrosso PA, et al. Response to a heptavalent conjugate *Streptococcus pneumoniae* vaccine in children with recurrent infections who are unresponsive to the polysaccharide vaccine. Pediatr Infect Dis J 1998; 17: 685–91.
33. Chan CY, Molrine DC, George S, et al. Pneumococcal conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin's disease. J Infect Dis 1996; 173: 256–8.
34. King JC Jr, Vink PE, Chang I, et al. Antibody titers eight months after three doses of a five-valent pneumococcal conjugate vaccine in HIV and non-HIV-infected children less than two years of age. Vaccine 1998; 16: 361–5.
35. Dagan R, Givon N, Yagupsky P, et al. Effect of a 9-valent pneumococcal vaccine conjugated to CRM_{197} (PncCRM9) on nasopharyngeal (NP) carriage of vaccine type and non-vaccine type *S. pneumoniae* (Pnc) strains among day care center (DCC) attendees [abstract G-52]. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA. Washington, DC: American Society for Microbiology, 1998: 299.
36. Obaro SK, Adegbola RA, Banya WA, Greenwood BM. Carriage of pneumococci after pneumococcal vaccination. Lancet 1996; 348: 271–2.
37. Klugman KP. Conjugate pneumococcal vaccine: current and future role. In: Program and abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy,

Toronto, Canada. Washington, DC: American Society for Microbiology, 1997: 395.
38. Black S, Shinefield H, Ray P, et al. Efficacy of heptavalent conjugate pneumococcal vaccine (Wyeth Lederle) in 37,000 infants and children: results of the Northern California Kaiser Permanente Efficacy Trial [abstract LB-9]. In: Program and abstracts (addendum) of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA. Washington DC: American Society for Microbiology, 1998: 23.
39. Rennels MB, Edwards KM, Keyserling HL, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM_{197} in United States infants. Pediatrics 1998; 101: 604–11.
40. Fedson DS. Influenza and pneumococcal vaccination of the *elderly:* newer vaccines and prospects for clinical benefits at the margin. Prev Med 1994; 23: 751–5.
41. Schutze M-P, Leclerc C, Jolivet TM, et al. Carrier-induced epitopic suppression, a major issue for future synthetic vaccines. J Immunol 1985; 135: 2319–22.
42. Peeters CCAM, Tenbergen-Meekers A-M, Poolman JT, et al. Effect of carrier priming on immunogenicity of saccharide-protein conjugate vaccine. Infect Immun 1991; 59: 3504–10.
43. Nesin M, Ramirez M, Tomasz A. Capsular transformation of a multidrug-resistant *Streptococcus pneumoniae* in vivo. J Infect Dis 1998; 177: 707–13.
44. Lock RA, Paton JC, Hansman D. Comparative efficacy of pneumococcal neuraminidase and pneumolysin as immunogens protective against *Streptococcus pneumoniae*. Microb Pathog 1988; 5: 461–7.
45. Tart RC, McDaniel LS, Ralph BA, Briles DE. Truncated *Streptococcus pneumoniae* PspA molecules elicit cross-protective immunity against pneumococcal challenge in mice. J Infect Dis 1996; 173: 380–6.
46. Sampson JS, O'Connor SP, Stinson AR, Tharpe JA, Russell H. Cloning and nucleotide sequence analysis of psaA, the *Streptococcus pneumoniae* gene encoding a 37-kilodalton protein homologous to previously reported *Streptococcus* sp. adhesins. Infect Immun 1994; 62: 319–24.
47. Alexander JE, Lock RA, Peeters C, et al. Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of *Streptococcus pneumoniae*. Infect Immun 1994; 62: 5683–8.
48. Canvin JR, Marvin AP, Sivakumaran M, et al. The role of pneumolysin and autolysin in the pathology of pneumonia and septicemia in mice infected with a type 2 pneumococcus. J Infect Dis 1995; 172: 119–23.
49. McDaniel LS, Sheffield JS, Delucchi P, Briles DE. PspA, a surface protein of *Streptococcus pneumoniae*, is capable of eliciting protection against pneumococci of more than one capsular type. Infect Immun 1991; 59: 222–8.
50. Briles DE, Hollingshead SK, Swiatlo E, et al. PspA and PspC: their potential for use as pneumococcal vaccines. Microb Drug Resist 1997; 3: 401–8.
51. Klein DL. Pneumococcal conjugate vaccines: review and update. Microb Drug Resist 1995; 1: 49–58.