Original Report

Serotypes of respiratory tract isolates of *Streptococcus pneumoniae* from Jamaican children

Upton D. Allen,[1] Sonia Thomas,[2] Jonathan Carapetis,[1,3] Sonia Henry,[2] Samia Wasfy,[1] Marguerite Lovgren,[4] Susan Richardson[1] and Donald E. Low[5]

Background: Data are lacking on the pneumococcal serotypes present in many developing regions, including the Caribbean. We examined the serotypes of nasopharyngeal (NP) isolates of pneumococci obtained from Jamaican children.

Methods: We obtained NP samples from children seen in the Emergency Department at the Bustamante Children's Hospital. The samples were transported to Canada for isolation and serotyping of pneumococci.

Results: We obtained 94 isolates from 276 children; median age 3.4 years. The majority (57%) had symptoms of acute respiratory infection at the time of sampling. The main serotypes carried were 6B (20.5%), 19F (14.5%), and 14 (8.4%). Non-typable isolates accounted for 10.8% of the isolates. Fifty-nine per cent of the serotypes were present among the 11 being considered for candidate pneumococcal conjugate vaccines (95% CI 48–70%); the corresponding proportion present in the recently licensed 7-valent vaccine was 57% (95% CI 45–67%). A significant proportion of the serotypes found is absent from those to be included in future conjugate vaccines ($P<0.0001$; reference=85% expected serotype representation). Less than 5% of isolates were non-susceptible to penicillin (3.2%), cefotaxime–ceftriaxone (3.2%) and cefuroxime (3.2%), while 8.4% and 1.1% of isolates were resistant to trimethoprim–sulfamethoxazole and erythromycin respectively. There were three isolates with resistance to two or more classes of drug. These isolates were all resistant to penicillin (MIC 2 μg/mL); the serotypes were 14, 23F, and 19F.

Conclusion: A significant proportion of the serotypes found is absent from those to be included in future conjugate vaccines.

Int J Infect Dis 2003; 7: 29–34

INTRODUCTION

Diseases due to *Streptococcus pneumoniae* (pneumococci) are of significant public health concern globally.[1] Each year, more than one million children less than 5 years of age die of pneumococcal diseases, and most of these deaths are in developing countries.[1] Among countries where the majority of the inhabitants are of African ancestry, the problem posed by *S. pneumoniae* is even greater, due to the relatively high prevalence of sickle-cell disease, a condition associated with significant morbidity and mortality from pnemococcoccal sepsis.[2]

It has been determined that the acquisition and carriage of *S. pneumoniae* are associated with the occurrence of acute otitis media,[3–5] bacteremia,[6–8] and pneumonia.[9] Knowledge of the prevalent serotypes and their association with antibiotic resistance is important for devising strategies to prevent and treat pneumococcal infections. In this context, the concern regarding drug-resistant *S. pneumoniae* has highlighted the importance of pneumococcal vaccination.[10] However, for many developing countries, data are lacking on the serotypes associated with infection in young children. Although sterile site isolates of *S. pneumoniae* provide a better means of evaluating disease-causing serotypes present in these countries, obtaining such isolates is often difficult. Evaluation of the serotypes associated with nasopharyngeal colonization provides some insight into the circulating serotypes, including those associated with antibiotic resistance.[10]

In the above context, the objectives of this study were to evaluate the distribution of serotypes associated with nasopharyngeal colonization among a population of Jamaican children, and to document the prevalence of drug resistance among the serotypes obtained.

[1]Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; [2]Bustamante Children's Hospital, Kingston, Jamaica; [3]Department of Paediatrics, Royal Children's Hospital, University of Melbourne, Melbourne, Australia; [4]National Centre for Streptococcus, Edmonton, Alberta, Canada; [5]Toronto Medical Laboratories and Mount Sinai Hospital, Department of Microbiology, University of Toronto, Toronto, Ontario, Canada.

Presented in part at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada. Supported in part by a grant from the Canadian Bacterial Diseases Network.

Address correspondence to Dr Upton D. Allen, Division of Infectious Diseases, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada. E-mail: upton.allen@ sickkids.ca

Corresponding Editor: Patricia Muñoz, Madrid, Spain

METHODS

Setting

The study was conducted at the Emergency Department of the Bustamante Children's Hospital, Kingston, Jamaica. This is the largest pediatric hospital in the English-speaking Caribbean. It is located in the nation's capital, Kingston, which has a population of over 250 000 people. The total population of Jamaica is approximately 2.2 million; the majority are of African descent. The patient population consists primarily of children less than 10 years of age who predominantly belong to a lower socioeconomic group. The vast majority of the children seen are from inner city urban areas.

Design and eligibility criteria

This was a point prevalence study that targeted all children presenting to the Emergency Department over a 5-day period in June 1999. Following verbal informed consent, a short questionnaire was administered to the accompanying caregiver. The information obtained included demographic data, reason(s) for the visit to hospital, underlying diseases, and current or recent antibiotic use.

Sample collection and laboratory processing

A sterile Dacron polyester-tipped swab (Hardwood Products Company, Guilford, ME, USA) was used to obtain a nasopharyngeal sample from each child. The swabs were taken by one of two physicians, immediately placed into skim milk–glucose-glycerol broth,[11] and frozen within 1–2 h at approximately −10°C.). The samples were taken to Toronto on dry ice and kept frozen at −70°C prior to laboratory processing. *S. pneumoniae* was isolated using standard laboratory techniques.[12] Samples were plated onto 5% sheep blood agar, and incubated in CO_2 at 35°C for 18–24 h. *S. pneumoniae* was identified by α-hemolysis, colony morphology, optochin susceptibility, and bile solubility.[12] In selecting colonies of pneumococci, three to five colonies were picked up for identification.

Susceptibility testing was performed by broth microdilution according to guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS), and interpretation was based on NCCLS breakpoints (1999).[13,14] In this regard, the breakpoints and interpretative standards for penicillin were as follows: ≤0.06 μg/mL=sensitive; 0.12–1 μg/mL=intermediate; and ≥2 μg/mL=resistant (non-susceptible=intermediate or resistant; susceptible=sensitive). In addition to penicillin, other antimicrobials tested included cefotaxime–ceftriaxone, erythromycin, doxycycline, trimethoprim–sulfamethoxazole, and chloramphenicol. Serotyping based on the Quellung reaction[15] using commercial antisera obtained from the Statens Seruminstitut, Copenhagen, Denmark was performed at the National Centre for Streptococcus, Edmonton, Canada.

Statistical analyses

Data management was facilitated by Epi Info software[16] using descriptive analyses. The one-sample test of binomial proportions was used to determine confidence intervals around point estimates and to compare the serotypes present among the study population with those covered by current pneumococcal conjugate vaccines.

RESULTS

Nasopharyngeal isolates were obtained from 276 children with a median age of 3.4 years (range 0.1–17.7 years). The male/female ratio was 1.1:1. Symptoms of acute respiratory tract infection were present in 57.4% of the subjects. Among 274 subjects with adequate information, 24.8% indicated that they had received antibiotics in the previous month. The single most frequent reason for the hospital visit was hyperreactive airway disease (14.3% of subjects). Less than 0.5% (1/276) had overt evidence of malnutrition, while 0.7% (2/276) had sickle-cell disease.

Ninety-four isolates of *S. pneumoniae* were obtained from the 276 children (34% carriage). These 94 isolates were from 93 different children. Eighty-three isolates were viable for serotyping. The relative frequencies of the most common serotypes were: 6B, 20.5%; 19F, 14.5%; 14, 8.4%; 23F, 7.2%; 6A, 7.2%; 16F, 4.8%; and 19A, 4.8%. One subject carried two serotypes (19F and non-typable). Figure 1 shows the distribution of the various serotypes, including 10.8% of the isolates that were non-typable.

We examined the proportion of the isolates represented in candidate 7- and 11-valent conjugate vaccines. Fifty-nine per cent of the serotypes were present among the 11 being considered for candidate pneumococcal conjugate vaccines (95% CI 48–70%). The proportion covered by the recently licensed 7-valent vaccine was 57% (95% CI 45–67%). A significant proportion of the serotypes found is absent from those to be included in future conjugate vaccines ($P<0.0001$; reference=85% expected serotype representation).

Susceptibility testing was performed on 94 isolates. Less than 5% of isolates were non-susceptible to penicillin (3.2%), cefotaxime–ceftriaxone, and cefuroxime (3.2%), while 8.4% of isolates were resistant to trimethoprim–sulfamethoxazole. Erythromycin resistance was low (1.1%). No isolates were resistant to doxycycline, while 2.1% were resistant to chloramphenicol. Three isolates showed resistance to two or more classes of drug. The penicillin minimum inhibitory concentration for each of these three isolates was 2 μg/mL). The serotypes of the above three isolates were 14, 23F, and 19F, respectively. While the numbers of resistant isolates were low, there was no indication that

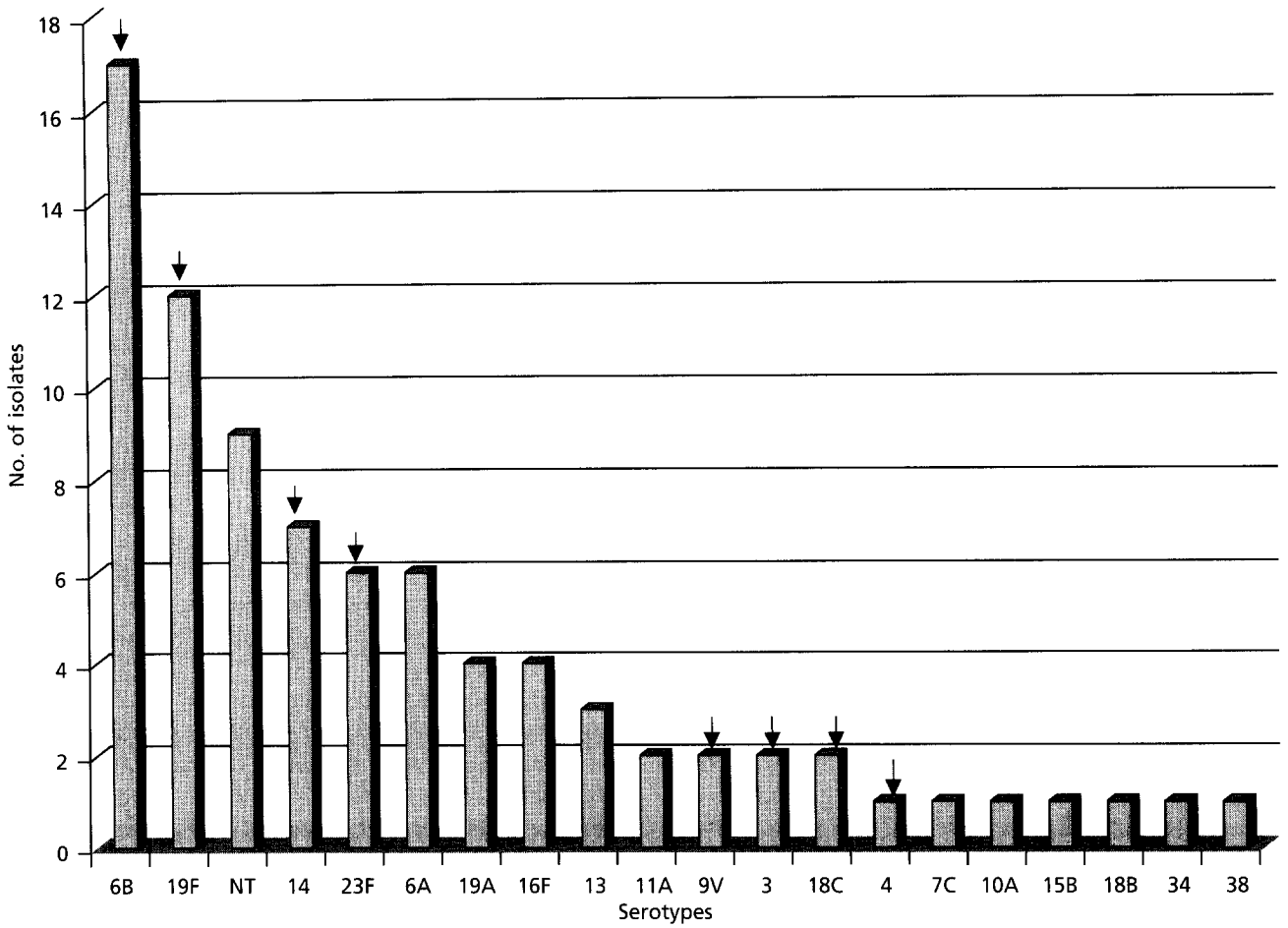

Figure 1. Serotypes of nasopharyngeal isolates of pneumococci—Jamaican children, 1999. Serotypes contained in the 7- and 11-valent conjugate vaccines are indicated by an arrow. NT denotes non-typable isolates.

current or recent antibiotic use was associated with carriage of such resistant isolates.

DISCUSSION

Invasive pneumococcal infections in North American children are most often caused by serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.[17] Serotypes 6B, 9V, 14, 19A, 19F and 23F are the isolates that are most frequently associated with resistance to penicillin.[18,19] Our results provide data on the serotypes associated with nasopharyngeal carriage in a group of Caribbean children. The main isolates were virtually identical to those identified in a similar study involving children attending pediatric practices in the private sector in Johannesburg,[20] where the predominant serotypes were 6B, 19F, 6A, 23F, 14, and 19A, compared with 6B, 19F, 14, 23F, 6A, 16F and 19A in the Caribbean study.

The serotypes present in 7-valent vaccines include 4, 6B, 9V, 14, 18C, 19F, and 23F.[21] The 11-valent formulation also includes types 1, 3, 5, and 7F.[22] In the developed countries, an 11-valent pneumococcal conjugate vaccine is expected to cover up to 85% of invasive isolates in young children.[22] While many of the serotypes that were identified in our study are included in candidate pneumococcal vaccines, a significant proportion is absent. This concurs with studies from other regions of the developing world, where in some cases less than two-thirds of carriage isolates belong to the serotypes included in current 7-valent or 9-valent vaccines.[23,24] The relevance of this would depend on the extent to which the isolates that we identified were associated with antibiotic resistance or diseases such as otitis media, meningitis, pneumonia, and bacteremia.

In the above context, with respect to the serotypes associated with specific diseases, additional studies are needed to address this issue in many developing regions of the world. Notwithstanding this, it has been shown that the vast majority of children with acute lower respiratory tract infection and pneumococcal bacteremia carry the same pneumococcal serotypes in their oropharynx.[25] Dagan and others have determined that, at least for acute otitis media, carriage of resistant *S. pneumoniae* in the community correlates with disease caused by the resistant strains.[10] With the exception of non-typable isolates, it is likely that the serotypes identified in our study reflect a significant proportion of those associated with pneumococcal disease.

With respect to the serotypes associated with resistance in the population studied, our study provides important preliminary data. The level of penicillin non-susceptibility among the serotypes was less than 5%. In

addition, the level of macrolide resistance was low. In 1993–94 (November to March), we conducted a survey in the same target population at the same institution, and found that the level of penicillin resistance was less than 5%.[26] However, in 1995 (summer) we observed that the level of resistance was just under 20%. In the 1993–94 and 1995 surveys, no macrolide-resistant isolates were observed. Seasonal differences in the proportion of resistant isolates would not explain these results, as the 1995 and 1999 isolates were collected during identical seasons (summer months). With respect to the level of resistance detected in 1995, we postulate that there may have been circulating clones of specific serotypes associated with resistance. In the current survey, we found that the multiresistant isolates belonged to serotypes 14, 23F, and 19F. This is consistent with the serotypes that are known to be most frequently associated with resistance.[18,19]

Overall, the level of pneumococcal antibiotic resistance is at the lower end of the range of prevalence documented worldwide.[27–31] With respect to developing countries in the region of the Americas, Kertesz et al obtained invasive pneumococcal isolates from six Latin American countries (Argentina, Brazil, Chile, Columbia, Mexico, and Uruguay).[28] Overall, 25% of isolates had diminished susceptibility to penicillin, with 8.3% showing high-level resistance. While it is well established that prior antibiotic use selects for carriage of resistant strains of *S. pneumoniae*,[31–43] it is unclear whether the level of resistance observed in our study is due to less antibiotic pressure in the study population compared with other regions. Over-the-counter antibiotics are not available in Jamaica. It is also possible that Jamaica may not yet have some of the major resistant clones that have become prevalent in some countries.

The pneumococcal conjugate vaccine, like the *Haemophilus influenzae*-type b conjugate vaccine, has been shown to decrease carriage of specific serotypes in the vaccine, including resistant serotypes.[41] Consequently, a vaccine that is effective against the limited number of serotypes associated with antibiotic resistance may decrease the prevalence of infections caused by antibiotic-resistant pneumococci. It is tempting to speculate that, at this time, there is a window of maximum benefit to be derived from the use of the conjugate vaccine in regions that currently have a low level of antibiotic resistance. In this way, the goal would be to see the conjugate vaccine as an important component of a strategy to limit the emergence and spread of antibiotic resistance; a strategy that would include measures to promote judicious antibiotic use. However, it should be noted that there are data showing that the reduction in number of serotypes following the use of the conjugate vaccine may be followed by replacement with other serotypes not included in the vaccine.[42] It is unknown whether the latter serotypes will become the ones predominantly responsible for disease and resistance in the future.[10]

We acknowledge the fact that the data presented in this study were collected over a relatively short period of time. However, the data are preliminary, and represent the only such data available from the target population at this point in time. Additional data will be obtained from a wider cross-section of children. Such data will include isolates obtained from sterile sites.

Our data support the need for the further development of pneumococcal vaccination, including pneumococcal protein vaccines. Proteins that are virulence factors for pneumococci have been identified. These include pneumococcal surface protein A (PspA), pneumococcal surface adhesion A protein (PsaA), and pneumolysin.[44,45] These proteins are being evaluated in experimental models singly and in combination.[44,45] It is possible that, in the future, the inclusion of these proteins in polysaccharide–protein conjugate vaccines will result in enhanced efficacy of the vaccines against pneumococcal disease and offer coverage against serotypes that are not included in the current pneumococcal vaccines.

In summary, we have generated baseline data on the circulating serotypes that are carried, including those that are associated with antibiotic resistance in a population of Jamaican children before the advent of the conjugate pneumococcal vaccine in that region. The occurrence of antibiotic-resistant serotypes in this study sample is relatively low. There is a significant proportion of serotypes present that are not included in current candidate pneumococcal conjugate vaccines. However, if the isolates identified are in concordance with disease-causing strains, the data suggest that the current 7-valent vaccine would provide similar coverage to an 11-valent formation.

ACKNOWLEDGEMENTS

We thank the many parents and children who participated. The study would not have been possible without the support of the many physicians and nurses at the Bustamante Children's Hospital, Jamaica. We give special thanks to Mrs Marlene Gris, Ms Corazon Kim, Ms Marguerita Castro, and Ms Fern Parisian, Hospital for Sick Children, Toronto.

REFERENCES

1. Anonymous. Pneumococcal vaccines: WHO position paper. Weekly Epidemiol Rec 1999; 74:177–183.
2. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, ed. 2000 Red Book: Report of the Committee on Infectious Diseases, 25th edn. Elk Grove Village, IL: American Academy of Pediatrics; 2000:452–460.
3. Long SS, Henretig FR, Teter MJ, McGowen KL. Nasopharyngeal flora and acute otitis media. Infect Immun 1983; 41:987–991.
4. Faden H, Duffy L, Wasielewski R, et al. Relationship between nasopharyngeal colonization and the develop-

ment of otitis media in children. J Infect Dis 1997; 175: 1440–1445.
5. Zenni MK, Cheatham SH, Thompson JM, et al. *Streptococcus pneumoniae* colonization in the young child: association with otitis media and resistance to penicillin. J Pediatr 1995; 127:533–537.
6. Matsro TD, Ghafoor A, Nomani NK, et al. Antimicrobial resistance of pneumococci in children with acute respiratory tract infection in Pakistan. Lancet 1991; 337:156–159.
7. Lehmann D, Gratten M, Montgomery J. Susceptibility of pneumococcal carriage isolates to penicillin provides a conservative estimate of susceptibility of invasive pneumococci. Pediatr Infect Dis J 1997; 16:297–305.
8. Lloyd-Evans N, O'Dempsey TJD, Baldeh I, et al. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. Pediatr Infect Dis J 1996; 15:866–871.
9. Gray BM, Converse GM III, Dillon HC, Jr. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage and infection during the first 24 months of life. J Infect Dis 1980; 142:923–933.
10. Dagan R, Fraser D. Conjugate pneumococcal vaccine and antibiotic-resistant *Streptococcus pneumoniae:* herd immunity and reduction of otitis media. Pediatr Infect Dis J 2000; 19:S79–S88.
11. Leech AJ, Shelby-James TM, Mayo M, et al. A prospective study of the impact of community-based azithromycin treatment of trachoma on carriage and resistance of *Streptococcus pneumoniae*. Clin Infect Dis 1997; 24:356–362.
12. Ruoff KL, Whiley RA, Beighton D, et al. Streptococcus. In: Murray PR, ed. Manual of clinical microbiology, 7th edn. Washington, DC: ASM Press, 1996:283–296.
13. National Committee for Clinical Laboratory Standards. Methods for dilution susceptibility tests for bacteria that grow anaerobically. Approved standard, 5th edn. NCCLS document M7-A4. Wayne PA: NCCLS, 2000.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. NCCLS document M100-S9, Vol. 19, No. 1. Wayne, PA: NCCLS, 1999:90–91.
15. Facklam RR, Washington JA II. Streptococcus and related catalase-negative Gram positive cocci. In: Balows A, Hausler WJ Jr, Herrmann KL, Isenberg HD, Shadom HJ, eds. Manual of clinical microbiology, 5th edn. Washington: American Society for Microbiology, 1991:238–257.
16. Centers for Disease Control and Prevention. Epi Info, Version 6. Atlanta, GA: CDC, 1994.
17. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, ed. 2000 Redbook. Report of the Committee on Infectious Diseases, 25th edn. Elk Grove Village, IL: American Academy of Pediatrics, 2000:452–460.
18. Butler JC, Hofmann J, Cetron MS, et al. The continued emergence of drug-resistant *Streptococcus pneumoniae* in the United States: an update from the Centers for Disease Control and Prevention's pneumococcal sentinel surveillance system. J Infect Dis 1996; 174:986–993.
19. Centers for Disease Control and Prevention. Defining the public health impact of drug-resistant *Streptococcus pneumoniae:* report of a working group. MMWR 1996; 45(RR-1):1–20.
20. Heubner RE, Wasas AD, Klugman KP, Pediatric Study Group. Prevalence of nasopharyngeal antibiotic-resistant pneumococcal carriage in children attending private paediatric practices in Johannesburg. South Afric Med J 2000; 90:1116–1121.
21. Fedson DS. Pneumococcal conjugate vaccination for adults: why it's important for children. Pediatr Infect Dis J 2000; 19:183–186.
22. Rubin LG. Pneumococcal vaccine. Pediatr Clin North Am 2000; 47:269–285.
23. Soewignjo S, Gessner BD, Sutanto A, et al. *Streptococcus pneumoniae* nasopharyngeal carriage prevalence, serotype distribution, and resistance patterns among children on Lombok Island, Indonesia. Clin Infect Dis 2001; 32:1039–1043.
24. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis 2000; 30:100–121.
25. Mastro TD, Nasreen KN, Ishaq Z, et al. Use of nasopharyngeal isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* from children in Pakistan for surveillance for antimicrobial resistance. Pediatr Infect Dis J 1993; 12:824–830.
26. Allen U, Johnson B, Henry S, Fuite L, Chan F. Antibiotic susceptibility of pneumococcal isolates among Jamaican children [Abstract E26]. In: Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco. Washington, DC: American Society for Microbiology, 1995.
27. Whitney CG, Fraley MM, Haldler J, et al. Increasing prevalence of multidrug resistant *Streptococcus pneumoniae* in the United States. N Engl J Med 2000; 343:1917–1927.
28. Kertesz DA, Di Fabio JL, de Cunto Brandileone MC, et al. Invasive *Streptococcus pneumoniae* infection in Latin American children; results of the Pan American Health Organization Surveillance study. Clin Infect Dis 1998; 26: 1355–1361.
29. Kellner JD, Ford-Jones EL. *Streptococcus pneumoniae* carriage in children attending 59 Canadian children care centers. Toronto Child Care Centre Study Group. Arch Pediatr Adolesc Med 1999; 153:495–502.
30. Skull S, Shelby-James T, Morris P, et al. *Streptococcus pneumoniae* antibiotic resistance in Northern Territory children in day care. J Pediatr Child Health 1999; 35:466–471.
31. Geslin P, Buu-Hoi A, Fremaux A, Acar JF. Antimicrobial resistance in *Streptococcus pneumoniae:* an epidemiological survey in France, 1970–1990. Clin Infect Dis 1992; 15:95–98.
32. Zenni MK, Cheatham SH, Thompson JM, et al. *Streptococcus pneumoniae* colonization in the young child: association with otitis media and resistance to penicillin. J Pediatr 1995; 127:533–537.
33. Dagan R, Melamed R, Muallem M, Piglansky L, Yagupsky P. Nasopharyngeal colonization in southern Israel with antibiotic-resistant pneumococci during the first 2 years of life: relation to serotypes likely to be included in the pneumococcal conjugate vaccines. J Infect Dis 1996; 174: 1352–1355.
34. Dagan R, Leibovitz E, Leiberman A, Yagupsky P. Clinical significance of antibiotic resistance in acute otitis media and implication of antibiotic treatment on carriage and spread of resistant organisms. Pediatr Infect Dis J 2000; 19:S57–S65.
35. Klugman KP, Koornof HJ, Kuhnle V. Clinical and nasopharyngeal isolates of unusual multiply resistant pneumococci. Am J Child 1986; 140:1186–1190.

36. Arason VA, Kristinsson KA, Sigurdsson JA, Stefamsddottir G, Molstad S, Gudmundsson S. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross-sectional prevalence study. BMJ 1996; 313: 387–391.
37. Brook I, Gober AE. Prophylaxis with amoxicillin or sulfisoxazole for otitis media: effect on the recovery of penicillin-resistant bacteria from children. Clin Infect Dis 1996; 22:143–145.
38. Abdel-Haq N, Abuhammour W, Asmar B, Thomas R, Dabbagh S, Gonzalez R. Nasopharyngeal colonization with *Streptococcus pneumoniae* in children receiving trimethoprim–sulfamethoxazole prophylaxis. Pediatr Infect Dis J 1999; 18:647–649.
39. Dagan R, Leibovitz E, Greenberg D, Yagupsky P, Fliss DM, Leiberman A. Dynamics of pneumococcal nasopharyngeal colonization during the first days of antibiotic treatment in pediatric patients. Pediatr Infect Dis J 1998; 17:880–885.
40. Melander E, Molstad S, Persson K, Hansson HB, Soderstrom M, Ekdahl K. Previous antibiotic consumption and other risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae* in children. Eur J Clin Microbiol Infect Dis 1990; 17:834–838.
41. Dagan R, Givon N, Yagpusky P, et al. Effect of a 9-valent pneumococcal vaccine on nasopharyngeal carriage of vaccine type and non-vaccine type *S. pneumoniae* strains among day care center attendees [Abstract G-52]. In: Program and Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego. Washington, DC: American Society for Microbiology, 1998.
42. Mbelle N, Huebner RE, Wasas AD, et al. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. J Infect Dis 1999; 180: 1171–1176.
43. Obarro SK, Adegbola RA, Banya WA, Greenwood BM. Carriage of pneumococci after pneumococcal vaccination [Letter]. Lancet 1996; 348:271.
44. Giebink GS. The prevention of pneumococcal disease in children. N Engl J Med 2001; 345:1177–1183.
45. Briles DE, Hollingshead SK, Nabors GS, Paton JC, Brooks-Walter A. The potential for using protein vaccines to protect against otitis media caused by Streptococcus pneumoniae. Vaccine 2000; 19(suppl 1):S87–S95.