Indian J Med Res 142, September 2015, pp 286-292
DOI:10.4103/0971-5916.166588

Pneumococcal serotypes associated with invasive disease in under five children in India & implications for vaccine policy

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Received October 31, 2013

***Background & objectives*: *Streptococcus pneumoniae* is a major cause of morbidity and mortality especially in children less than five years, particularly in India. We present data on *S.pneumoniae* infections in children less than five years age group, with response to its serotype distribution, antibiotic resistance profile and available vaccines expected coverage.**

***Methods*: Children aged less than five, who were suspected for invasive pneumococcal disease were included in the study and their sterile body fluids were investigated for the presence of *S. pneumoniae*. Invasive *S. pneumoniae* isolates from sterile body fluids were identified by bile solubility and optochin susceptibility test. Pneumococcal serotyping was performed with co-agglutination technique and reconfirmed with multiplex PCR.**

***Results*: The most common pneumococcal serotypes causing invasive infections in children less than five years of age were 14, 19F, 5, 6A and 6B. Of the 114 *S. pneumoniae* isolates studied, 110 (96.4%) were non-susceptible to co-trimoxazole and 30 per cent were non-susceptible to erythromycin, 5.2 per cent of the isolates were non-susceptible to penicillin and only 0.8 per cent was non-susceptible to cefotaxime.**

***Interpretation & conclusions*: Our results indicate that PCV-10 can protect against 64 per cent of serotypes causing invasive pneumococcal infections. Use of PCV-13 in this region can provide increase in protection upto 74.6 per cent against serotypes causing invasive pneumococcal infections. Incorporating PCV-13 in the Universal Immunization Programme may provide incremental protection against IPD serotypes in the southern region of the country.**

Key words Invasive pneumococcal disease - PCV - pneumococcal serotypes - vaccine coverage

Pneumococcal disease is a serious global problem with an estimated 14.5 million episodes of invasive pneumococcal disease (IPD) and approximately 500,000 deaths each year in children under five years of age, almost all from low- and middle-income countries[1]. A review of more than 70 studies has shown that only 10 serogroups are responsible for most paediatric infections, serogroups 1, 6, 14, 19, and 23 are the major encountered serogroups in each continent around the world in paediatric age group[2].The impact of the seven-valent pneumococcal conjugate vaccine (PCV-7) that targets 4, 6B, 9V, 14, 18C, 19F, 23F

serotypes has been dramatic in most countries where it was introduced. In the United States alone, three years after introduction of PCV-7 the overall decline of IPD was 75 per cent with a decrease of 94 per cent in the rate of IPD in children <5 years of age[3]. At the time of licensure, PCV-7 covered 80 per cent of pneumococcal serotypes causing invasive disease in most developed countries. However, in India and other developing countries, PCV-7 was found to have a protective efficacy of only around 50 per cent due to differences in the prevalent serotypes[4].

After introduction of PCV-7, surveillance studies from the US showed a decrease in cases of IPD due to vaccine serotypes and an increase in cases due to non-vaccine serotypes, the "replacement phenomenon"[5]. Among non PCV-7 serotypes, 1 and 5 cause significant pneumococcal disease in India[6] as well as in other developing countries[7]. Serotype 3 usually causes non-invasive disease but can also cause IPD which is associated with increased mortality[8]. Serotypes 6A and 19A were not included in PCV-7 as it was thought that cross-protection would be provided by the immune response to serotypes 6B and 19F[9]. Though some cross-protection was observed for serotype 6A, but no significant clinical cross-protection was observed against serotypes 6C and 19A[10,11]. Serotype 19A which is prevalent worldwide causes disease in all age groups and is highly multidrug resistant[12-14]. Inadequate coverage of serotypes by PCV-7 has led to the formulation of PCV-10 that provides protection against 1, 5 and 7F and PCV-13 which protects against 3, 6A and 19A, in addition to protection against PCV-7 serotypes.

PCV-7 was originally recommended as an optional vaccine by the Indian Academy of Pediatrics (IAP)[15] but was removed from market since 2010. PCV-10 and PCV-13 have also recently been licensed for use in India and are now recommended for use as routine vaccines under the IAP schedule[16].

Here we present collated information on serotypes causing invasive pneumococcal disease in under five children from 2007 - June 2013 in a tertiary care hospital in south India, to predict the benefits we expect to get on using PCV-10 and PCV-13 in Indian children.

Material & Methods

Study setting and sample population: This study was carried out at the Christian Medical College and Hospital (CMCH), a multi speciality tertiary care, 2082 bedded hospital, situated at Vellore district in Tamil Nadu, India. Children aged 60 days to five years who attained medical help for suspected invasive pneumococcal diseases in CMCH during January 2007 through June 2013 were screened for this study. During the study period 2,42,211 children (in 60 days to 5 year age group) attained medical help.

The standard case definition used to set up inclusion and exclusion criteria to screen children has been described elsewhere[6]. Informed written consent was obtained from their parents or guardians and the study protocol was approved by the Institutional Review Board (IRB) of CMCH, Vellore.

Laboratory methods: Sample collection (blood, CSF and pleural fluid) and laboratory investigations were performed as a part of routine clinical practice of patient care. Blood specimen (2-4 ml) was collected and bed side inoculation was made aseptically in paediatric BacT/ALERT bottle (biomerieux, France) and transferred immediately to microbiology laboratory and loaded in BacT/ALERT 3D system (Automated blood culture system from biomerieux, France) until a positive signal was observed. If no positive signal was observed for seven days, the blood culture was ruled out as negative. Positive blood cultures were further processed for microbiological confirmation of *S. pneumoniae*. Briefly, broth from positive BacT/ALERT bottles were removed aseptically using sterile syringe and plated onto 10 per cent sheep blood agar and incubated at CO_2 incubator at 37°C. Alpha haemolytic colonies suspected to be of *S. pneumoniae* were further sub-cultured onto 10 per cent sheep blood agar and further characterized with bile soluble test and optochin susceptibility test[17]. In case of CSF and pleural fluid specimens, care was taken to process the specimen as early as possible. Briefly, CSF and fluid samples were centrifuged and sediments were plated directly onto 10 per cent sheep blood agar and incubated at CO_2 incubator at 37°C. If alpha haemolytic colonies were identified, further characterization of *S. pneumoniae* was performed as described earlier[17]. *S. pneumoniae* isolates were serogrouped/typed by co-agglutination technique[18] with Neufeld antisera, Pool A-I, 25 types and 21 groups corresponding with specific set of factor sera were obtained from Statens Serum Institut (Copenhagen, Denmark)[18]. Detailed schematic diagramme of co-agglutination technique is described in Fig. 1. The serogroup/type was reconfirmed by multiplex PCR following Centers for Disease Control and Prevention (CDC) protocol[19]. Technical training, molecular reagents and all *S. pneumoniae,* serogroup/

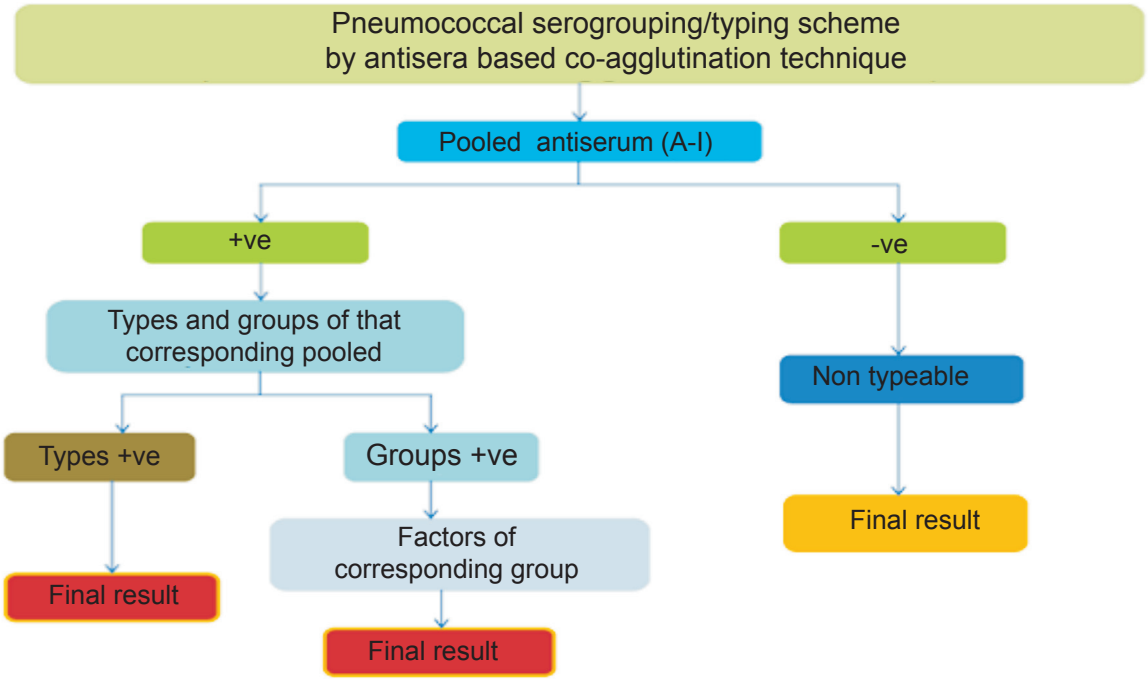

Fig. 1. Schematic diagram of co-agglutination technique.

type control strains were obtained from *Streptococcus* Laboratory, CDC, Atlanta, USA, as a part of World Health Organization supporting Invasive Bacterial Disease surveillance activity. Antimicrobial susceptibility testing (AST) for 60 isolates from 2007-2010 was done by agar dilution method[20] for the following antibiotics: penicillin, cefotaxime, erythromycin, chloramphenicol and co-trimoxazole. For the remaining 54 isolates from 2011-June 2013, AST was done by Vitek system 2 (biomerieux, France) for the antibiotics penicillin, cefotaxime, ceftriaxone, levofloxacin, erythromycin, clindamycin, linezolid, vancomycin, tetracycline and co-trimoxazole. Antimicrobial susceptibility tests were interpreted based on the Clinical Laboratory Standards Institute (CLSI) recommendations[21].

Results

A total of 114 *S. pneumoniae* isolates were obtained from children less than five years. Overall, the prevalent serotypes causing IPD in children <5 yr in descending order were 14 (17.5%), 19F (12.2%), 5 (8.7%), 6A (8.7%), 6B (8.7%), covering up to 56 per cent of the total isolates (Fig. 2). PCV-13 targeted serotypes were responsible for 74.6 per cent of the IPD in this study group. Serotype 3 was included in PCV-13, but none of our study isolates had serotype 3 (Figs 2 and 3).

Antimicrobial susceptibility testing revealed that 110 (96.4%) isolates were non-susceptible to co-trimoxazole, 35 isolates (30%) were non-susceptible to erythromycin. Six isolates (5.2%) were non-susceptible to penicillin, of which three isolates were non-susceptible to erythromycin as well. The one isolate that was non-susceptible to cefotaxime was also non-susceptible to erythromycin (Fig. 4).

Majority of the antibiotic resistance was caused by serotypes targeted by 13 valent pneumococcal vaccine (PCV-13). Of the 85 isolates causing IPD that would have been covered by PCV-13, 31 isolates (36.4%) were non-susceptible to erythromycin. All six of the penicillin non-susceptible isolates and the one isolate not susceptible to cefotaxime also fell into this group (Fig. 5). The non-vaccine targeted serotypes two each of 11A, 15B, 22A, 27, 33C, 33F, 23A, 47F, and one each of 6C, 8, 9N, 10A, 10F, 15C, 17F, 18A, 19B, 24B, 24F, 29, 39 were relatively susceptible to penicillin and cefotaxime. Only four (13.7%) of the non vaccine serotypes 8, 11A, 18A, 24F were non-susceptible to erythromycin.

Discussion

It should be taken into consideration that in most cases the PCV coverage is estimated only with culture positive *S.pneumoniae* isolates, as widely used

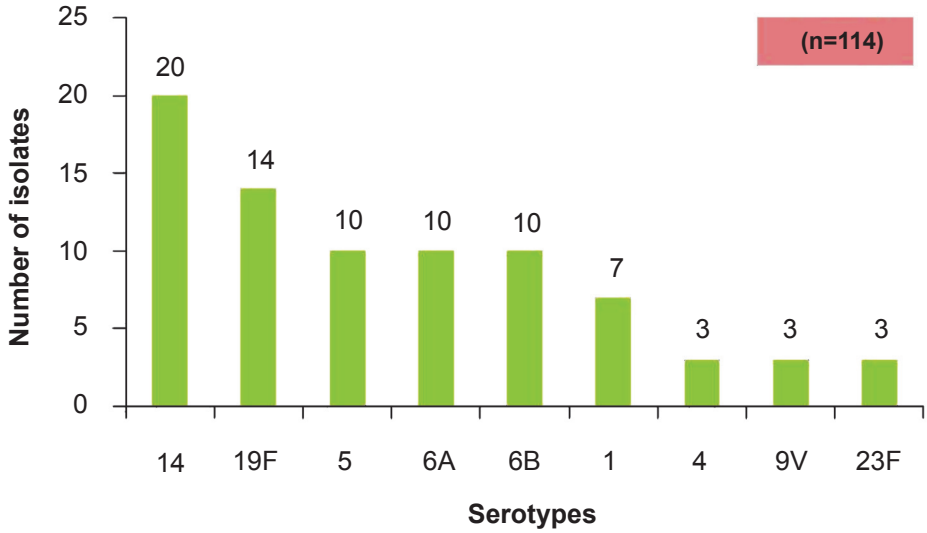

Fig. 2. Invasive *S. pneumoniae* serotype distribution among children less than five years from 2007 - June 2013. Not shown : Two isolates each of 18C, 19A, 11A, 15B, 22A, 27, 33C, 33F, 23A, 47F and one isolate each of 6C, 7F, 8, 9N, 10A, 10F, 15C, 17F, 18A, 19B, 24B, 24F, 29, 39.

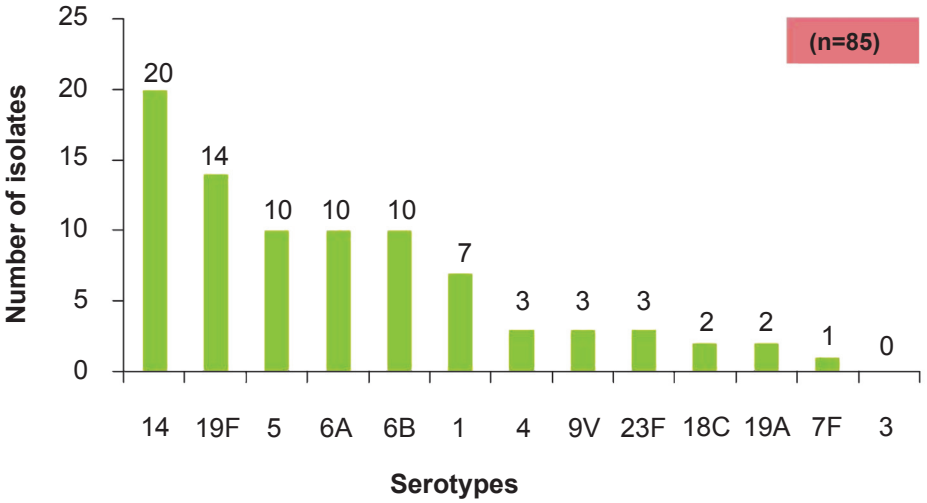

Fig. 3. Prevalence of PCV-13 serotypes among invasive *S. pneumoniae* isolates in children less than five years from 2007-June 2013.

serotyping methods require culture positive isolates, but in India and other developing countries most cases of clinically suspected IPD are culture negatives due to lack of laboratory facilities and/or initiation of therapy before collection of samples for culture. Hence the true burden of the disease and the predominant serotypes are not clear and estimation of vaccine protective efficacy with available data may not reflect true nature. Pre antibiotic use limits the invasive pneumococcal incidence detection around 39[22] to 60 per cent[23], but serotype distribution remains almost similar in both culture positive and culture negative IPD.

Our data showing only 48.2 per cent coverage by PCV-7 against IPD serotypes (data not shown) is in concordance with similar low coverage from other Asian countries such as Bangladesh (31%)[24], and Nepal (15%)[25]. In contrast, 61 per cent of serotypes causing IPD in Sri Lanka were covered by PCV-7[26]. This difference in serotypes prevalence within neighbouring countries can create bias in predicting the vaccine

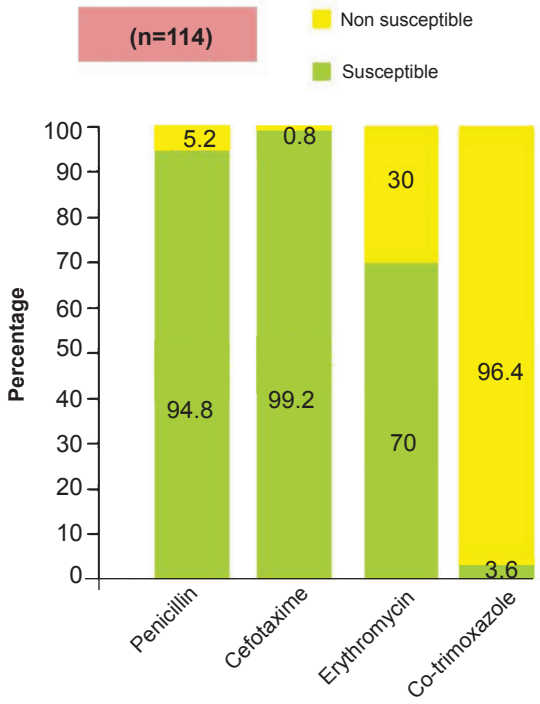

Fig. 4. Antimicrobial susceptibility profile for invasive *S.pneumoniae* isolates under children less than five years from 2007-June 2013.

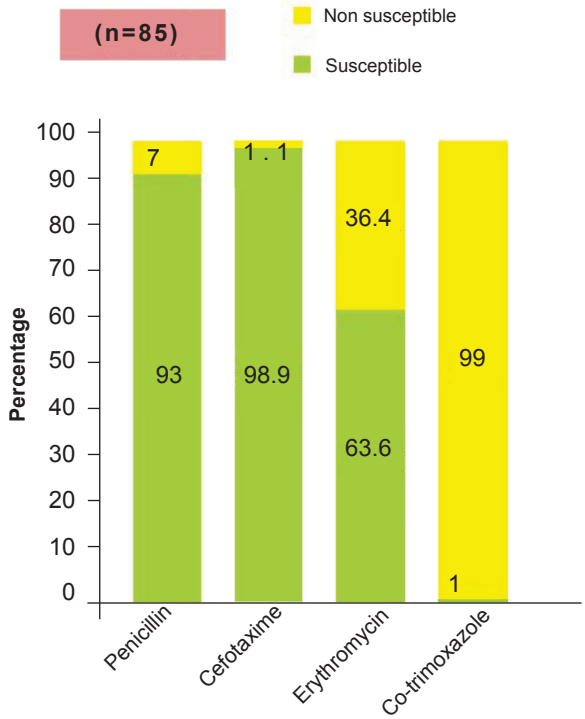

Fig. 5. Antimicrobial susceptibility profile for PCV-13 targeted *S. pneumoniae* isolates under children less than five years from 2007-June 2013.

coverage in a particular continent. Use of PCV-10 and PCV-13 would provide coverage of 64 and 74.6 per cent, respectively (Fig. 6) against serotypes associated with IPD from our centre. Inclusion of serotypes 1, 5 and 6A in PCV-13 contributes to 23.6 per cent increase in coverage compared to PCV-7 in this study group. Serotype 19A, although a prominent cause of IPD among non PCV-7 serotypes has been reported in developing[27] and developed countries[28,29], however, we found only two isolates of 19A serotypes causing IPD in our study.

Although non-vaccine serotypes accounted for 25.4 per cent of IPD from our centre, individual number of isolates were equally divided as follows, two each of 11A, 15B, 22A, 27, 33C, 33F, 23A, 47F, and one each of 6C, 8, 9N, 10A, 10F, 15C, 17F, 18A, 19B, 24B, 24F, 29, 39. This makes it difficult to suggest any among the non-vaccine serotypes for inclusion in vaccine formulation. There is a need to establish more sentinel surveillance centres all over the country to gather sufficient data about the importance of non-PCV13 serotypes in IPD.

Drug resistant *S.pneumoniae* is a growing concern. Within the Southeast Asian Region, Sri Lanka has reported more than 90 per cent penicillin non-susceptibility in *S. pneumoniae* in children under five years of age[26]. Among Asian countries, India has the lowest incidence of penicillin non-susceptible *S. pneumoniae*[30]. However, non-susceptibility to penicillin among IPD serotypes has shown an increase from 0 per cent in 1995 to 1.3 per cent in 1999[27] and to 5.2 per cent non-susceptibility in the present study. This shows the increase of non-susceptibility to penicillin (8%) in the >5 yr age group children within the same time period (unpublished data). Increase in erythromycin non-susceptibility is also a concern in IPD treatment. Erythromycin non-susceptibility was only 4.2 per cent in 1999[6], but in the present study 30 per cent isolates were non-susceptible to erythromycin. A study from Sri Lanka has reported high erythromycin non-susceptibility with 60.8 per cent[26], but non-susceptibility to erythromycin was low in Nepal (7.1%)[25]. High percentage of non-susceptibility for co-trimoxazole seen in neighbouring countries like Bangladesh (>76%)[24], and Sri Lanka (around 73.9%)[26], was in concordance with present data.

In conclusion, our findings show that inclusion of PCV-13 in the National immunization schedule under Universal Immunization Programme of India

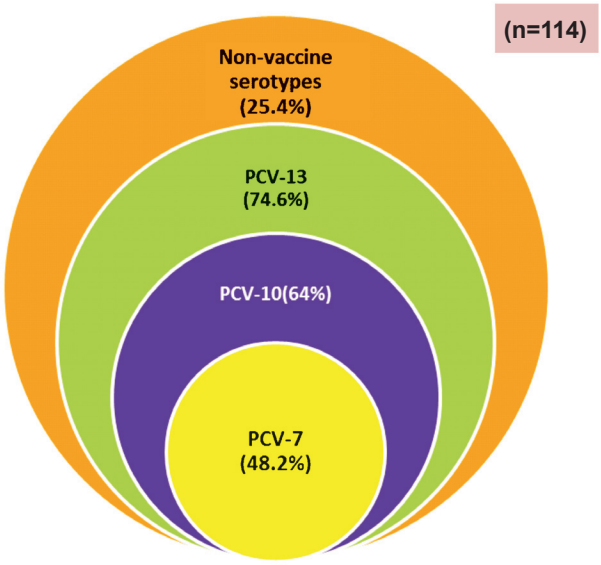

Fig. 6. Expected protective coverage for pneumococcal congugate vaccine-13 (PCV-13) against invasive pneumococcal disease (IPD) in India.

would provide incremental coverage against serotypes causing IPD in southern India. Vaccination in children indirectly provides protection to the older population against pneumococcal disease, and would contribute to a reduced carriage of drug resistant and multi-drug resistant serotypes.

References

1. Centers for Disease Control and Prevention (CDC). Progress in introduction of pneumococcal conjugate vaccine - worldwide, 2000-2012. *MMWR Morb Mortal Wkly Rep* 2013; *62* : 308-11.
2. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis* 2005; *5* : 83-93.
3. Centers for Disease Control and Prevention (CDC). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease - United States, 1998-2003. *MMWR Morb Mortal Wkly Rep* 2005; *54* : 893-7.
4. Bravo LC, Asian Strategic Alliance for Pneumococcal Disease Prevention (ASAP) Working Group. Overview of the disease burden of invasive pneumococcal disease in Asia. *Vaccine* 2009; *27* : 7282-91.
5. Byington CL, Samore MH, Stoddard GJ, Barlow S, Daly J, Korgenski K, *et al*. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clin Infect Dis* 2005; *41* : 21-9.
6. Invasive Bacterial Infection Surveillance (IBIS) Group, International Clinical Epidemiology Network (INCLEN). Prospective multicentre hospital surveillance of *Streptococcus pneumoniae* disease in India. *Lancet* 1999; *353* : 1216-21.
7. Le CF, Mohd Yusof MY, Shamala D, Sekaran SD. Current trends in pneumococcal serotype distribution in Asia. *J Vaccines Vaccin* 2011; *S2* : 001.
8. Martens P, Worm SW, Lundgren B, Konradsen HB, Benfield T. Serotype-specific mortality from invasive *Streptococcus pneumoniae* disease revisited. *BMC Infect Dis* 2004; *4* : 21.
9. Paradiso PR. Advances in pneumococcal disease prevention: 13-valent pneumococcal conjugate vaccine for infants and children. *Clin Infect Dis* 2011; *52* : 1241-7.
10. Park IH, Moore MR, Treanor JJ, Pelton SI, Pilishvili T, Beall B, *et al*. Differential effects of pneumococcal vaccines against serotypes 6A and 6C. *J Infect Dis* 2008; *198* : 1818-22.
11. Hausdorff WP, Hoet B, Schuerman L. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? *BMC Pediatr* 2010; *10* : 4.
12. Kaplan SL, Barson WJ, Lin PL, Stovall SH, Bradley JS, Tan TQ, *et al*. Serotype 19A Is the most common serotype causing invasive pneumococcal infections in children. *Pediatrics* 2010; *125* : 429-36.
13. Reinert R, Jacobs MR, Kaplan SL. Pneumococcal disease caused by serotype 19A: review of the literature and implications for future vaccine development. *Vaccine* 2010; *28* : 4249-59.
14. Hulten KG, Kaplan SL, Lamberth LB, Barson WJ, Romero JR, Lin PL, *et al*. Changes in Streptococcus pneumoniae serotype 19A invasive infections in children from 1993 to 2011. *J Clin Microbiol* 2013; *51* : 1294-7.
15. Indian Academy of Pediatrics Committee on Immunization (IAPCOI). Consensus recommendations on immunization, 2008. *Indian Pediatr* 2008; *45* : 635-48.
16. Indian Academy of Pediatrics Committee on Immunization (IAPCOI). Consensus recommendations on immunization and IAP immunization timetable 2012. *Indian Pediatr* 2012; *49* : 549-64.
17. Castillo D, Harcourt B, Hatcher C, Jackson M, Katz L, Mair R, *et al*. Laboratory Methods for the Diagnosis of Meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae,* WHO manual, 2nd ed. Geneva: World Health Organization; 2011.
18. Lalitha MK, Pai R, John TJ, Thomas K, Jesudason MV, Brahmadathan KN, *et al*. Serotyping of *Streptococcus pneumoniae* by agglutination assays: a cost-effective technique for developing countries. *Bull World Health Organ* 1996; *74* : 387-90.
19. Centers for Disease Control and Prevention (CDC). Protocol for multiplex PCR - *S. pneumoniae* stereotyping - clinical specimens and pneumococcal isolates - African set. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2014. Available from: *http://www.cdc.gov/streplab/downloads/pcr-africa-clinical-specimens.pdf*, accessed on January 19, 2014.
20. Zhang SX, Rawte P, Brown S, Lo S, Siebert H, Pong-Porter S, *et al*. Evaluation of CLSI agar dilution method and Trek Sensititre broth microdilution panel for determining antimicrobial susceptibility of *Streptococcus pneumoniae*. *J Clin Microbiol* 2011; *49* : 704-6.

21. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI document M100-S23. Wayne, PA: CLSI; 2013.

22. Rhodes J, Hyder JA, Peruski LF, Fisher C, Jorakate P, Kaewpan A, *et al*. Antibiotic use in Thailand: quantifying impact on blood culture yield and estimates of pneumococcal bacteremia incidence. *Am J Trop Med Hyg* 2010; *83* : 301-6.

23. Saha SK, Darmstadt GL, Baqui AH, Hossain B, Islam M, Foster D, *et al*. Identification of serotype in culture negative pneumococcal meningitis using sequential multiplex PCR: implication for surveillance and vaccine design. *PLoS One* 2008; *3* : e3576.

24. Arifeen SE, Saha SK, Rahman S, Rahman KM, Rahman SM, Bari S, *et al*. Invasive pneumococcal disease among children in rural Bangladesh: results from a population-based surveillance. *Clin Infect Dis* 2009; *48* (Suppl 2) : S103-13.

25. Shah AS, Knoll MD, Sharma PR, Moisi JC, Kulkarni P, Lalitha MK, *et al*. Invasive pneumococcal disease in Kanti Children's Hospital, Nepal, as observed by the South Asian Pneumococcal Alliance network. *Clin Infect Dis* 2009; *48* (Suppl 2) : S123-8.

26. Batuwanthudawe R, Karunarathne K, Dassanayake M, de Silva S, Lalitha MK, Thomas K, *et al*. Surveillance of invasive pneumococcal disease in Colombo, Sri Lanka. *Clin Infect Dis* 2009; *48* (Suppl 2) : S136-40.

27. Kim SH, Song J-H, Chung DR, Thamlikitkul V, Yang Y, Wang H, *et al*; ANSORP Study Group. Changing trends in antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Antimicrob Agents Chemother* 2012; *56* : 1418-26.

28. Tan TQ. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. *Clin Microbiol Rev* 2012; *25* : 409-19.

29. Weil-Olivier C, van der Linden M, de Schutter I, Dagan R, Mantovani L. Prevention of pneumococcal diseases in the post-seven valent vaccine era: a European perspective. *BMC Infect Dis* 2012; *12* : 207.

30. Veeraraghavan B, Kurien T. Penicillin resistant *Streptococcus pneumoniae* in India: effects of new clinical laboratory standards institute breakpoint and implications. *Indian J Med Microbiol* 2011; *29* : 317-8.

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