Epidemiol. Infect. (2002), **128**, 21–27. © 2002 Cambridge University Press
DOI: 10.1017/S0950268801006331 Printed in the United Kingdom

Non-invasive pneumococcal disease and antimicrobial resistance: vaccine implications

M. H. KYAW[1,3]*, S. CLARKE[2], I. G. JONES[3] AND H. CAMPBELL[1]

[1] *University of Edinburgh, Public Health Sciences, Edinburgh*
[2] *Scottish Meningococcus and Pneumococcus Reference Laboratory, Glasgow*
[3] *Scottish Centre for Infection and Environmental Health, Glasgow*

(*Accepted 30 September 2001*)

SUMMARY

We reviewed laboratory data on non-invasive pneumococcal isolates reported from all diagnostic laboratories in Scotland during the period 1988–99. Of 4491 isolates from hospitalized patients, 654 (64·7%) were from sputum, 79 (7·8%) from the nasopharynx and 278 (27·5%) from other superficial sites. The serogroups included in the 23-valent polysaccharide vaccine caused 96–9% of all non-invasive disease in all age groups. The 7-, 9-, and 11-valent conjugated vaccine serogroups were responsible for 87–94%, 85–93%, 74–81% and 75–84% of non-invasive disease respectively in age groups < 2 years, $\leqslant 5$ years, $\geqslant 65$ years and all ages. The coverage of non-susceptible penicillin and erythromycin non-invasive isolates was $> 99\%$ and $> 95\%$ with the 23-valent polysaccharide and 7–11-valent conjugate vaccines respectively. The eight most common serogroups were 23, 9, 6, 19, 14, 3, 15 and 11 (in descending order). The serogroups associated with antimicrobial resistance in non-invasive disease were similar to those found in invasive disease. The finding of a similar serogroup distribution in both invasive and non-invasive disease (regardless of the site of clinical isolate), is consistent with serogroups colonizing non-sterile sites and having the potential to invade. The availability of conjugated vaccines reinforces the importance of systematic surveillance to determine accurately and regularly the coverage of pneumococcal serogroups and types causing both invasive and non-invasive disease.

INTRODUCTION

The pneumococcus is a major pathogen in the young and the elderly and in those with underlying chronic medical disorders [1]. In addition, it is a leading cause of non-invasive diseases such as community-acquired pneumonia [2] and otitis media [3], and can be associated with considerable morbidity and economic burden [4]. It poses an important public health problem in the UK.

Although there are 90 known serotypes of pneumococci, the majority of pneumococcal disease is associated with fewer than 11 serotypes in infants and children and fewer than 23 serotypes in adults [5–7]. Colonization with pneumococcal serogroups 6, 14, 19 and 23 is most common in young children and adults [8–10]. These serogroups are also the dominant cause of disease and antibiotic resistance worldwide [11–14]. The available evidence shows that pneumococcal conjugated vaccines induce mucosal immunity [15]. Studies in South Africa and Israel showed a reduction in carriage of vaccine-related pneumococcal serotypes, especially antibiotic resistant strains [16, 17]. Thus, widespread use of pneumococcal conjugate vaccine could limit the spread of pneumococci and decrease the prevalence of antibiotic resistant strains.

Knowledge of the coverage of non-invasive sero-

* Author for correspondence: Scottish Centre for Infection and Environmental Health, Clifton House, Clifton Place, Glasgow, G3 7LN.

groups for 7–11-valent conjugated vaccines and the 23-valent pneumococcal polysaccharide vaccine in various age groups and from different clinical sites is important for vaccine formulation and recommendations for usage. In addition, data on the distribution of pneumococcal serogroups isolated from non-sterile site specimens may help to determine which serogroups are important in the development of invasive disease. We report here the prevalence of the major serogroups causing non-invasive disease and the coverage of polysaccharide and conjugated vaccines for non-invasive pneumococcal serogroups from population-based laboratory surveillance in Scotland, during the period 1988–99.

METHODS

Data from this study were obtained from the Scottish Centre for Infection and Environmental Health (SCIEH) and the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL). Both organizations provide the national surveillance and monitoring for diseases caused by these organisms in Scotland (estimated 5 million population). The establishment of SMPRL in 1992 has improved the data on both invasive and non-invasive pneumococcal disease, particularly for serogroup/type distribution and antimicrobial resistance in Scotland. Although SMPRL encourages all diagnostic laboratories to send isolates from body fluid of patients with suspected pneumococcal infection, it is likely that some laboratories may refer isolates thought to be antimicrobial resistant. Enhanced pneumococcal surveillance is currently underway in Scotland and will improve population-based data on this disease before and after the implementation of any pneumococcal conjugate vaccine strategy. Detailed background information and methods of this study have been described in a previous report [18]. This earlier study evaluated the prevalence of antibiotic resistance, serogroup/type distribution, and coverage of polysaccharide and conjugate pneumococcal vaccines related-serotypes/groups for invasive isolates. This report encompasses isolates from non-sterile sites from patients who were admitted to hospital mainly with acute respiratory infections and who were diagnosed as having pneumococcal infection. Serogrouping and serotyping of pneumococcal isolates were performed by coagglutination testing [19], using antisera obtained from the Statens Serum Institut (Copenhagen, Denmark). Isolates included those from sputum, nasopharynx, ear, eye, urine and other non-sterile sites. Antimicrobial susceptibility testing against penicillin and erythromycin was performed on selected isolates using the E-test method (Cambridge Diagnostics, Cambridge, UK). Isolates showing minimum inhibitory concentrations (MICs) of $\leqslant 0{\cdot}06\ \mu$g/ml, $0{\cdot}1$–$1\ \mu$g/ml and $\geqslant 2\ \mu$g/ml were considered sensitive, intermediate and highly resistant respectively, to penicillin. Erythromycin sensitivity and resistance were defined by MICs of $< 1\ \mu$g/ml and $> 1\ \mu$g/ml respectively. Intermediate resistant and highly resistant isolates were considered as non-susceptible isolates. Multiple isolates from the same patient were excluded from the study. Laboratory reports included demographic and limited clinical information. Data analysis was performed using SPSS version 10.

RESULTS

There was a total of 4491 non-invasive isolates in 1988–99. Of these, 1262 (28·1%) were from females, 1692 (37·7%) from males and in 1537 (34·2%) cases, the patient gender was unknown. Serological information was available for 1011 (22·5%) isolates. Of these serogrouped/typed isolates, 654 (64·7%) isolates were from sputum, 79 (7·8%) from the nasopharynx and 278 (27·5%) from other non-invasive sites. Serogroup/type information was available for 186 (18·4%) isolates from those aged less than 2 years, 209 (20·7%) isolates from those aged 5 years or less, and 375 (37·1%) isolates from those aged 65 years or more. Of the 1011 isolates with serological information, 524 had specific serotype information and 833 had serogroup information (Table 1).

Patient ages ranged from 1 year to 99 years, with a mean and median age of 36 years and 35 years respectively. Susceptibility to penicillin and erythromycin was tested in 60·4% (611/1011) and 50% (505/1011) of all isolates which were serogrouped/typed. The prevalence of non-susceptible isolates was 373/611 (61%) for penicillin in 1992–9 and 70/505 (14%) for erythromycin in 1994–9 for the isolates for which there was serogroup/type information.

Potential vaccine coverage in different age groups

Serogroups contained in the 23-valent vaccine accounted for 99% of non-invasive isolates in age groups

https://doi.org/10.1017/S0950268801006331 Published online by Cambridge University Press

Table 1. *Distribution of pneumococcal serotypes and serogroups*

	No.	Percent		No.	Percent
Serogroups			**Serotypes** (*cont.*)		
Groups			Types		
23	224	26·9	27	3	0·6
9	166	19·9	29	3	0·6
6	150	18	4	2	0·4
19	138	16·6	20	2	0·4
15	36	4·3	22A	2	0·4
11	35	4·2	34	2	0·4
33	18	2·2	42	2	0·4
7	15	1·8	5	1	0·2
10	15	1·8	16F	1	0·2
18	13	1·6	24F	1	0·2
17	6	0·7	37	1	0·2
12	5	0·6	Total	524	100
16	5	0·6			
22	3	0·4	**Serogroups/types**		
24	3	0·4	Groups/types		
41	1	0·1	23	224	22·2
Total	833	100	9	166	16·4
			6	150	14·8
Serotypes			19	138	13·6
Types			14	69	6·8
23F	77	14·7	3	54	5·3
23A	12	2·3	15	36	3·6
19F	57	10·9	11	35	3·5
19A	18	3·4	33	18	1·8
14	69	13·2	7	15	1·5
6A	32	6·1	10	15	1·5
6B	30	5·7	8	13	1·3
9V	36	6·9	18	13	1·3
9N	13	2·5	1	12	1·2
15B	16	3·1	35	9	0·9
15C	6	1·1	17	6	0·6
15A	3	0·6	12	5	0·5
15F	3	0·6	16	5	0·5
11A	33	6·3	31	5	0·5
11C	1	0·2	22	3	0·3
33F	16	3·1	24	3	0·3
10A	14	2·7	27	3	0·3
8	13	2·5	29	3	0·3
1	12	2·3	4	2	0·2
18C	12	2·3	20	2	0·2
7F	9	1·7	34	2	0·2
7C	1	0·2	42	2	0·2
35	7	1·3	5	1	0·1
17F	6	1·1	37	1	0·1
31	5	1	41	1	0·1
12F	3	0·6	Total	1011	100

< 2 years and $\leqslant 5$ years and 96% in age groups $\geqslant 65$ years and all ages. The coverage of 7, 9 and 11-valent conjugate vaccine in these age groups was 87–94%, 85–93%, 74–81% and 75–84% of non-invasive isolates respectively. Overall, coverage of 7- to 11-valent conjugate vaccine serogroups was between 85% and 94% for age groups < 2 years and $\leqslant 5$ years but their coverage reduced to 74–84% in age groups $\geqslant 65$ years and all ages. Overall, the 23-valent polysaccharide vaccine serogroups accounted

https://doi.org/10.1017/S0950268801006331 Published online by Cambridge University Press

Table 2. *Coverage of pneumococcal vaccines for penicillin and erythromycin susceptible and non-susceptible isolates*

	Penicillin*				Erythromycin†		
	No. (%) of vaccine related serotype				No. (%) of vaccine related serotype		
Vaccine	Sensitive	Intermediate	Resistant	Total	Sensitive	Resistant	Total
7-valent	158 (66·4)	347 (95·6)	10 (100)	515 (84·3)	376 (86·4)	68 (97·1)	444 (87·9)
9-valent	166 (69·7)	348 (95·9)	10 (100)	524 (85·8)	382 (87·8)	68 (97·1)	450 (89·1)
11-valent	194 (81·5)	353 (97·2)	10 (100)	557 (91·2)	405 (93·1)	68 (97·1)	473 (93·7)
23-valent	228 (95·8)	360 (99·2)	10 (100)	598 (97·9)	423 (97·2)	70 (100)	493 (97·6)
Overall	238 (100)	363 (100)	10 (100)	611 (100)	435 (100)	70 (100)	505 (100)

* Sensitive MIC $\leqslant$ 0·06 μg/ml, intermediate MIC 0·12–1·0 μg/ml, resistant MIC $\geqslant$ 2 μg/ml, † Sensitive MIC $<$ 1 μg/ml, resistant MIC $>$ 1 μg/ml (7-valent vaccine serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F), (9-valent vaccine serotypes: 7-valent vaccine serotypes with serotypes 1 and 5), (11-valent vaccine serotypes: 9-valent vaccine serotypes with serotypes 3 and 7F), (23-valent vaccine serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F).

Table 3. *Penicillin and erythromycin susceptible and non-susceptible non-invasive pneumococcal serogroups/types*

	No. (%) of isolates						
	Penicillin				Erythromycin		
Serogroups/ types	Sensitive (MIC $\leqslant$ 0·06)	Intermediate (MIC = (0·12–1·0))	Resistance (MIC $\geqslant$ 2)	Total	Sensitive (MIC $<$ 1)	Resistance (MIC $>$ 1)	Total
23	72	78	5	155	110	24	134
9	14	115	4	133	113	5	118
6	24	61	0	85	53	19	72
19	35	45	0	80	55	8	63
14	12	46	1	59	43	11	54
Others*	81	18	0	99	61	3	64
Total†	157 (66)	345 (95)	10 (100)	512 (83·8)	374 (86)	67 (95·7)	441 (87·3)
Total‡	238 (100)	363 (100)	10 (100)	611 (100)	435 (100)	70 (100)	505 (100)

* Others (sensitive = serogroups/types 11, 15, 1, 7, 10, 31, 33, 16, 4, 5, 22, 24, 29, 34, 35, 42) (intermediate = serogroups/types 8, 15, 7, 29, 1, 4, 18, 35) in descending order for penicillin. * Others (sensitive = serogroups/types 8, 15, 1, 7, 29, 31, 16, 4, 5, 18, 22, 24, 33, 34, 35, 42) (resistant = serogroups/types 4, 11, 15) in descending order for erythromycin. Non-susceptible isolates = intermediate and resistant isolates.
† Total isolates for serogroups/types (23, 9, 6, 19, 14).
‡ Overall total isolates.

for 96–9% of non-invasive disease respectively in all age groups.

Potential vaccine coverage for penicillin and erythromycin non-susceptible isolates

7–9-, 11-valent vaccine

The 7–11-valent vaccine serogroups accounted for $>$ 95% and $>$ 97% of penicillin and erythromycin non-susceptible non-invasive isolates respectively (Table 2).

23-valent vaccine

The coverage for non-susceptible penicillin and erythromycin non-invasive isolates was $>$ 99% and 95% respectively with 23-valent vaccine (Table 2).

Most prevalent non-invasive pneumococcal serogroups, 1988–99

The eight most prevalent pneumococcal serogroups (in descending rank order) were 23, 9, 6, 19, 14, 3, 15 and 11. These eight serogroups were responsible for

https://doi.org/10.1017/S0950268801006331 Published online by Cambridge University Press

over 86·3% of non-invasive disease regardless of specimen type.

Antibiotic susceptibility

Serogroups 23, 9, 6, 19 and 14 were associated with the majority of penicillin and erythromycin non-susceptibility (Table 3).

DISCUSSION

Our data indicate that the serogroups included in the 23-valent polysaccharide and 7–11-valent conjugated pneumococcal vaccines caused the majority of non-invasive pneumococcal disease in all age groups in the study period in Scotland. In addition, serogroups associated with antibiotic resistance were found to be very similar in both non-invasive and invasive disease in Scotland [18]. In this study, data on coverage of vaccines were based on serogroups, assuming that there is cross-reactivity of serotype-specific protection within a serogroup (e.g. 6A *vs.* 6B, 9A *vs.* 9V) [20]. However, since there are limited data on cross-reactivity of vaccine serotypes within a serogroup [21], our data may have overestimated the potential coverage of vaccines for non-invasive disease. Penicillin resistant isolates are likely to be over-represented in this as a result of selectively higher referral by diagnostic laboratories. Also, only 60% and 50% of the total serogrouped/typed isolates were tested for penicillin and erythromycin susceptibility respectively in the study period. For these two reasons, data on antibiotic sensitivity should be interpreted with considerable caution.

Serogroups 23, 9, 6 and 19 were the predominant causes of non-invasive pneumococcal diseases, in keeping with earlier reports from the United Kingdom [22–4] and from other developed and developing countries [12, 25]. In common with other reports [12, 26], the coverage of 7–9-valent conjugated vaccine serogroups was lower for the elderly and the 'all age' group than the age groups < 2 years and $\leqslant 5$ years. The conjugated vaccine-related serogroups were the cause of over 80% of non-invasive disease in children under 2 years of age. Earlier studies [27, 28] reported a higher nasopharyngeal carriage with both vaccine serotypes and non-vaccine serotypes than in adults. Since there is an association between carriage and the spread of disease, children may be a major source of pneumococci and pneumococcal disease in the community. If so, universal vaccination of young children may extend protection from pneumococcal disease to non-vaccinated individuals by herd immunity.

The finding of a similar serogroup distribution in both invasive and non-invasive disease (regardless of the site of clinical isolate), is consistent with serogroups colonizing non-sterile sites and having the potential to cause invasive disease. Acquisition and carriage of *S. pneumoniae* is associated with the development of both invasive and non-invasive disease [28–30]. Data from Papua New Guinea suggest that serogroups/types causing upper respiratory tract infection (URTI) could be used to obtain a conservative estimate of susceptibility to invasive pneumococci [31]. Therefore, targeting vaccination on serogroups/types associated with non-invasive disease could reduce the risk of invasive disease. The efficacy of 7-valent pneumococcal conjugated vaccines in preventing pneumonia and otitis media has been documented: 73% (95% CI 38–88) against clinically diagnosed pneumonia confirmed with chest radiograph (pulmonary consolidation $\geqslant 2{\cdot}5$ cm) in a US study [32] and 57% (95% CI 44–67) against otitis media caused by vaccine serotypes in a Finnish study [33]. Studies from Europe and the United States reported that the estimated incidence rate of pneumococcal pneumonia was 5–9 per 1000 children under 5 years of age [34–36]. In addition, the incidence rate of pneumococcal otitis media has been estimated at 0·56 episodes per child under 2 years of age [3, 37]. Therefore, it appears that pneumococcal conjugated vaccination could reduce non-invasive disease caused by *S. pneumoniae* significantly. Additional studies are required to determine its efficacy in preventing disease in adults, the elderly and in immunocompromised patients.

Pneumococci carried in the upper respiratory tract are more often resistant to antibiotics than invasive strains [23, 38]. Serogroups 23 and 9 were frequently associated with penicillin and erythromycin non-susceptibility in this study. Our previous study on invasive pneumococcal isolates found that serotype 14 was the most common serotype associated with penicillin and erythromycin non-susceptibility [18]. In accordance with data from other countries [11], we found that serogroups 23, 9, 6, 19 and 14 were responsible for $\geqslant 95\%$ of non-invasive pneumococcal antibiotic resistance in Scotland. All these serogroups are represented in the 7-valent conjugated vaccine. Existing data suggest a reduction of antibiotic resistant pneumococcal serotypes [17] and use of antibiotics (5·3%) in pneumococcal conjugated vac-

https://doi.org/10.1017/S0950268801006331 Published online by Cambridge University Press

cine recipients [39]. Thus, widespread use of pneumococcal conjugated vaccine could prevent the spread of antibiotic resistant pneumococcal isolates and thereby reduce antibiotic consumption. However, serotype exchange and capsular switching by pneumococci leading to an increase in virulence has been observed [40, 41]. It is possible that in the future, conjugated vaccine-induced pressure could lead to replacement of vaccine serotypes with non-vaccine serotypes of increased virulence, leading to increased disease and antibiotic resistance [42, 43]. Thus, it will be necessary to monitor the epidemiological, microbiological and immunological characteristics of the pneumococcal population worldwide. Our data highlight the importance of improved pneumococcal surveillance in Scotland in order to inform effective local public health strategies.

ACKNOWLEDGEMENTS

We thank the staff at microbiology laboratories and the Scottish Centre for Infection and Environmental Health for reporting and entering the data. We also thank Dr Peter Christie for helpful discussion and two reviewers for helpful comments. We are also grateful to Professor K. A. V. Cartwright for careful editing of the paper.

REFERENCES

1. Anonymous. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 1997; **46**: 1–24.
2. MacFarlane J. The clinical impact of pneumococcal disease. In: Mayon-White RT, ed. The clinical impact of pneumococcal disease and strategies for its prevention. Royal Society of Medicine International Congress and Symposium Series 1995; 210: London, Royal Society Medicine: 9–17.
3. Klein J. Otitis media. Clin Infect Dis 1994; **19**: 823–33.
4. Nguyen-Van-Tam J, Neal KR. Clinical effectiveness, policies, and practices for influenza and pneumococcal vaccines. Semin Resp Infect 1999; **14**: 184–95.
5. Lee C-J, Banks SD, Li JP. Virulence, immunity, and vaccine related to *Streptococcus pneumoniae*. Crit Rev Microbiol 1991; **18**: 89–114.
6. Sniadack DH, Schwartz B, Lipman H, et al. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children – implications for vaccine strategies. Pediatr Infect Dis J 1995; **14**: 503–10.
7. Klein DL. Pneumococcal disease and the role of conjugate vaccines. Microb Drug Resist 1999; **5**: 147–57.
8. Klein JO. The epidemiology of pneumococcal disease in infants and children. Rev Infect Dis 1981; **3**: 246–53.
9. Scott J, Hall A, Dagan R, Dixon J, et al. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7000 episodes of invasive disease. Clin Infect Dis 1996; **22**: 973–81.
10. Ghaffar F, Friedland IR, McCracken GH. Dynamics of nasopharyngeal colonization by *Streptococcus pneumoniae*. Pediatr Infect Dis J 1999; **18**: 638–46.
11. Appelbaum P. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin Infect Dis 1992; **15**: 77–83.
12. Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, Part II. Clin Infect Dis 2000; **30**: 122–40.
13. 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**: 100–21.
14. Schrag S, Beall B, Dowell S. Limiting the spread of resistant pneumococci: biological and epdemiologic evidence for the effectiveness of alternative interventions. Clin Microbiol Rev 2000; **13**: 588–601.
15. Eskola J, Anttila M. Pneumococcal conjugate vaccines. Pediatr Infect Dis J 1999; **18**: 543–51.
16. 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 pneumococcal conjugate vaccines. J Infect Dis 1996; **174**: 1352–5.
17. Mbelle N, Huebner RE, Wasas AD, et al. Immunogenecity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. J Infect Dis 1999; **180**: 1171–6.
18. Kyaw MH, Clarke S, Edwards G, Jones IG, Campbell H. Serotypes/groups distribution and antimicrobial resistance of invasive pneumococcal isolates: implication for vaccine strategies. Epidemiol Infect 2000; **125**: 561–72.
19. Smart LE. Serotyping of *Streptococcus pneumoniae* strains by coagglutination. J Clin Pathol 1986; **39**: 328–31.
20. Robbins JB, Austrian R, Lee C-J, et al. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within serogroups. J Infect Dis 1983; **148**: 1136–59.
21. Yu X, Gray B, Chang Swei-ju, Ward JI, Edwards KM, Nahm MH. Immunity to cross-reactive serotypes induced by pneumococcal conjugate vaccines in infants. J Infect Dis 1999; **180**: 1569–76.

22. Smart L, Dougall A, Girdwood R. New 23-valent pneumococcal vaccine in relation to pneumococcal serotypes in systemic and non-systemic disease. J Infect 1987; **14**: 209–15.
23. Ridgway E, Tremlett C, Allen K. Capsular serotypes and antibiotic sensitivity of *Streptococcus pneumoniae* isolated from primary-school children. J Infect 1995; **30**: 245–51.
24. Colman G, Cooke E, Cookson B, Cooper P, Efstratiou A, George R. Pneumococci causing invasive disease in Britain 1982–1990. J Med Microbiol 1998; **47**: 17–27.
25. Jamal F. Epidemiological data on pneumococcal infections in Asian countries. Vaccine 1999; **17**: S75–8.
26. Butler J, Breiman R, Lipman H, Hofmann J, Facklam R. Serotypes distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. J Infect Dis 1995; **171**: 885–9.
27. Hendley J, Sande M, Stewart P, Gwaltney MJ. Spread of *Streptococcus pneumoniae* in families. Carriage rates and distribution of types. J Infect Dis 1975; **132**: 55–61.
28. Gray B, Converse G, Dillion HJ. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980; **142**: 923–33.
29. Mastro T, Ghafoor A, Nomani N, et al. Antimicrobial resistance of pneumococci in children with acute lower respiratory tract infection in Pakistan. Lancet 1991; **337**: 156–9.
30. Craig AS, Erwin PC, Schaaffner W, et al. Carriage of multi-resistant *Streptococcus pneumoniae* and impact of chemoprophylaxis during an outbreak of meningitis at a day care center. Clin Infect Dis 1999; **29**: 1257–64.
31. 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.
32. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenecity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J 2000; **19**: 187–95.
33. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001; **344**: 403–9.
34. Murphy T, Henderson F, Clyde W, et al. Pneumonia: an eleven year study in a pediatric practice. Am J Epidemiol 1981; **113**: 12–21.
35. Heiskanen-Kosma T, Korppi M, Jokinen C, Kurki S. Etiology of childhood pneumonia: serologic results of a prospective, population based study. Pediatr Infect Dis J 1998; **17**: 986–91.
36. Djuretic T, Ryan M, Miller E, Fairley C, Goldblatt D. Hospital admissions in children due to pneumococcal pneumonia in England. J Infect Dis 1998; **37**: 54–8.
37. Teele D, Klein J, Rosner B, et al. Epidemiology of otitis media during the first seven years of life in children in Greater Boston: a prospective, cohort study. J Infect Dis 1989; **160**: 83–94.
38. Montgomery J, Lehmann D, Smith T, et al. Bacterial colonization of the upper respiratory tract and its association with acute respiratory tract infections in Highland children of Papua New Guinea. Rev Infect Dis 1990; **12** (Suppl 8): S1006–16.
39. Black S. The Kaiser Permanente Efficacy Trial: update otitis media (presentation). Second International Symposium of Pneumococci and Pneumococcal Diseases, 2000. Presentation available at ⟨http://216.247.185.154/isppd00/is0b0810/maib0810.htm⟩. Accessed 21 December 2000.
40. Kelly T, Dillaard J, Yother J. Effect of genetic switching of capsular type on virulence of *Streptococcus pneumoniae*. Infect Immun 1994; **62**: 1813–9.
41. Nesin M, Ramirez M, Tomasz A. Capsular transformation of a multidrug-resistant *Streptococcus pneumoniae* in vivo. J Infect Dis 1998; **177**: 707–13.
42. Lipsitch M. Bacterial vaccines and serotypes replacement: lessons from *Haemophilus influenzae* and prospects for *Streptococcus pneumoniae*. Emerg Infect Dis 1999; **5**: 336–45.
43. Spratt BG, Greenwood BM. Prevention of pneumococcal disease by vaccination: does serotype replacement matter? Lancet 2000; **365**: 1210–11.