

New Strategy Is Needed to Prevent Pneumococcal Meningitis

Reshmi Mukerji, MPH,* and David E. Briles, PhD†

Background: Polysaccharide conjugate vaccines (PCVs) target the pneumococcal capsular types that most commonly cause fatal pneumonia and sepsis. Because these types were eliminated by the vaccines, it became apparent that in immunized populations, most invasive pneumococcal diseases, including bacteremia, sepsis and complicated pneumonia, were greatly reduced. However, the protective effects of PCVs against another invasive disease, meningitis, has shown much less or no decrease in disease incidence.

Methods: References were identified through searches of PubMed for articles published from January 1930 to the present by use of specific search terms. Relevant articles were also identified through searches in Google and Google Scholar. Relevant references cited in those articles were also reviewed.

Results: Even in the presence of the PCVs, meningitis rates in children have been reported globally to be as high as 13 per 100,000 annually. Widespread use of vaccines resulted in the emergence of a broad diversity of replacement non-PCV type strains. These strains generally failed to cause sepsis, but caused meningitis of comparable severity and levels similar to, or in excess of, prior pneumococcal meningitis rates. This is probably because these non-PCV type strains do not survive well in the blood, therefore possibly entering the brain through nonhematogenous routes.

Conclusions: Because virtually all cases of pneumococcal meningitis lead to either permanent neurologic sequelae or death, it would be well worth the effort to develop a new vaccine capable of preventing pneumococcal meningitis regardless of capsular type. Such a vaccine would need to protect against colonization with most, if not all, pneumococci.

Key Words: meningitis, *Streptococcus pneumoniae*, polysaccharide conjugate vaccine

(*Pediatr Infect Dis J* 2020;39:298–304)

Despite the use of polysaccharide conjugate vaccines (PCVs), *Streptococcus pneumoniae* remains the leading cause of bacterial pediatric meningitis after the neonatal period.^{1–4} Bacterial meningitis is difficult to treat, has a high case fatality rate and generally leaves patients with long-term sequelae.⁵ The successful introduction of PCV7 followed by PCV13 in national immunization programs resulted in a sharp decrease in rates of invasive pneumococcal disease (IPD), both through protection against disease caused by serotypes present in the vaccines and through herd

immunity against those serotypes.⁶ However, recent reports have consistently shown that the rates of pediatric and adult pneumococcal meningitis have either remained stable or increased, mainly due to the increase in carriage and subsequent meningitis caused by nonvaccine type (NVT) strains.^{2–4,7–11} The capsule types of meningitis strains now are more representative of present carriage types than PCV types.⁶ Here we discuss the evidence for serotype replacement in meningitis and possible limitations and alternatives to the current pneumococcal vaccine.¹²

SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified through searches of PubMed for articles published from January 1930 to the present by use of the terms “*Streptococcus pneumoniae*,” “meningitis,” “PCV,” “serotype replacement,” “capsule type,” “capsule dependent disease” and “nasopharynx to brain transmission.” Relevant articles were also identified through searches in Google and Google Scholar. Articles resulting from these searches and relevant references cited in those articles were also reviewed. Only articles written in English were included.

CHANGING EPIDEMIOLOGY OF PNEUMOCOCCAL MENINGITIS

The introduction of PCVs has led to a changing epidemiology of pneumococcal serotypes with a virtually complete replacement of PCV types in carriage and an incomplete replacement in invasive disease.^{13–15} Several studies have shown the impact of PCVs on serotype replacement. A Swedish study showed that before the introduction of PCV7 and PCV13, 38% and 18% of carriage isolates were non-PCV7 and non-PCV13 types, respectively, but after the introduction of the vaccines, the respective values were 95% and 89%.¹⁶ The primary emergent strains in IPD were serotypes 22F, 23A, 11A and 35F, and the distribution of serotypes in IPD reflected those in carriage.¹⁶ A recent South African study showed that children extensively immunized with PCV13 still carry PCV13 type strains as well as NVT strains.¹⁷ The most frequently carried PCV13 type strains were 19F, 9V, 19A and 6A (accounting for 22% of all isolates), while the most common NVT isolates were 15B/C, 21, 10A, 16F, 35B, 9N and 15A, which include some of the most frequently carried serotypes globally, post-PCV13 use.^{17,18} A large systematic review examining serotype replacement in IPD worldwide has shown that non-PCV13 serotypes were responsible for 42.2% (95% confidence interval: 36.1 ± 49.5%) of IPD in children.¹⁹ However, there were notable regional differences, with the highest serotype replacement rates reported from the European region (71.9%) and North America (57.8%).¹⁹

Effects of PCVs on Pediatric Meningitis Rates and Types

Meningitis is classified within the broader category of IPD. Distinct changes in serotypes causing meningitis, both in children and adults, have been reported worldwide, with annual disease rates as high as 13/100,000 for children 1–59 months of age²⁰ caused mainly by NVT emergent strains. Moreover, although the PCVs have led to a 35.4% reduction in cases of pneumococcal pneumonia, the reduction in meningitis cases is only 18.5% worldwide.^{20,21} Although all the contributors to strain replacement are not known,

Accepted for publication December 23, 2019.

From the *Department of Microbiology and †Department of Microbiology and Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama.

This study was supported by the National Institutes of Health Grant (NIH R01AI118805). D.E.B. confirms that he had full access to all the data in the study and had final responsibility for the decision to submit for publication. The authors have no conflicts of interest to disclose.

The University of Alabama Research Foundation holds a patent on the use of PRD in vaccines. R.M. and D.E.B. are among the inventors on this patent.

Address for correspondence: David E. Briles, PhD, 658 BBRB, Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294. E-mail: dbriles@uab.edu.

Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

ISSN: 0891-3668/20/3904-0298

DOI: 10.1097/INF.0000000000002581

the open colonization niche as a result of PCV vaccination is thought to be critical, while prevaccine carriage of some of the replacement serotypes in children and antibiotic use may all play a role.²²

An early analysis of IPD trends following PCV13 introduction in the United States, carried out using data from 8 hospitals between 2009 and 2011, showed that although there was a decline in IPD cases including bacteremia, pneumonia and mastoiditis, there was hardly a decline in meningitis cases.¹¹ A smaller scale study from Alabama following PCV introduction showed that nearly 40% of IPD among children in the state was caused by non-PCV13 type strains and that meningitis was more strongly associated with NVT strains than other IPDs.²³ Studies from France have shown a pronounced increase in meningitis mainly caused by NVT strains.^{2,7,24} Alexandre et al² showed that in France, there was a 6.5-fold increase in pneumococcal meningitis cases in children <2 years of age between 2005 and 2008, of which 82% was caused by non-PCV7 serotypes in 2008. Levy et al²⁴ analyzed large-scale French surveillance data showing that during the study period, there was a decline in PCV13 type pediatric meningitis cases, but NVT case rates remained stable. Of the meningitis cases, 67.6% of cases were caused by NVT strains, mainly 12F, 24F, 22F and 15B/C.²⁴ Similar results were reported by Olarte et al²⁵ on the impact of PCV13 on pediatric meningitis among US children, showing a 50% increase in pediatric meningitis cases in the post-PCV13 period. The most common serotypes isolated were 19A, 22F and 35B, with 73% of isolates being non-PCV13 serotypes.²⁵

A recent time-series analysis from France examined the incidence of pediatric meningitis over a 16-year period.⁷ The results showed a decline in the monthly incidence of pediatric meningitis from 0.12/100,000 in the pre-PCV13 period to 0.07 in 2014. However, a strong rebound in monthly meningitis rates was seen in the 2015–2016 period to 0.13/100,000, driven mainly by the emergence of serotype 24F (18% in the early PCV13 period and 74% in late PCV13 period).⁷ Ben-Shimol et al⁸ also found similar increases in NVT cases of IPD among Israeli children, with a 3.6-fold increase in rates of NVT cases of meningitis, driven largely by the emergent type 12F strain, but also by types 27, 15B/C, 33F and 24F.

In Brazil, the distribution of pneumococcal serotypes was studied before and 5 years after the introduction of PCV10. Here too a shift in serotypes toward NVT was noted with increasing number of meningitis cases attributed to serotypes 3, 6A, 6C and 19A.²⁶ A study from Burkina Faso, which is in the meningitis belt, showed that PCV13 led to reductions in vaccine type disease except that caused by serotype 1, which did not show a decline. For children <1 year of age, the main reported serotypes causing meningitis were 12F/12A/12B/44/46 (17%), 1 (12%) and 5 (10%).²⁷ The emergence of a range of different serotypes in different countries shows that there is no 1 or 2 NVTs that predominantly cause meningitis globally (Fig. 1A). From the small group of 7 post-PCV studies examined here, there were a total of 34 different non-PCV capsular types that caused meningitis (Fig. 1B). Three non-PCV types (10A, 12F and 15B/C) were reported in the top 50% of meningitis types in 5 or more of the 7 studies. Sixteen types caused meningitis in 2–6 studies, and 15 types were reported in only 1 or 2 studies. This capsule diversity makes adding enough new conjugates to PCVs to cover meningitis problematic.²⁸ Adding the 3 most common meningitis capsular types to the PCV vaccine might be possible but would likely just open the niche for expansion of other non-PCV types that can also cause meningitis.

Effect of PCV Vaccination of Children on Meningitis Rates in Adults

The changing epidemiology and the rising rates of pediatric meningitis are part of an overall picture of increasing number of pneumococcal meningitis cases. Although a large number of

studies examine the impact of PCVs on the rate of pediatric meningitis, studies on meningitis rates in adults' post-PCVs are rare. A 2015 study from Israel reporting on the herd immunity effect of PCV vaccination on adult meningitis showed an increased incidence and proportion of pneumococcal meningitis among all adult IPD post-PCV.⁹ A more recent study from the same group showed that although there was a 70% decline in vaccine type meningitis among Israeli adults, the overall incidence increased due to a significant increase in adult meningitis caused by the emergence of NVT strains, particularly serotypes 23A and 23B, among others (24F, 15B/C and 6C).⁴ This provides evidence of large-scale strain replacement in adult meningitis, which has become a cause for concern. A study by Alari et al²⁹ in France showed that for adults over 64 years of age, meningitis episodes caused by NVT strains were mainly due to serotypes 6C (10%), 23B (9%) and 24F (6%) in the years 2012–2014. A study from Utah in the United States has shown that 47% of meningitis cases were due to NVT strains compared with 18% for other IPD.¹⁰ Another large study using data collected from 8 sites in the United States between 1998 and 2005 showed that there was a 68.1% increase in NVT meningitis for the 40- to 64-year age group.³ This examination of the changing epidemiology of pneumococcal meningitis in both children and adults emphasizes the need to develop novel strategies to address serotype replacement in disease.

EVIDENCE OF DIRECT INVASION OF THE CENTRAL NERVOUS SYSTEM THROUGH THE NASOPHARYNX

Because meningitis appears to be readily caused by non-PCV strains whose capsule types (Fig 1B, lower circle) do not allow them to commonly cause bacteremia and sepsis in humans, it seems likely that they may be reaching the brain through a nonhematogenous route (Fig. 2). Indeed, several studies have supported the expectation that pneumococcal meningitis can be caused by a non-hematogenous route of infection, whereby the bacteria can travel directly from the nasopharynx or from ear infections to the brain.³⁰

Clinical studies of bacterial meningitis in humans have shown that there are a significant number of cases where bacteria were isolated from the cerebrospinal fluid of patients but not from blood.^{31–33} One study of Kenyan children presenting with impaired consciousness found that there were 10 cases of positive cerebrospinal fluid cultures with concomitant negative blood cultures, of which *S. pneumoniae* was the causative agent in 5.³¹ Several studies have shown that 15%–55% of neonates presenting with culture-confirmed meningitis had negative blood cultures.^{12,33–39} A study using retrospective data, from neonatal patients, further supported this finding by showing that 38% of culture-confirmed cases of bacterial meningitis had negative blood cultures.³² While antibiotic use could in some cases explain the lack of organisms in the blood, the evidence from animal experimental models indicates that pneumococci can travel directly to the brain using a nonhematogenous route.

Rake⁴⁰ was the first to show that pneumococci could travel directly to the brain from the nasopharynx via the olfactory mucosa, thus skipping the well-established hematogenous route. Later studies confirmed this finding when van Ginkel et al⁴¹ showed that initial nasopharyngeal infection was followed by isolation of high number of bacteria from olfactory epithelium, brain, olfactory bulbs and trigeminal ganglia in the absence of bacteremia. A later study used *in vivo* imaging to show that pneumococci are able to directly localize to the olfactory bulb and the brain, in the absence of detectable bacteremia.⁴² Marra and Brigham⁴³ were able to show, using a mutant of *S. pneumoniae* that is unable to survive in blood, that bacteria were able to directly disseminate to the brain from the lungs or the ears, in the absence of bacteremia. One of the proposed mechanisms

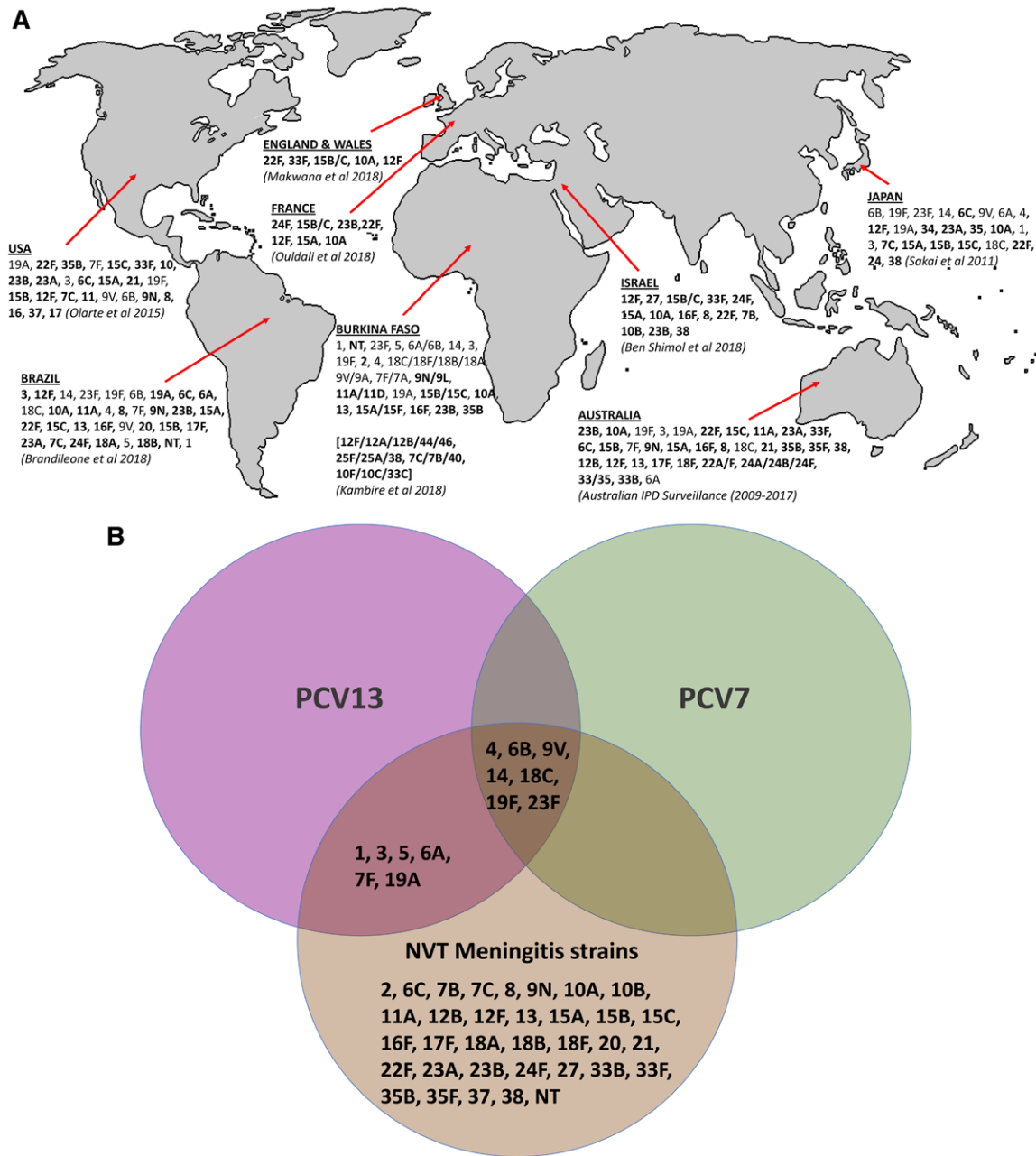


FIGURE 1. Pneumococcal meningitis has been reported to be caused by 35 capsular types not covered by PCV13, and one non-typable strain of pneumococci. These strains comprise 23 different capsular types/groups. A: Worldwide distribution of pediatric meningitis strains. Each continent is represented by data from one country except the European region where data from England/Wales and France are shown. The strains are listed according to those causing most to least meningitis post-PCV13 introduction. Because there were no published data from Australia, data from the Australian IPD Surveillance dataset were analyzed by enumerating the number of cases of pediatric meningitis caused by each serotype for the years 2012–2017 (post-PCV13 period). There were no data available from Japan that listed strains causing pediatric meningitis in children post-PCV13; hence, the data shown represent strains from the pre-PCV period that caused meningitis in Japan. Data from France, United Kingdom and Israel only provided information for non-PCV type strains. North America (United States), Africa (Burkina Faso) and Australia show both VT and NVT strains causing meningitis after the introduction of PCV13, while that from South America (Brazil) show both VT and NVT strains post-PCV10 introduction. Bolded strains represent NVT strains, while nonbolded strains are VT strains. The choice of these representative studies was based on study size and recent data. B: PCV type and non-PCV type strains that are reported to cause meningitis in the PCV era. In this figure, PCV7 and PCV13 strains are shown in green and purple circles, respectively. The brown circle shows non-PCV type strains reported to cause meningitis. The overlapping regions of the circles represent PCV type strains that have been reported to cause some meningitis post-PCV use. The serotype data shown here come from Figure 1A except that the data from Japan were excluded as that study reported only on serotypes causing meningitis in the pre-PCV era. NT represents pneumococci of unknown capsular type. VT indicates vaccine type. [full color online](#)

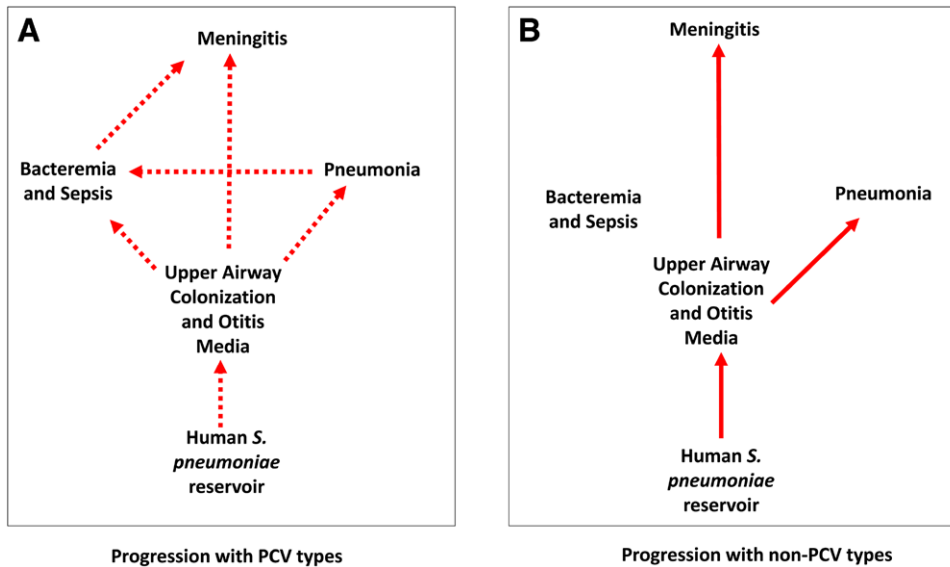


FIGURE 2. Model to explain our view of how pneumococcal meningitis largely escapes protection by PCV immunization. A: The dotted red arrows follow PCV capsule type strains from acquisition to their disease manifestations. They colonize the upper airway and can spread in some cases to the middle ear where they cause otitis media. From the upper airway, they can spread to the lung to cause pneumonia, which in some cases leads to detectable bacteremia and serious sepsis. In infants, they can also cause bacteremia without a primary focus of infection. The classic view has been that meningitis is the result of blood–brain transmission. This view is likely to be true in many/most cases for meningitis caused by PCV type strains because they are able to invade the blood. B: The non-PCV strains (solid red arrows) appear to be less likely to cause bacteremia, sepsis and complicated pneumonia than are the PCV strains. This finding is consistent with the view that these capsular structures are not compatible with survival in the blood. However, the non-PCV type strains are still able to cause pneumococcal pneumonia. The relative inability of these strains to cause bacteremia and sepsis even although they cause most of the meningitis in PCV immune populations strongly suggest that they reach the brain through a nonhematogenous route. In the text, we have summarized data from humans and animals supporting a route of invasion of the CNS that follows olfactory and auditory nerves into the brain. If non-PCV strains can reach the brain through a nonhematogenous route, then it would seem likely that pneumococci of many of the PCV capsular types could probably also reach the brain in this manner. CNS indicates central nervous system. [full color online](#)

by which pneumococci are able to invade the brain through the olfactory ensheathing cells.^{44,45} Macedo-Ramos et al⁴⁴ showed that pneumococci are able to suppress the immune function of olfactory ensheathing cells thus helping them evade the immune system and travel directly to the brain. Furthermore, it was shown that infection with *S. pneumoniae* potentially activated neurotropic factors that interfered with the activation of microglia, thereby allowing invasion of the central nervous system in the absence of bacteremia.⁴⁵

Given the evidence that meningitis likely requires prior colonization of the nasopharynx, a vaccine that eradicates carriage may be able to largely prevent pneumococcal meningitis.

STRATEGIES TO PREVENT PNEUMOCOCCAL MENINGITIS

Much of the pneumococcal meningitis that occurred post-PCVs was caused by capsular types, which could colonize, but generally failed to cause as much bacteremia, sepsis and complicated pneumonia as had the PCV strains in the past. Hence, the most effective strategy to significantly reduce rates of pneumococcal meningitis would be to greatly reduce carriage. There may be several ways to reduce or eliminate carriage. Because PCV use led to almost complete elimination of carriage by vaccine types, one possibility may be to continue to increase the number of capsular types in the conjugate vaccine until all types that can colonize and cause meningitis are covered. The problem with this approach is that because there are >98 different capsular types,⁴⁶ it might

become too cumbersome and expensive to include the majority of them in a vaccine. Moreover, present studies already reveal that as the number of polysaccharide antigens has increased, the antibody responses to each appear to decrease.^{47–49}

Another option, which has been widely proposed, may be to include broadly cross-reactive pneumococcal proteins in a vaccine to reduce rates of carriage. Proteins such as pneumococcal surface adhesin A, pneumococcal surface protein A, pneumococcal surface protein C, neuraminidase A, immunoglobulin A1 protease and possibly pneumolysin⁵⁰ (Fig. 3), all play roles in colonization in animals. Immunization with pneumococcal surface protein C, pneumococcal surface adhesin A, neuraminidase A, pneumococcal surface protein A, the pneumococcal histidine triad family of proteins, polyamine transport protein D and/or pneumococcal protective protein A are all effective against carriage in animal models.^{52,53} Furthermore, it has been shown that naturally acquired immunity to pneumococci in humans is to pneumococcal surface proteins rather than to capsular antigens.⁵⁴ Hence, the above-mentioned protein antigens and other potentially broadly cross-protective antigens might be used singly or in combination for an optimally effective vaccine against pneumococcal carriage and meningitis. Some worry that elimination of pneumococcal carriage might affect the respiratory biome in deleterious ways.⁵⁵ This seems unlikely to be a problem, since at least in developed countries, most citizens carry pneumococci only a minority of the time^{56,57} without harmful consequences. In animals, the strongest protection against carriage is elicited by mucosal

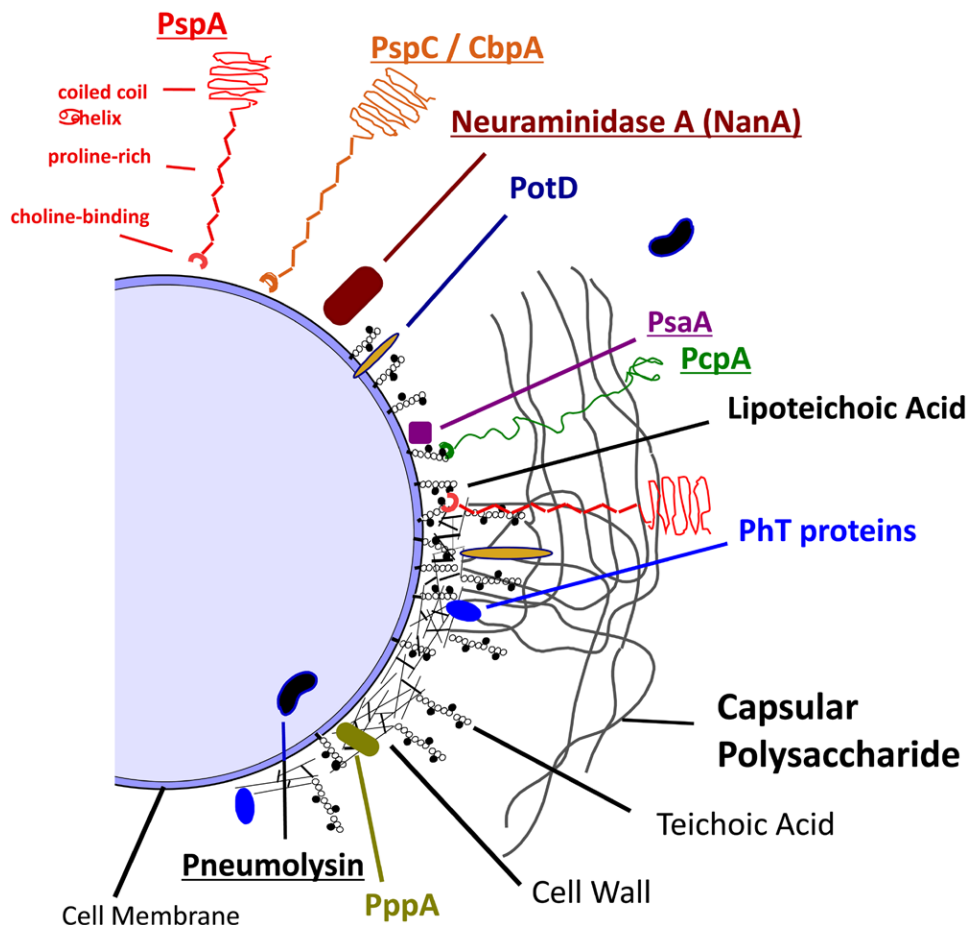


FIGURE 3. Model of the pneumococcal surface showing surface proteins that are being investigated as vaccine candidates. All of the molecules shown in color are proteins that have been reported to elicit protection against colonization. Pneumolysin has been reported in some cases to play a role in colonization, but its ability to elicit protection against colonization is not clear. Figure modified from Briles et al.⁵¹ NanA indicates neuraminidase A; CbpA, choline binding protein A; PcpA, pneumococcal choline binding protein A; Pht, pneumococcal histidine triad; PotD, polyamine transport protein D; pppA, pneumococcal protective protein A; PsaA, pneumococcal surface adhesin A; PspA, pneumococcal surface protein A; PspC, pneumococcal surface protein C. [full color online](#)

immunization of the upper airway.⁵⁸ The need to eliminate carriage might be avoided if an immunogenic pneumococcal molecule is identified that is essential for their transport by nerves.

Because PCVs are already available, human trials of the new vaccines would need to include the PCV with or without the protein antigen(s). Because efficacy testing could initially use as its endpoint, carriage of NVT strains, the initial trials could be relatively small. Eventually, tests to look at protection against a meningitis endpoint would be important but might be possible postlicensure if all individuals received PCV. Another strategy could be to vaccinate with the whole-cell vaccine, which has been shown to reduce rates of carriage in animals.^{53,59} However, concerns about injecting the whole organism coupled with a preference for purified products have slowed down its clinical development, although this would be an inexpensive strategy to save millions of lives worldwide. The multiple antigen vaccine which is prepared from pneumococcal lysates enriched for protein antigens and heat shock proteins has shown protection against pneumonia and sepsis in mouse models of infection. This vaccine strategy should be further tested for protection against carriage and ultimately meningitis.⁶⁰

Other options include prophylactic use of antibiotics that eliminate all carriage of pneumococci or the identification of

competitive flora that would take over the niche occupied by pneumococci in the human nasopharynx and eliminate pneumococcal carriage.⁶¹ These last 2 options, while promising, may have unintended consequences. For instance, the prophylactic use of antibiotics would likely lead to acquisition of antibiotic resistance in multiple common pathogens and could lead to problems with the microbiome. Despite these concerns, there is a need to investigate alternative strategies, given the known morbidity and mortality caused by pneumococcal meningitis. To assist the development of such protocols, it will be important to have epidemiologic studies of rates of colonization and meningitis with each capsular type in the same well-defined population, to identify which colonization types most efficiently cause meningitis.

CONCLUSIONS

Pneumococcal meningitis continues to cause morbidity and mortality among children and adults despite widespread use of PCVs in several countries around the globe. In those countries, pneumococcal meningitis is caused by non-PCV type strains that have occupied the niche created by the almost complete elimination of PCV type strains in the human nasopharynx. Because the PCVs result in a major reduction in bacteremia, sepsis and complicated pneumonia, it

is unlikely that the non-PCV type strains can generally survive well in the blood and therefore probably enter the brain through nonhematogenous routes. The high serotype diversity of these new replacement strains makes it problematic to expand the PCVs with enough capsular types to stem strain replacement and prevent the majority of pneumococcal meningitis. One way to prevent pneumococcal meningitis is to completely eradicate pneumococcal colonization. This might be best done with a vaccine that targets the important pneumococcal virulence factors essential for colonization.

ACKNOWLEDGMENTS

The authors dedicate this manuscript to the memory of Basudeb Mukherjee whose values encouraged many of the threads woven into it and Richard M. Krause for his commitment to public health as shown in this manuscript.

REFERENCES

- Thigpen MC, Whitney CG, Messonnier NE, et al; Emerging Infections Programs Network. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med*. 2011;364:2016-2025.
- Alexandre C, Dubos F, Courouble C, et al; Hospital Network for Evaluating the Management of Common Childhood Diseases. Rebound in the incidence of pneumococcal meningitis in northern France: effect of serotype replacement. *Acta Paediatr*. 2010;99:1686-1690.
- Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med*. 2009;360:244-256.
- Regev-Yochay G, Reisenberg K, Katzir M, et al. Pneumococcal meningitis in adults after introduction of PCV7 and PCV13, Israel, July 2009-June 2015(1). *Emerg Infect Dis*. 2018;24:1275-1284.
- Baraff LJ, Lee SI, Schriger DL. Outcomes of bacterial meningitis in children: a meta-analysis. *Pediatr Infect Dis J*. 1993;12:389-394.
- Pilishvili T, Lexau C, Farley MM, et al; Active Bacterial Core Surveillance/ Emerging Infections Program Network. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis*. 2010;201:32-41.
- Ouldali N, Levy C, Varon E, et al; French Pediatric Meningitis Network. Incidence of paediatric pneumococcal meningitis and emergence of new serotypes: a time-series analysis of a 16-year French national survey. *Lancet Infect Dis*. 2018;18:983-991.
- Ben-Shimol S, Givon-Lavi N, Grisaru-Soen G, et al; Israel Bacteremia and Meningitis Active Surveillance Group. Comparative incidence dynamics and serotypes of meningitis, bacteremic pneumonia and other-IPD in young children in the PCV era: Insights from Israeli surveillance studies. *Vaccine*. 2018;36:5477-5484.
- Regev-Yochay G, Paran Y, Bishara J, et al; IAIPD group. Early impact of PCV7/PCV13 sequential introduction to the national pediatric immunization plan, on adult invasive pneumococcal disease: a nationwide surveillance study. *Vaccine*. 2015;33:1135-1142.
- Kendall BA, Dascomb KK, Mehta RR, et al. Early Streptococcus pneumoniae serotype changes in Utah adults after the introduction of PCV13 in children. *Vaccine*. 2016;34:474-478.
- Kaplan SL, Barson WJ, Lin PL, et al. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2013;32:203-207.
- Hendricks-Muñoz KD, Shapiro DL. The role of the lumbar puncture in the admission sepsis evaluation of the premature infant. *J Perinatol*. 1990;10:60-64.
- Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet*. 2011;378:1962-1973.
- Fleming-Dutra KE, Conklin L, Loo JD, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type nasopharyngeal carriage. *Pediatr Infect Dis J*. 2014;33(suppl 2):S152-S160.
- Loo JD, Conklin L, Fleming-Dutra KE, et al. Systematic review of the indirect effect of pneumococcal conjugate vaccine dosing schedules on pneumococcal disease and colonization. *Pediatr Infect Dis J*. 2014;33(suppl 2):S161-S171.
- Galanis I, Lindstrand A, Darenberg J, et al. Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm, Sweden. *Eur Respir J*. 2016;47:1208-1218.
- Dube FS, Ramjith J, Gardner-Lubbe S, et al. Longitudinal characterization of nasopharyngeal colonization with Streptococcus pneumoniae in a South African birth cohort post 13-valent pneumococcal conjugate vaccine implementation. *Sci Rep*. 2018;8:12497.
- Richter SS, Diekema DJ, Heilmann KP, et al. Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-valent conjugate vaccine in the United States. *Antimicrob Agents Chemother*. 2014;58:6484-6489.
- Balsells E, Guillot L, Nair H, et al. Serotype distribution of Streptococcus pneumoniae causing invasive disease in children in the post-PCV era: a systematic review and meta-analysis. *PLoS One*. 2017;12:e0177113.
- Wahl B, O'Brien KL, Greenbaum A, et al. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000-15. *Lancet Glob Health*. 2018;6:e744-e757.
- O'Brien KL, Wolfson LJ, Watt JP, et al; Hib and Pneumococcal Global Burden of Disease Study Team. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *Lancet*. 2009;374:893-902.
- Lepoutre A, Varon E, Georges S, et al; Microbiologists of Epibac; ORP Networks. Impact of the pneumococcal conjugate vaccines on invasive pneumococcal disease in France, 2001-2012. *Vaccine*. 2015;33:359-366.
- Croney CM, Coats MT, Nahm MH, et al. PspA family distribution, unlike capsular serotype, remains unaltered following introduction of the heptavalent pneumococcal conjugate vaccine. *Clin Vaccine Immunol*. 2012;19:891-896.
- Levy C, Varon E, Picard C, et al. Trends of pneumococcal meningitis in children after introduction of the 13-valent pneumococcal conjugate vaccine in France. *Pediatr Infect Dis J*. 2014;33:1216-1221.
- Olarte L, Barson WJ, Barson RM, et al. Impact of the 13-valent pneumococcal conjugate vaccine on pneumococcal meningitis in US children. *Clin Infect Dis*. 2015;61:767-775.
- Brandileone MC, Almeida SCG, Minamisava R, et al. Distribution of invasive Streptococcus pneumoniae serotypes before and 5 years after the introduction of 10-valent pneumococcal conjugate vaccine in Brazil. *Vaccine*. 2018;36:2559-2566.
- Kambiré D, Soeters HM, Ouédraogo-Traoré R, et al; MenAfriNet Consortium. Early impact of 13-valent pneumococcal conjugate vaccine on pneumococcal meningitis-Burkina Faso, 2014-2015. *J Infect*. 2018;76:270-279.
- Hulten KG. The changing epidemiology of pneumococcal diseases. *Lancet Infect Dis*. 2018;18:929-930.
- Alari A, Chaussade H, Domenech De Cellès M, et al. Impact of pneumococcal conjugate vaccines on pneumococcal meningitis cases in France between 2001 and 2014: a time series analysis. *BMC Med*. 2016;14:211.
- Dando SJ, Mackay-Sim A, Norton R, et al. Pathogens penetrating the central nervous system: infection pathways and the cellular and molecular mechanisms of invasion. *Clin Microbiol Rev*. 2014;27:691-726.
- Berkley JA, Mwangi I, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM*. 1999;92:151-157.
- Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics*. 2006;117:1094-1100.
- Wiswell TE, Baumgart S, Gannon CM, et al. No lumbar puncture in the evaluation for early neonatal sepsis: will meningitis be missed? *Pediatrics*. 1995;95:803-806.
- Bell AH, Brown D, Halliday HL, et al. Meningitis in the newborn—a 14 year review. *Arch Dis Child*. 1989;64:873-874.
- Hack M, Horbar JD, Malloy MH, et al. Very low birth weight outcomes of the national institute of child health and human development neonatal network. *Pediatrics*. 1991;87:587-597.
- Hristeva L, Booy R, Bowler I, et al. Prospective surveillance of neonatal meningitis. *Arch Dis Child*. 1993;69(1 Spec No):14-18.
- Shattuck KE, Chonmaitree T. The changing spectrum of neonatal meningitis over a fifteen-year period. *Clin Pediatr (Phila)*. 1992;31:130-136.
- Tessin I, Trollfors B, Thiringer K. Incidence and etiology of neonatal septicemia and meningitis in western Sweden 1975-1986. *Acta Paediatr*. 1990;79:1023-1030.
- Visser VE, Hall RT. Lumbar puncture in the evaluation of suspected neonatal sepsis. *J Pediatr*. 1980;96:1063-1067.
- Rake G. The rapid invasion of the body through the olfactory mucosa. *J Exp Med*. 1937;65:303-315.

41. van Ginkel FW, McGhee JR, Watt JM, et al. Pneumococcal carriage results in ganglioside-mediated olfactory tissue infection. *Proc Natl Acad Sci U S A*. 2003;100:14363–14367.
42. Hatcher BL, Hale JY, Briles DE. Free sialic acid acts as a signal that promotes streptococcus pneumoniae invasion of nasal tissue and nonhematogenous invasion of the central nervous system. *Infect Immun*. 2016;84:2607–2615.
43. Marra A, Brigham D. Streptococcus pneumoniae causes experimental meningitis following intranasal and otitis media infections via a nonhematogenous route. *Infect Immun*. 2001;69:7318–7325.
44. Macedo-Ramos H, Ruiz-Mendoza S, Mariante RM, et al. Streptococcus pneumoniae resists intracellular killing by olfactory ensheathing cells but not by microglia. *Sci Rep*. 2016;6:36813.
45. Ruiz-Mendoza S, Macedo-Ramos H, Santos FA, et al. Streptococcus pneumoniae infection regulates expression of neurotrophic factors in the olfactory bulb and cultured olfactory ensheathing cells. *Neuroscience*. 2016;317:149–161.
46. Geno KA, Saad JS, Nahm MH. Discovery of novel pneumococcal serotype 35D, a natural WciG-deficient variant of serotype 35B. *J Clin Microbiol*. 2017;55:1416–1425.
47. Andrews NJ, Waight PA, Burbidge P, et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. *Lancet Infect Dis*. 2014;14:839–846.
48. Weinberger R, van der Linden M, Imöhl M, et al. Vaccine effectiveness of PCV13 in a 3+1 vaccination schedule. *Vaccine*. 2016;34:2062–2065.
49. Spijkerman J, Veenhoven RH, Wijmenga-Monsuur AJ, et al. Immunogenicity of 13-valent pneumococcal conjugate vaccine administered according to 4 different primary immunization schedules in infants: a randomized clinical trial. *JAMA*. 2013;310:930–937.
50. Hotomi M, Yuasa J, Briles DE, et al. Pneumolysin plays a key role at the initial step of establishing pneumococcal nasal colonization. *Folia Microbiol (Praha)*. 2016;61:375–383.
51. Briles DE, Tart RC, Swiatlo E, et al. Pneumococcal diversity: considerations for new vaccine strategies with emphasis on pneumococcal surface protein A (PspA). *Clin Microbiol Rev*. 1998;11:645–657.
52. Darrieux M, Goulart C, Briles D, et al. Current status and perspectives on protein-based pneumococcal vaccines. *Crit Rev Microbiol*. 2015;41:190–200.
53. Miyaji EN, Oliveira ML, Carvalho E, et al. Serotype-independent pneumococcal vaccines. *Cell Mol Life Sci*. 2013;70:3303–3326.
54. Wilson R, Cohen JM, Reglinski M, et al. Naturally acquired human immunity to pneumococcus is dependent on antibody to protein antigens. *PLoS Pathog*. 2017;13:e1006137.
55. McDaniel LS, Swiatlo E. Should pneumococcal vaccines eliminate nasopharyngeal colonization? *MBio*. 2016;7:e00545–16.
56. Zhou JY, Isaacson-Schmid M, Uttersson EC, et al. Prevalence of nasopharyngeal pneumococcal colonization in children and antimicrobial susceptibility profiles of carriage isolates. *Int J Infect Dis*. 2015;39:50–52.
57. Hussain M, Melegaro A, Pebody RG, et al. A longitudinal household study of Streptococcus pneumoniae nasopharyngeal carriage in a UK setting. *Epidemiol Infect*. 2005;133:891–898.
58. Kong IG, Sato A, Yuki Y, et al. Nanogel-based PspA intranasal vaccine prevents invasive disease and nasal colonization by Streptococcus pneumoniae. *Infect Immun*. 2013;81:1625–1634.
59. Lu YJ, Leite L, Gonçalves VM, et al. GMP-grade pneumococcal whole-cell vaccine injected subcutaneously protects mice from nasopharyngeal colonization and fatal aspiration-sepsis. *Vaccine*. 2010;28:7468–7475.
60. Chan WY, Entwisle C, Ercoli G, et al. A novel multiple-antigen pneumococcal vaccine protects against lethal Streptococcus pneumoniae challenge. *Infect Immun*. 2019;87:e00846–18.
61. Bernstein JM, Haase E, Hasse E, et al. Bacterial interference of penicillin-sensitive and -resistant Streptococcus pneumoniae by Streptococcus oralis in an adenoid organ culture: implications for the treatment of recurrent upper respiratory tract infections in children and adults. *Ann Otol Rhinol Laryngol*. 2006;115:350–356.