International Journal of Infectious Diseases 16 (2012) e753–e757

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Nasopharyngeal carriage of *Streptococcus pneumoniae*: prevalence and risk factors in HIV-positive children in Tanzania

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ARTICLE INFO

Article history:
Received 5 December 2011
Received in revised form 15 May 2012
Accepted 16 May 2012

Corresponding Editor: Timothy Barkham, Tan Tock Seng, Singapore

Keywords:
Nasopharyngeal carriage
Streptococcus pneumoniae
HIV
Child
Risk factor

SUMMARY

Background: Pneumococcal colonization of the nasopharynx is especially common in young children and is a pre-requisite for pneumococcal disease. Those with immunosuppression, such as HIV, are at higher risk of colonization and disease, especially at older ages. Currently, vaccination schedules are only offered to children under 6 months of age, despite the large impact of pneumococcal disease in older unvaccinated children with HIV. We conducted a study to assess the prevalence of, and risk factors for, pneumococcal carriage in HIV-positive children aged <15 years.

Methods: We collected a single nasopharyngeal swab from 142 HIV-infected children aged 1–14 years over a 2-month period. To detect carriage of pneumococcus, these samples were cultured and serotyped; PCR was performed on negative samples. We also collected epidemiological data via survey and medical records.

Results: The overall carriage rate was 81% and was at least 76% in those aged 5–14 years. The 7-, 10-, and 13-valent pneumococcal vaccines would cover 37%, 37%, and 49% of children with carriage, respectively. In the multivariate analysis, we identified increase in weight since last visit ($p = 0.028$) and the existence of care-givers who had respiratory symptoms in the past week ($p = 0.022$) as risk factors for carriage. Weight gain was also significantly associated with antiretroviral use ($p = 0.002$).

Conclusions: These data illuminate the little known area of pneumococcal carriage in older HIV-infected children as well as finding novel risk factors for pneumococcal carriage, namely the association with household members who have respiratory symptoms and with an increase in the child's weight prior to swabbing. Weight gain may be due to an increase in health enabling more mobility and increasing the risk of acquiring carriage. The carriage rate observed (81%) is one of the highest recorded. Further research should address whether vaccination can prevent the acquisition of carriage and so protect against disease.

1. Introduction

Streptococcus pneumoniae is a Gram-positive bacterium with over 90 serotypes identified. Pneumococci colonize the nasopharynx asymptomatically, and usually transiently, for weeks or up to months.[1] Carriage is a prerequisite for invasive disease, such as pneumonia, meningitis, or septicemia to develop.[2] The World Health Organization (WHO) reports that invasive pneumococcal disease causes up to one million childhood deaths per year.[3,4]

The association between *S. pneumoniae* and pneumonia was described in 1883, while the association of pneumococcal capsular

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1201-9712/$36.00 – see front matter
http://dx.doi.org/10.1016/j.ijid.2012.05.1037

polysaccharide with virulence and human disease were first described in 1915.[5]

Bacterial disease is a leading cause of mortality and morbidity in children in the developing world,[4,6,7] especially those with HIV.[8,9] HIV-positive children aged <2 years have about a 40 times greater risk of invasive pneumococcal disease compared to healthy children; these infections are more likely to be fatal,[9,10] and importantly the incidence does not decline with age as in healthy children.[9–12]

Mortality and morbidity in children with HIV may be reduced by vaccination against pneumococcal disease.[8] Antiretroviral therapy also reduces disease, but long-term delivery is logistically challenging. It was recently estimated that up to 90% of Tanzanians in need of antiretrovirals (ARVs) were not receiving them.[13] A pneumococcal vaccination program, on the other hand, is relatively cheaper, and pneumococcal vaccines are both well tolerated and immunogenic in HIV-infected infants.[13] It has been shown that pneumococcal vaccination has prevented death, invasive disease, and pneumonia in African infants with and without HIV infection.[8,14,15]

Research has concentrated on looking at the effectiveness of the vaccine in infants vaccinated when only a few months old. Vaccination has already been shown to be immunogenic in older children.[15] Thus, before a policy decision is implemented regarding immunization of older children (>1 year) living with HIV (2.3 million[16]), it is important to better examine the prevalence of, and risk factors for, pneumococcal carriage in this population.

This paper aimed to examine the prevalence of, and risk factors for, *S. pneumoniae* carriage in HIV-positive children, to identify the serotypes circulating in this population (without vaccine pressure), and to estimate the theoretical coverage offered by different conjugate vaccines. Identifying risk factors for colonization can inform methods to better control transmission and ultimately reduce the incidence of invasive disease. Pneumococcal conjugate vaccine (PCV) has been shown to reduce vaccine-type carriage, although this may require higher serum antibody concentrations than is needed to prevent pneumococcal disease.[17]

2. Methods

The study was conducted at The Diana Centre, Teule Hospital in Muheza, a rural town in Tanzania, which has a leading HIV treatment clinic. The Centre receives funding from the government, plus private and non-governmental organization (NGO) sources. It is an outpatient-based service that both educates and treats people with HIV.

2.1. Recruitment

Inclusion criteria for children of The Diana Centre required all three of the following: informed consent from the primary care-giver (i.e., usually the mother, but in a small minority from a guardian, usually an aunt), HIV-positive, and age between 1 and 14 years. Exclusion criteria were: refusal to participate, presence of contraindications to a nasopharyngeal swab (such as thrombocytopenia), presence of severe disease (any condition requiring immediate medical treatment as determined by medical personnel or researchers), or previous receipt of pneumococcal vaccination.

2.2. Data and swab collection

A questionnaire was administered to care-givers at recruitment which collected epidemiological data including recent respiratory symptoms in the household and previous HIV treatment. Health data including CD4+ count were obtained from medical records. Detailed data on risk factors were collected from children, primary care-givers, and other household members (Table 1).

Swabs (Medical Wire and Equipment, UK) were collected and processed according to the WHO working group standard methods for detecting upper respiratory carriage of *S. pneumoniae*.[18,19] The serotype of isolates from culture-positive specimens was determined by the Quellung reaction, using antisera supplied by the Statens Serum Institut (Copenhagen, Denmark).

Culture-negative samples were tested by *lyt*A PCR (specific for *S. pneumoniae* identification) using a TaqMan probe and primers as described by McAvin et al.,[20] except that the result was read by spectrofluorometry and interpreted as described by Poddar and Le.[21] Briefly, a 25-μl PCR reaction containing 1.5 mM $MgCl_2$, 200 μM dNTPs, 200 nM of each primer, 120 nM probe, 1.23 U HotStar*Taq* DNA polymerase, and 10 μl of total nucleic acid, yielded a 101-bp product. The PCR cycling conditions were: initial denaturation at 95 °C for 15 min, followed by 45 cycles at 96 °C for 10 s, 63 °C for 1 min, and a final extension step of 72 °C for 2 min. The end-point results were analyzed by calculating the post- to pre-read ratio. Samples with ratios of ≥2.78 were reported as positive and confirmed using gel electrophoresis on a 2% gel, at 200 V for 40 min. Samples with ratios of ≤1.21 were reported as negative. The limit of detection of the assay was 6 colony-forming units (CFU)/ml.

2.3. Pneumococcal serotype identification

All samples in which *S. pneumoniae* was detected by PCR were examined by multiplex PCR reverse line assay (mPCR/RLB) to

Table 1
Comparison (univariate analyses and final model of multivariate analysis) of characteristics between HIV-infected children who were pneumococcal carriers or non-carriers

Characteristic	Carriers (*n* = 115)	Non-carriers (*n* = 27)	OR (95% CI)	*p*-Value
Univariate analysis				
Age, years, mean (SD)	6.39 (3.18)	7.54 (3.18)	Not applicable	0.100
Female, *n* (%)	57/109 (52%)	14/26 (54%)	0.94 (0.40–2.22)	0.887
Number of household members diagnosed with HIV, mean (SD)	0.89 (0.82)	0.50 (0.72)	Not applicable	0.053
Children exposed to passive smoking in the house, *n* (%)	36/110 (33%)	7/25 (28%)	1.25 (0.48–3.27)	0.647
Children sleeping in a room where cooking is done, *n* (%)	53/110 (48%)	13/26 (50%)	0.93 (0.40–2.19)	0.868
Children currently attending school, *n* (%)	52/107 (49%)	17/26 (65%)	0.50 (0.21–1.22)	0.124
Children with a recent (in the past week) respiratory infection, *n* (%)	82/109 (75%)	18/26 (69%)	1.35 (0.53–3.45)	0.531
Latest CD4 count, mean (median)	752 (540)	733 (441)	Not applicable	0.879
Signs of malnutrition, *n* (%)	12/109 (11%)	4/26 (15%)	0.68 (0.20–2.31)	0.535
Gaining weight (compared with last outpatient visit), *n* (%)	54/83 (65%)	8/22 (36%)	3.26 (1.22–8.67)	0.015
Antiretrovirals taken, *n* (%)	61/95 (64%)	11/26 (42%)	2.45 (1.01–5.92)	0.044
Number of household members, mean (SD)	5.02 (1.85)	5.19 (2.12)	Not applicable	0.680
Children whose care-giver had respiratory symptoms in past week, *n* (%)	50/110 (45%)	5/25 (20%)	3.33 (1.17–9.52)	0.019
Final model of multivariate analysis				
Gaining weight (compared with last outpatient visit), *n* (%)	54/83 (65%)	8/22 (36%)	3.62 (1.21–10.89)	0.028
Children whose care-giver had respiratory symptoms in past week, *n* (%)	50/110 (45%)	5/25 (20%)	2.97 (1.12–7.86)	0.022

OR, odds ratio; CI, confidence interval; SD, standard deviation.

identify serotypes individually or in small groups of related serotypes, as previously described.[22,23] If serogroup 6 was identified, serotype-specific PCRs targeting *wci*P, to distinguish serotypes 6A and 6C from 6B, and the *wci*N single nucleotide polymorphism (SNP), which distinguishes serotype 6A from 6C,[24] were performed. Samples that gave no signals in mPCR/RLB (n = 33) (result recorded as 'below detection level' (BDL)) and those in which only the SP-positive control probes targeting *ply* or *lyt*A produced signals ('non-typeable' (NT)) were further tested, if sufficient DNA remained, by PCR and sequencing of the *cps*A–B region of the capsular polysaccharide synthesis (*cps*) gene cluster, as previously described and validated.[25]

2.4. Statistical analysis

To assess the association between pneumococcal carriage and each risk factor, the Chi-square test was employed for univariate analysis, which provided odds ratio (OR) and 95% confidence intervals (CIs). Multiple logistic regression was applied using risk factors with $p < 0.25$ on univariate analysis to produce the final model containing only significant variables. All the statistical data analyses were performed using SPSS version 19 (IBM, USA).

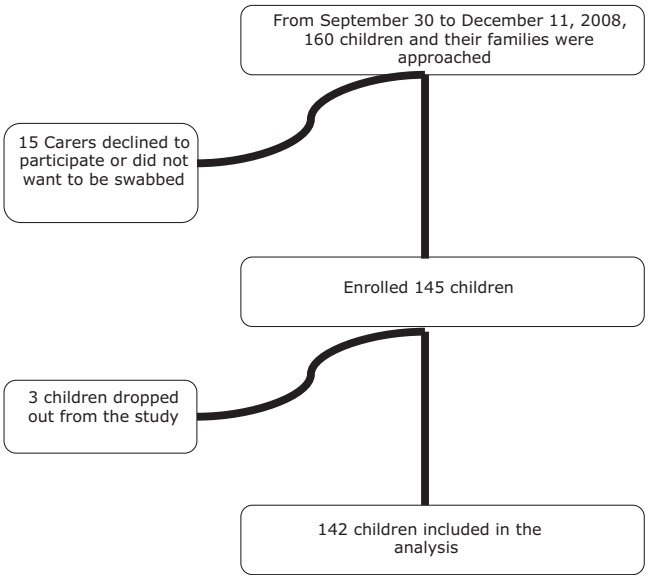

Figure 1. Flow of study participants.

3. Results

Between September 30 and December 11, 2008, 160 children and their families were approached; 148 initially agreed to take part but six children refused to be swabbed (Figure 1). Pneumococci were isolated (or detected by PCR) from the nasopharyngeal swab of 115/142 (81%) children: 93 by culture and 22 by PCR.

The age was recorded for 138 of the 142 children; three of the four children of uncertain age had positive swabs. The age-specific carriage prevalences were: 88% in those aged 1–4 years (46/52), 77% in those aged 5–9 years (47/61), and 76% in those aged 10–14 years (19/25, $p = 0.13$). All swabs yielded a single pneumococcal serotype with the exception of one from a 2.5-year-old child who was colonized with two serotypes (19A and 9 V).

We detected 31 serotypes among the 115 positive samples; 13 positive samples were BDL. We assessed the frequency of each serotype (Figure 2). The most common were 19F (11%) and 11A (9%). The 7-, 10-, and 13-valent vaccines covered 43/115 (37%), 43/115 (37%), and 56/115 (49%) of the colonizing serotypes, respectively (Table 2).

We compared several characteristics between the carriers and non-carriers (Table 1). On multivariate analysis, we found two factors associated with the increased risk of an HIV-infected child being a carrier: weight gain in the child since the last visit to the centre ($p = 0.028$) and the care-giver having a respiratory infection in the past week ($p = 0.022$). We found no differences in carriage rates by age, school attendance, number of other people with HIV in the household, or recent health and treatment of the child (i.e., presence of nasal mucus, use of ARVs, and CD4+ count). For example, of 130 children with complete data on respiratory symptoms, 58 had one or more symptoms (47 were carriers, 81%), while 72 had no symptoms (59 carriers, 82%) (Table 1).

There was however a significant association between ARV use and weight gain ($p = 0.002$). However, weight gain was not associated with the child being on ARV medication, comparing more than 1 year treatment with less than a year.

4. Discussion

As carriage is a prerequisite for invasive disease, being able to moderate risk factors for carriage may assist in controlling disease. Of the variables we examined, we found two significant predictive risk factors: carriage was more likely if the child had gained weight since their last visit to the clinic and if the care-giver had had a respiratory infection in the past week.

Weight gain is probably associated with the children being healthier, therefore more mobile and more likely to mix with other children and adults, thus increasing the chance of transmission by contact. There are data that support the horizontal transmission of carriage within families, specifically between siblings.[26] However

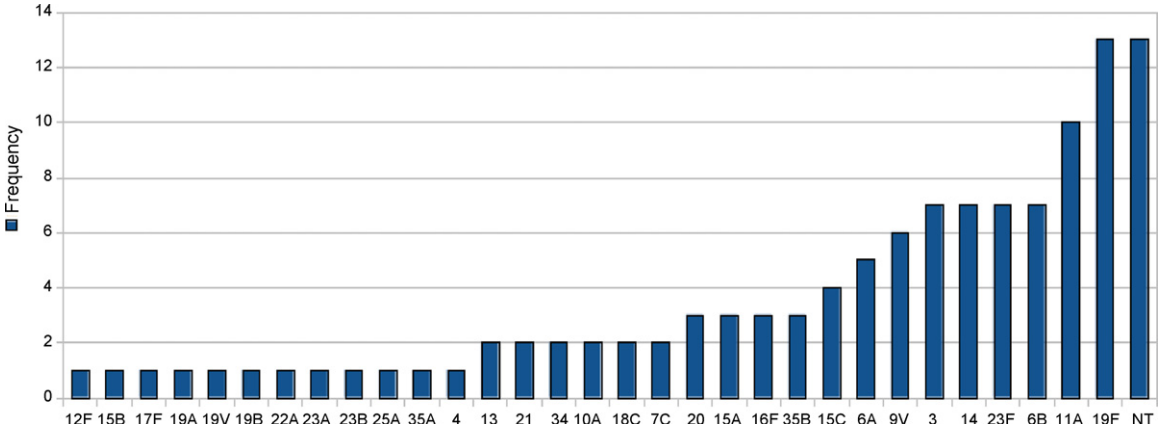

Figure 2. Frequency of pneumococcal serotypes isolated from nasopharyngeal swabs from HIV-infected children (NT = no serotype isolated).

Table 2
Serotypes carried and proportion covered by vaccines

Serotypes	*n*	7v	10v	13v
3	7			7
4	1	1	1	1
6A	5			5
6B	7	7	7	7
7C	2			
9V	6	6	6	6
13	2			
14	7	7	7	7
10A	2			
11A	10			
12F	1			
15A	3			
15B	1			
15C	4			
16F	3			
17F	1			
18C	2	2	2	2
19A	1			1
19V	1			
19B	1			
19F	13	13	13	13
20	3			
21	2			
22A	1			
23A	1			
23B	1			
23F	7	7	7	7
25A	1			
34	2			
35A	1			
35B	3			
Negative	27			
Below detection level	13			
Total	142	43	43	56
Denominator is 115 carriers		37%	37%	49%

the indirect effect of PCV on pneumococcal carriage among unvaccinated contacts is controversial. A study by Cheung et al.[27] showed that in areas with high carriage rates, vaccination of a child using 9-valent PCV may not provide herd immunity to siblings, suggesting they are acquiring carriage in the community. Cheung's finding, however, was counter to the results of a household carriage study by Millar et al.,[28] which demonstrated an indirect protection against vaccine-type pneumococcal carriage in the unvaccinated household members living with a 7-valent PCV vaccinee.

Our study showed that weight gain had a significant association with pneumococcal carriage, although we were not able to record the exact weight change of subjects at each study visit. Thus, we suggest that future studies should endeavor to increase granularity on the measurement of recent weight change.

There is evidence supporting community transmission such as in day-care centers. Studies of childcare transmission show attendance is associated with greater pneumococcal carriage[29–31] and disease.[32] This may have a threshold effect; a study by Pebody et al.[33] only found an association when the child attended for more than 20 h a week.

Attending for treatment of disease may also help explain why weight gain is associated with greater carriage. Although we found no statistically significant association between ARV use and carriage, we did find an association between ARV use and increase in weight ($p = 0.003$). Carriage was not associated with duration of ARV use (comparing treatment for <1 year vs. ≥ 1 year). ARV use may also be associated with more effective parenting or better access to medical care. Children may acquire carriage at the same time they are being treated (in a crowded clinic).

The significant association between carriage in a child and a household member who has recently had symptoms of an upper respiratory tract infection (URTI) may be explained by the child passing an infection to the care-giver or by carriage acquisition in the child from a coughing household member. In the wake of the impressive herd effect seen after pneumococcal conjugate vaccination was introduced in the USA,[34–36] there were studies associating carriage in children with invasive disease in care-givers.[37] As far as we can tell, there are no data on the association between respiratory illness in adult household members and pneumococcal carriage (or disease) in children. Another explanation could be that the URTI (in the care-giver) is passed to the child causing damage to the respiratory tract, which increases the chance of acquiring pneumococcus. It has been shown that the inflammatory milieu increases susceptibility to pneumococcal infection.[38]

The point prevalence of 81% for pneumococcal carriage in children with HIV in Muheza is one of the highest rates recorded to date. This high rate is partly a reflection of our comprehensive approach to testing, using both culture and PCR. Children aged less than 5 years had a carriage prevalence rate of 88%; in children aged 5–9 years, 77% were carriers and the rate among those aged 10–14 years was still high at 76%. Although the report by Hill et al.[39] also found a very high prevalence (80–90%), only infants younger than 18 months of age were studied. High rates have also been found in other developing countries,[40] but ours is the highest documented for school-age children. Unfortunately, we do not have data on carriage in HIV-negative children. It is important to note that the high carriage rate is maintained in older children (5–14 years) who have an HIV infection; carriage is normally inversely associated with age.[11,12] This underlines how much of a burden pneumococcal carriage (and secondary disease) may be in older HIV-positive children. It also suggests how important vaccination could be to this population, especially given that about half of carriers had serotypes covered by the 13-valent pneumococcal vaccine. It should also be remembered that serotypes 1, 5, and 7 are covered by the vaccine, but colonization was not demonstrated; these serotypes are known to be rarely isolated from children's nasopharynges, but, by contrast, disease due to these serotypes can be common. Thus, the vaccine may have more benefits (personal communication with Ben Amos).

Acquisition of some serotypes may be diminished by conjugate vaccines as the vaccine can induce a strong antibody response. Conjugate vaccines consist of capsular polysaccharides, chemically linked with a highly immunogenic protein. They induce both B- and T-cell responses, and can result in mucosal immunity to the vaccine serotypes covered by the vaccine. However, it takes a higher antibody concentration in the blood to prevent mucosal colonization than to prevent invasive disease.[17] This suggests that the prevention of nasopharyngeal carriage can be used as a surrogate for vaccine effectiveness, although it will give a more conservative estimate.[41] However, of concern, prevention of carriage may lead to other serotypes (not in the vaccine) filling the vacated ecological niche, i.e. colonizing and then potentially causing replacement disease. The 13-valent conjugate pneumococcal vaccine, were it 100% effective in preventing colonization, could have prevented about half of the serotyped colonizing episodes we detected. It is important to note that about 10% of all pneumococcal isolates were BDL and also about 50% of the typeable isolates are not covered by the 13-valent vaccine. For these, other non-vaccine measures are required to prevent acquisition of carriage.

In conclusion, we have identified novel risk factors that point to possible opportunities for reducing carriage. There are few data on carriage rates in children with HIV and fewer still in school-age children with HIV. The rate of colonization observed in older children of 76% is the highest we know of. Vaccination could help prevent acquisition and thereby reduce invasive disease. Nowhere

is the need for fast efficient roll out of the pneumococcal vaccine as important as in HIV-positive populations like this one in Tanzania.

Acknowledgements

We gratefully thank the members of the Joint Malaria Project, The Confederation of Meningitis Organisations Inc. (CoMO), Tanga, Tanzania, for assistance with the study, and all the participants in the project.

Funding: This study was made possible by a grant from Wyeth (now Pfizer) pharmaceuticals.

Ethics committee approval: This study was approved by the Ethics Committee of The Royal Alexandra Hospital for Children.

Conflict of interest: Gwendolyn L Gilbert's department has received funding from GSK to perform laboratory testing for sponsored pneumococcal empyema studies.

Peter C. Richmond has been a member of vaccine advisory boards for Wyeth and Baxter, has received funding for investigator-initiated research from GlaxoSmithKline Biologicals, and has received travel support from Pfizer and Baxter to present study data at international meetings.

Robert Booy has received funding from CSL, Roche, Sanofi, GlaxoSmithKline (GSK) and Wyeth to conduct sponsored research or attend and present at scientific meetings; any funding received is directed to a research account at the Children's Hospital at Westmead.

The other authors declared no conflict of interest.

References

1. Smith T, Lehmann D, Montgomery J, Gratten M, Riley ID, Alpers MP. Acquisition and invasiveness of different serotypes of *Streptococcus pneumoniae* in young children. *Epidemiol Infect* 1993;**111**:27–39.
2. Ghaffar F, Friedland IR, McCracken Jr GH. Dynamics of nasopharyngeal colonization by *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 1999;**18**:638–46.
3. PneumoADIP. GAVI's PneumoADIP surveillance and research report. Baltimore, MD: PneumoADIP; 2007. Available at: http://www.preventpneumo.org/results/pneumoadip_activities/surveillance_and_research/upload/RS-Report-Final1207.pdf (Accessed July 20, 2012).
4. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* 2005;**352**:39–47.
5. US Centers for Disease Control and Prevention. Vaccines pink book. 12th ed. 2012. Chapter 16. Pneumococcal disease. Atlanta, GA: CDC; Available at: http://www.cdc.gov/vaccines/pubs/pinkbook/pneumo.html (accessed April 28, 2012).
6. Mulholland EK, Adegbola RA. Bacterial infections—a major cause of death among children in Africa. *N Engl J Med* 2005;**352**:75–7.
7. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:417–32.
8. Bliss SJ, O'Brien KL, Janoff EN, Cotton MF, Musoke P, Coovadia H, Levine OS. The evidence for using conjugate vaccines to protect HIV-infected children against pneumococcal disease. *Lancet Infect Dis* 2008;**8**:67–80.
9. Madhi SA, Petersen K, Madhi A, Wasas A, Klugman KP. Impact of human immunodeficiency virus type 1 on the disease spectrum of *Streptococcus pneumoniae* in South African children. *Pediatr Infect Dis J* 2000;**19**:1141–7.
10. Heffernan RT, Barrett NL, Gallagher KM, Hadler JL, Harrison LH, Reingold AL, et al. Declining incidence of invasive *Streptococcus pneumoniae* infections among persons with AIDS in an era of highly active antiretroviral therapy, 1995–2000. *J Infect Dis* 2005;**191**:2038–45.
11. Nilsson P, Laurell MH. Carriage of penicillin-resistant *Streptococcus pneumoniae* by children in day-care centers during an intervention program in Malmo, Sweden. *Pediatr Infect Dis J* 2001;**20**:1144–9.
12. Uzuner A, Ilki A, Akman M, Gundogdu E, Erbolukbas R, Kokacya O, et al. Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* in healthy children. *Turk J Pediatr* 2007;**49**:370–8.
13. World Health Organization/Joint United Nations Programme on HIV/AIDS. Country profiles—Tanzania. WHO/UNAIDS. 2005; Available at: http://www.who.int/hiv/HIVCP_TZA.pdf (accessed July 20, 2012).
14. Cutts FT, Zaman SM, Enwere G, Jaffar S, Levine OS, Okoko JB, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005;**365**:1139–46.
15. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003;**349**:1341–8.
16. The Joint United Nations Programme on HIV/AIDS. Global Report UNAIDS Report on The Global AIDS Epidemic. 2010. Available at: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf (accessed July 21, 2012).
17. Millar EV, O'Brien KL, Bronsdon MA, Madore D, Hackell JG, Reid R, Santosham M. Anticapsular serum antibody concentration and protection against pneumococcal colonization among children vaccinated with 7-valent pneumococcal conjugate vaccine. *Clin Infect Dis* 2007;**44**:1173–9.
18. O'Brien KL, Bronsdon MA, Dagan R, Yagupsky P, Janco J, Elliott J, et al. Evaluation of a medium (STGG) for transport and optimal recovery of *Streptococcus pneumoniae* from nasopharyngeal secretions collected during field studies. *J Clin Microbiol* 2001;**39**:1021–4.
19. O'Brien KL, Nohynek H. Report from a WHO working group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 2003;**22**:133–40.
20. McAvin JC, Reilly PA, Roudabush RM, Barnes WJ, Salmen A, Jackson GW, et al. Sensitive and specific method for rapid identification of *Streptococcus pneumoniae* using real-time fluorescence PCR. *J Clin Microbiol* 2001;**39**:3446–51.
21. Poddar SK, Le CT. *Bordetella pertussis* detection by spectrofluorometry using polymerase chain reaction (PCR) and a molecular beacon probe. *Mol Cell Probes* 2001;**15**:161–7.
22. Wang Y, Kong F, Yang Y, Gilbert GL. A multiplex PCR-based reverse line blot hybridization (mPCR/RLB) assay for detection of bacterial respiratory pathogens in children with pneumonia. *Pediatr Pulmonol* 2008;**43**:150–9.
23. Zhou F, Kong F, Tong Z, Gilbert GL. Identification of less-common *Streptococcus pneumoniae* serotypes by a multiplex PCR-based reverse line blot hybridization assay. *J Clin Microbiol* 2007;**45**:3411–5.
24. Jin P, Xiao M, Kong F, Oftadeh S, Zhou F, Liu C, Gilbert GL. Simple, accurate, serotype-specific PCR assay to differentiate *Streptococcus pneumoniae* serotypes 6A, 6B, and 6C. *J Clin Microbiol* 2009;**47**:2470–4.
25. Kong F, Wang W, Tao J, Wang L, Wang Q, Sabananthan A, Gilbert GL. A molecular-capsular-type prediction system for 90 *Streptococcus pneumoniae* serotypes using partial *cps*A–*cps*B sequencing and *wzy*- or *wzx*-specific PCR. *J Med Microbiol* 2005;**54**:351–6.
26. Givon-Lavi N, Fraser D, Porat N, Dagan R. Spread of *Streptococcus pneumoniae* and antibiotic-resistant *S. pneumoniae* from day-care center attendees to their younger siblings. *J Infect Dis* 2002;**186**:1608–14.
27. Cheung YB, Zaman SM, Nsekpong ED, Van Beneden CA, Adegbola RA, Greenwood B, Cutts FT. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian children who participated in a 9-valent pneumococcal conjugate vaccine trial and in their younger siblings. *Pediatr Infect Dis J* 2009;**28**:990–5.
28. Millar EV, Watt JP, Bronsdon MA, Dallas J, Reid R, Santosham M, O'Brien KL. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. *Clin Infect Dis* 2008;**47**:989–96.
29. Kellner JD, Scheifele D, Vanderkooi OG, Macdonald J, Church DL, Tyrrell GJ. Effects of routine infant vaccination with the 7-valent pneumococcal conjugate vaccine on nasopharyngeal colonization with *Streptococcus pneumoniae* in children in Calgary, Canada. *Pediatr Infect Dis J* 2008;**27**:526–32.
30. Regev-Yochay G, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. *Clin Infect Dis* 2004;**38**:632–9.
31. Sa-Leao R, Nunes S, Brito-Avo A, Alves CR, Carrico JA, Saldanha J, et al. High rates of transmission of and colonization by *Streptococcus pneumoniae* and *Haemophilus influenzae* within a day care center revealed in a longitudinal study. *J Clin Microbiol* 2008;**46**:225–34.
32. Siedler A, Reinert RR, Toschke M, Al-Lahham A, von Kries R. Regional differences in the epidemiology of invasive pneumococcal disease in toddlers in Germany. *Pediatr Infect Dis J* 2005;**24**:1114–5.
33. Pebody RG, Morgan O, Choi Y, George R, Hussain M, Andrews N. Use of antibiotics and risk factors for carriage of *Streptococcus pneumoniae*: a longitudinal household study in the United Kingdom. *Epidemiol Infect* 2009;**137**:555–61.
34. Metlay JP, Fishman NO, Joffe M, Edelstein PH. Impact of pediatric vaccination with pneumococcal conjugate vaccine on the risk of bacteremic pneumococcal pneumonia in adults. *Vaccine* 2006;**24**:468–75.
35. O'Brien KL, Dagan R. The potential indirect effect of conjugate pneumococcal vaccines. *Vaccine* 2003;**21**:1815–25.
36. Pletz MW, Maus U, Krug N, Welte T, Lode H. Pneumococcal vaccines: mechanism of action, impact on epidemiology and adaption of the species. *Int J Antimicrob Agents* 2008;**32**:199–206.
37. Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, Breiman RF. Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. *N Engl J Med* 2000;**342**:681–9.
38. Sigaud S, Goldsmith CA, Zhou H, Yang Z, Fedulov A, Imrich A, Kobzik L. Air pollution particles diminish bacterial clearance in the primed lungs of mice. *Toxicol Appl Pharmacol* 2007;**223**:1–9.
39. Hill PC, Townend J, Antonio M, Akisanya B, Ebruke C, Lahai G, et al. Transmission of *Streptococcus pneumoniae* in rural Gambian villages: a longitudinal study. *Clin Infect Dis* 2010;**50**:1468–76.
40. Hill PC, Cheung YB, Akisanya A, Sankareh K, Lahai G, Greenwood BM, Adegbola RA. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian infants: a longitudinal study. *Clin Infect Dis* 2008;**46**:807–14.
41. Mohle-Boetani JC, Ajello G, Breneman E, Deaver KA, Harvey C, Plikaytis BD, et al. Carriage of *Haemophilus influenzae* type b in children after widespread vaccination with conjugate *Haemophilus influenzae* type b vaccines. *Pediatr Infect Dis J* 1993;**12**:589–93.