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Focus on pneumococcal vaccines and nasopharyngeal carriage*

Focos em vacinas pneumocócicas e o portador na nasofaringe

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Abstract
The vaccination is the only available tool to prevent disease caused by *Streptococcus pneumoniae*. In this review emphasis was given on pneumococcal conjugate vaccine (PCV) and *S. pneumoniae* nasopharyngeal carriage, as nasopharynx colonization precede invasive disease. To optimize the development of future conjugate vaccines and to evaluate their efficacy, it is necessary to understand the serogroup specific epidemiology of pneumococci and their associated disease types. Continuous monitoring of *S. pneumoniae* serotypes is essential since it has been shown that the incidence of types responsible for invasive disease can change over time. The extended protection increases the cost effectiveness of PCV and should clearly encourage its use in poorly resourced countries. However, the accumulated experience also shows that the herd immunity, due to PCV, is partly offset by replacement of the vaccine serotypes by other, nonvaccine serotypes. Owing to the general reduced virulence of the latter, this has only had a modest effect on disease, but the possibility of more virulent nonvaccine serotypes arising cannot be ignored and should be the focus of continued surveillance
Keywords: Pneumococcal vaccine – Nasopharyngeal carriage – Vaccines – *Streptococcus pneumoniae*.

Resumo
A vacinação é a única ferramenta disponível para prevenir as doenças causadas pelo Streptococcus pneumoniae*. Nesta revisão, foi dada ênfase na vacina conjugada pneumocócica (PCV) e o portador de S. pneumoniae de nasofaringe, uma vez que a colonização da nasofaringe precede a doença invasiva. Para otimizar o desenvolvimento de futuras vacinas conjugadas e avaliar a sua eficácia, é necessário compreender a epidemiologia e os sorotipos específicos de pneumococos e os tipos associados à doença. A monitoração contínua dos sorotipos de S. pneumoniae é essencial, uma vez que foi demonstrado que os tipos responsáveis pela doença invasiva podem mudar ao longo do tempo. A cobertura da PCV demonstrada justifica a sua utilização em países com poucos recursos. No entanto, a experiência acumulada mostra também que ocorre imunidade do rebanho devido a PCV, bem como substituição dos sorotipos da vacina por outros sorotipos não-incluídos na vacina. Devido à sua reduzida virulência, só se observou um efeito modesto sobre a doença, mas a possibilidade de ocorrer sorotipos não-vacinais mais virulentos não pode ser ignorada e devem ser monitorados de forma contínua.*
***Palavras-chave**: Vacina pneumocócica – Portador na nasofaringe – Vacinas – Streptococcus pneumoniae.*

INTRODUCTION

Streptococcus pneumoniae, is a Gram positive alpha-haemolytic encapsulated diplococcus. The polysaccharide capsule defines the serotype and at present, 91 distinct serotypes were identified[1]. The major virulence factor of *S. pneumoniae* is the polysaccharide capsule, which prevents phagocytosis of the bacteria by macrophages. Most *S. pneumoniae* serotypes have been shown to cause serious disease, but only a few serotypes produce the majority of pneumococcal infections. The reservoir for pneumococci is presumably the nasopharynx (NP) of asymptomatic human carriers. The rate of *S. pneumoniae* nasopharyngeal carriage varies with age, geographical location, socioeconomic status and in households with children.[2,3]

The pneumococcal colonization of the NP occurs early in life depending on the local epidemiology. A single serotype usually is carried for extended periods (45 days to 6 months). Carriage does not consistently induce local or systemic immunity sufficient to prevent later reacquisition of the same serotype. The colonization is the starting point for all relevant aspects of this pathogen[4]. As a result of vaccination, a decrease in carriage of vaccine serotypes and a significant increase of nonvaccine serotypes occurs in immunized children, probably due to replacement of serotypes or unmasking of minority populations of *S. pneumoniae* present in the NP since multiple serotypes can colonize simultaneously[5]. Although the biology of *S. pneumoniae* carriage is not well understood, the human NP is the reservoir of pneumococcal and studies suggest that NP colonization can reflect the epidemiological aspects of pneumococcal disease in the community.[6]

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During the past 4 decades, serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F constituted the majority of invasive isolates in children in the United States and other developed countries. Of these, serotypes 6B, 9V, 14, and 19F frequently have reduced susceptibility to penicillin. Rates of pneumococcal carriage peak during the first two years of life and decline gradually thereafter. Carriage rates are highest in institutional settings and during the winter, and rates are lowest in summer. Nasopharyngeal carriage of pneumococci is common among young children attending day care with rates of 61.3% in point prevalence estimates.[7]

PNEUMOCOCCAL VACCINES

Pneumococcal polysaccharide vaccine

The first polysaccharide pneumococcal vaccine was licensed in the United States in 1977. It contained purified capsular polysaccharide antigen from 14 different types of pneumococcal bacteria. In 1983, a 23valent polysaccharide vaccine (PPSV23) was licensed and replaced the 14valent vaccine, which is no longer produced. The PPV23 is a polysaccharide vaccine composed of capsular polysaccharide antigens purified from the 23 most prevalent pneumococcal 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 that cause 88% of bacteremic pneumococcal disease.[8]

The PPV23 is primarily designed for use in older children and adults who are at risk for pneumococcal disease. It is not licensed for use in children <2 years of age. In some countries it is recommended by the public health authorities for all adults at the age of 60 years or older. It is not effective for children under 2 years of age because the immune response in this age group is reduced with low production of specific antibodies and the memory phenomenon does not occur.[9]

Pneumococcal conjugate vaccine

Employment of pneumococcal conjugate vaccine is particularly successful in young children vaccination. Capsular polysaccharides of PCV7 are conjugated to highly immunogenic cross reactive material 197 (CRM197), a nontoxic diphtheria toxoid protein. The CRM197specific type 2 helper T (Th2) cells interact with B cells that have bound and internalized the polysaccharideCRM197 complex via polysaccharide specific.

IgM and subsequently present the processed CRM197 protein along with MHC II to effector T cells. This type of adaptive immune response is characterized by antibody isotype switching and the generation of memory B cells.[9] Prevention of pneumococcal nasopharyngeal colonization is the first step in the infection cycle that has important consequences: it reduces chances of spread of the infection and indirectly protects from disease. Through these indirect effects, the protection afforded by the vaccine extends to the whole population, including those not vaccinated (herd immunity).[10]

After the introduction of PCV7 in USA in the infant vaccination program, it was observed a reduction of 76% in the overall incidence of invasive pneumococcal disease (IPD), and 94% for vaccine serotypes in children younger than five years old [11]. This PCV7 came onto the market in Europe in 2002.

The PCV7 available in Brazil was licensed for use in February 2001 but was not introduced in the National Immunization Program (NIP). The estimated cover of PCV7 is 73,9% for invasive serotypes [3]. Furthermore, it could also protect against serotypes that are cross reactive antigenic similarity: 6A, 9A, 9L, 18B and 18F [12]. At the present time the development of PCVs against more than seven serotypes continues undiminished and different types of carrier protein are under development. Pneumococcal conjugate vaccine 10valent (PCV10) includes the PCV7 serotypes plus serotypes 1, 5, and 7F, conjugated with the protein D from nontypeable *H. influenzae*, and has already been licensed for use in Brazil. The pneumococcal conjugate vaccine PCV13 contains the PCV10 3, 6A, and 19A, and has recently been approved by the regulatory agencies of European communities (EMEA), Chile and Canada. The PCV13 is under evaluation in Brazil by ANVISA. Studies carried out with PCV13 showed that the vaccine was well tolerated and more immunogenic than the polysaccharide to the most serotypes contained in the two vaccines.[13]

Since the introduction of PCV7 in the USA there is a need to expand coverage to include other serotypes. The potential coverage of PCV7, PCV10 and PCV13 against IPD, acute otitis media (AOM), acute conjunctivitis (AC) and patients in southern Israel, before the introduction of PCV7 in the period 2000-2004 were evaluated. A total of 5497 samples were collected from children <36 months: 189 from blood or cerebral spinal fluid (CSF), 3197 from the middle ear secretion, 348 from the conjunctiva, and 1763 from the NP of healthy children. According to the serotypes detected, PCV7 coverage for IPD, AOM, AC, and carriage would be 44%, 54%, 37% and 46%, respectively. The PCV10 extended coverage primarily to IPD, whereas the addition of serotypes 6A and 19A in PCV13 increased coverage in all entities (84%, 79%, 54% and 67% in IPD, AOM, AC, and carrier, respectively).[14]

Conjugate vaccines are protective against pneumococcal disease; however the vaccination resulted in selective pressure for replacement with nonvaccine *S. pneumoniae* serotypes in both invasive disease and asymptomatic carriage [15]. In almost all clinical trials among vaccinated children has been observed a reduction in NP colonization of vaccine serotypes and an increase in nonvaccine serotypes[16]. The clinical significance of change in the microbiota and consequent replacement of serotypes is unclear, but the replacement with potentially virulent strains and development of antibiotic resistance in nonvaccine type pneumococci are theoretically possible and should encourage more

prudent use of antiinfectives to reduce antibiotic pressure[17]. Analysis of colonizing serotypes among healthy children in communities provides critical data on changes in serotype distribution and antimicrobial susceptibility, particularly of emerging nonvaccine strains[18]. A prospective study to assess the impact of the PCV7 in resistant pneumococci colonizing the NP of healthy children in day care aged 6 months to 6 years was conducted, and was observed a reduction in colonization with penicillin resistant serotypes vaccine related, and could notice resistance increase in nonvaccine serotype[19]. Pediatric vaccination with PCV7 has also resulted in decreased PCV7type pneumococcal carriage among adults and helps to explain recent decreases in the rate of PCV7type invasive pneumococcal disease among adults.[20]

A significant reduction in colonization rate of penicillin resistant pneumococci, especially in children under 36 months, and also an increase in colonization with serotypes not contained in the vaccine, which were susceptible to penicillin after the PCV9 vaccination was observed [21]. The carrier state of resistant pneumococci remained low, concluding that the rate *S. pneumoniae* resistant was high in childcare and that the conjugate vaccines appear to be an important tool to reduce the antibiotic resistant pneumonia rate in nursery.

Several questions are open to debate especially those related to NP colonization, antimicrobial resistance, vaccination, and cross reaction of serotype replacement, although many of these issues can be addressed and possibly resolved by mass vaccination, accompanied by an accurate monitoring. In the state of the art, it is clear that the conjugate vaccine significantly reduces pneumococcal disease in vaccinated individuals and indirectly in nonvaccinated contacts, and is generally well tolerated and safe.[22]

IMPORTANCE OF MONITORING *S. pneumoniae*

High rates of pneumococcal carriage are detected in day care centers with higher prevalence of multidrugresistant strains, which emphasize the role of these institutions as the focus of selection and spread of resistant isolates[23]. Thus, the monitoring of children with resistant pneumococci should be established, to control the spread of resistant clones, especially in institutionalized children. Although a limited number of serotypes cause the majority of invasive pneumococcal disease and multiple serotypes can colonize the NP. The identification of these serotypes is important in monitoring programs and to evaluate the vaccination effect on the carrier status. This dynamic of replacing serotypes, the impact of vaccination, can result in major changes in the epidemiology of invasive pneumococcal disease [24]. The presence of multiple strains of pneumococci in substantially lower levels in the NP may have an important role in the dynamics of population shift that occurs when the balance of pneumococcal NP is changed by interventions such as the introduction of vaccines, antimicrobial treatment or improvements in infection control measures.[25]

In Brazil, few studies have been conducted to assess the NP colonization and distribution of pneumococcal serotypes in children younger than five years [26,27,28,29,30,31]. The distribution of serotypes and resistance of pneumococci colonizing the NP should be used as one of the predictors of invasive disease[2]. However, there is difficulty in identifying multiple serotypes in a single carrier. Strategies should be developed to evaluate the impact of conjugate vaccines on the pneumococcal ecology in the NP. There is no single method available to identify multiple serotypes of a simultaneous original sample without the step of culture, since specific primers for all capsular types are not available [32]. The high cost of antisera, the subjectivity in the interpretation and technical skill is serious disadvantages of the classical method of agglutination for serological determination. The development of genotyping techniques based on multiplex PCR system could be a simple and effective technique to infer serotypes in large numbers of isolates [32]. The conventional multiplex PCR method to serotype pneumococcal has shown good sensitivity and specificity[24,32,33] and represent a valuable tool in investigations of surveillance of pneumococci.[33]

A total of 446 pneumococci isolated from children NP of attending day care centers in Lisbon (Portugal) were serotyped by classic immunological techniques and by conventional multiplex PCR. The capsular typing results obtained by PCR were consistent with the results obtained by the classic serotyping with accuracy and cost effective[34]. A system for capsular typing of pneumococcal, comprising 29 serotypes, with seven sequential reactions, based on the sequence of multiplex PCR were outlined.[33] A total of 421 *S. pneumoniae* from Active Bacterial Core Surveillance (ABC) were randomly selected to validate the technique. In 229 (54.4%) capsular typing, the results showed complete agreement with the results obtained by classic serotyping (Quellung reaction). A total of 172 (40.9%) was characterized in pneumococcal serogroup and 20 (were rare serotypes or nontypeable) not included in the reaction. The multiplex PCR technique represents a valuable tool in investigations of pneumococcal surveillance. The conventional multiplex PCR method [35] was adapted to include the most prevalent serotypes in Latin America and 139/147 (94.6%) of the isolates were demonstrating the efficiency and accuracy of the method[36]. The same strategy was used of adapting the multiplex PCR reactions according to the serotype prevalence in Mozambique for pneumococcal serotype, and also demonstrated the efficiency and accuracy of the technique. It is important to demonstrate the flexibility of the method, by altering the combinations of specific serotypes to achieve the strains diversity in different countries.[37]

A rapid pneumococcal serotyping called multibead test, based in a multiplex immunoassay type inhibition

for 36 capsular polysaccharides was highly specific and could differentiate the 90 pneumococcal serotypes represented. To validate this test *S. pneumoniae* were serotyped by the agglutination test in reference laboratories in their countries of origin and the lysate of each strain were coded and sent to the USA for the multibead test. The method showed agreement in 89% of the results and discrepancies that have persisted in only eight isolates involving serotypes 6A, 11A and 18C. Later studies showed that the discrepancies were due to technical problems (reagents) used in multibead or agglutination test for these three serotypes. The test has been considered appropriate for epidemiological studies since it is simple, low cost, fast and accurate [38]. The conventional multiplex PCR was employed to evaluate the secretion of NP in children with otitis media. The most frequent pneumococcal serogroups and serotypes were 6, 19F and 23F. The technique allowed a rapid characterization of pneumococcal serotypes and resistance genes [39]. The technique of multiplex PCR was also used to detect *S. pneumoniae* directly in 279 NP swabs and a greater number of serotypes were detected when compared with conventional methods such as culture, latex agglutination and Quellung reaction.[40]

The knowledge of cocolonization with multiple serotypes of pneumococci is very important in clarifying the replacement and change as a result of vaccination. Cocolonization has been reported in more than 30% of patients, especially in populations with high rates of colonization[41]. The technique of multiplex PCR was applied in 50 primary cultures of NP samples and identified a second serotype in 20% of patients [42]. An important gap in knowledge about pneumococcal refers to the genetic diversity of these bacteria in a single clinical specimen. However, one of the recommendations of the Vaccines Department from the World Health Organization (WHO) is the development of standardized methods for the study of multiple serotypes of pneumococci colonizing the nasopharynx in order to detect the dynamics of colonization of this bacterium in the population after introduction of conjugate vaccines.[43]

The question to ask is: How to validate the isolation of a single colony for surveillance of patients with *S. pneumoniae*? Most surveillance studies characterize only a single colony when seeking average prevalences of serotype/serogroups or antibiotic susceptibility. Therefore to study the diversity of pneumococcal colonization and to identify which serotypes are circulating in the population the methodology of multiple colonies should be applied[44]. New evidence for between strain competition among pneumococci have been reported, suggesting that the essential mechanism of competition works in acquisition rather than in clearance of carriage.[45]

CONCLUSION

Pneumococcal conjugate vaccine has a significant impact on pneumococcus epidemiology with a remarkable decline in invasive disease rates in young children. It also reduces the nasopharyngeal carriage of vaccine related serotypes that result in herd immunity effect benefits on unvaccinated children and adults. There is also a decrease in the antibiotics resistance in the pneumococcal isolates in the vaccinated communities. *S. pneumoniae* nasopharyngeal carriage of children reflect the infection causing strains currently circulating in the community, and so studies of the prevalence of different pathogens and their resistance patterns can provide useful indications for more rational therapeutic and preventive strategies.

REFERENCES

1. PARK, I.H. et al. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. J. Clin. Microbiol., Washington, DC, v.45, p.1225-1233, 2007.
2. BOGAERT, D.; GROOT, T.R.; HERMANS, W.H. *Streptococcus pneumoniae* colonization: the key to pneumococcal disease. Lancet Infect. Dis., New York, v.4, p.144-154, 2004.
3. ORGANIZAÇÃO PAN-AMERICANA DA SAÚDE. Informe Regional SIREVA II, 2007: datos por países y por grupos de edad sobre las características de los aislamientos de *Streptococcus pneumoniae, Haemophilus influenzas y Neisseria meningitidis* en procesos invasores. Washington, DC, 2008.
4. DAGAN, R.E. et al. Dynamics of pneumococcal nasopharyngeal colonization during the first days of antibiotic treatment in pediatric patients. Pediatr. Infect. Dis. J., Baltimore, v.17, p.880-885, 1998.
5. DE LENCASTRE, H.; TOMASZ, A. From ecological reservoir to disease: the nasopharynx, daycare centers and drug resistant clones of *Streptococcus pneumoniae*. J. Antimicrob. Chemother., London, v.50, p.75-81, 2002.
6. KAPLAN, S.L. et al. Three year multicenter surveillance of systemic pneumococcal infections in children. Pediatrics, Elk Grove Village, v.102, p.538-545, 1998.
7. RODRIGUES, F. et al. *Streptococcus pneumoniae* nasopharyngeal carriage in children attending daycare centers in the central region of Portugal, in the era of 7valent pneumococcal conjugate vaccine. Microb. Drug Resist., Larchmont, v.15, n.4, p.269-277, 2009.
8. PREVENTING pneumococcal disease among infants and young children: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep., Atlanta, v.49, n.RR-9, p.1-35, 2000.
9. PLETZ, M.W. et al. Pneumococcal vaccines: mechanism of action, impact on epidemiology and adaption of the species. Int. J. Antimicrob. Agents, Amsterdam, v.32, p.199-206, 2008.
10. KÄYHTY, H. et al. Nasopharyngeal colonization: a target for pneumococcal vaccination. Expert Rev. Vaccines, London, v.5, p.651-667, 2006.
11. PILISHVILI, T. et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J. Infect. Dis., Chicago, v.201, p.32-41, 2010.
12. ESKOLA, J.; ANTILLA, M. Peumococcal conjugate vaccines. Pediatr. Infect. Dis. J., Baltimore, v.18, p.543-551, 1999.
13. SCOTT, D.A. et al. Phase 1 trial of a 13valent pneumococcal conjugate vaccine in healthy adults. Vaccine, Amsterdam, v.25, p.6164-6166, 2007.
14. SHOUVAL, D.S. et al. Serotype coverage of invasive and mucosal pneumococcal disease in Israeli children younger than 3 years by various pneumococcal conjugate vaccines. Pediatr. Infect. Dis. J., Baltimore, v.28, p.277-282, 2009.
15. BLACK, S.; SHINEFIELD, H.; FIREMAN, B. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr. Infect. Dis. J., Baltimore, v.19, p.187-195, 2001.

16. SPRATT, B.G.; GREENWOOD, B.M. Prevention of pneumococcal disease by vaccination: does serotype replacement matter? Lancet, London, v.356, p.1210-1211, 2000.
17. COHEN, R.; LEVY, C.; DE LA ROCQUE, F. Impact of pneumococcal conjugate vaccine and of reduction of antibiotic use on nasopharyngeal carriage of nonsusceptible pneumococci in children with acute otitis media. Pediatr. Infect. Dis. J., Baltimore, v.25, p.1001-1007, 2006.
18. FRAZAO, N. et al. Effect of the seven valent conjugate pneumococcal vaccine on carriage and drug resistance of *Streptococcus pneumoniae* in healthy children attending daycare centers in Lisbon. Pediatr. Infect. Dis. J., Baltimore, v.24, p.243-252, 2005.
19. MILLAR, E.V. et al. Indirect effect of 7valent pneumococcal conjugate vaccine on pneumococal colonization among unvaccinated household members. Clin. Infect. Dis., Chicago, v.47, p.989-996, 2008.
20. HAMMITT, L.L. et al. Indirect effect of conjugate vaccine on adult carriage of *Streptococcus pneumoniae*: an explanation of trends in invasive pneumococcal disease. J. Infect. Dis., Chicago, v.193, n.11, p.1487-1494, 2006.
21. DAGAN, R. et al. Reduction of nasopharyngeal carriage of *Streptococcus pneumoniae* after administration of a 9valent pneumococcal conjugate vaccine to toddlers attending day care centers. J. Infect. Dis., Chicago, v.185, p.927-936, 2002.
22. OOSTERHUIS-KAFEJA, F.; BEUTELS, P.; VAN DAMME, P. Immunogenicity, efficacy, safety and effectiveness of pneumococcal conjugate vaccines (1998-2006). Vaccine, Amsterdam, v.25, n.12, p.2194-2212, 2007.
23. LAUDERDALE, T.L. et al. High carriage rate of high level penicillin resistant *Streptococcus pneumoniae* in Taiwan kindergarten associated with a case of pneumococcal meningitis. BMC Infect. Dis., London, v.5, p.96, 2005.
24. MORENO, J. et al. Detection and serotyping of *Streptococcus pneumoniae* from nasopharyngeal samples by PCR based multiplex assay. J. Clin. Microbiol., Washington, DC, v.43, p.6152-6154, 2005.
25. HUEBNER, R.E. et al. Lack of utility of serotyping multiple colonies for detection of simultaneous nasopharyngeal carriage of different pneumococcal serotypes. Pediatr. Infect. Dis. J., Baltimore, v.19, p.1017-1019, 2000.
26. WOLF, B. et al. Molecular epidemiology of penicillin resistant *Streptococcus pneumoniae* colonizin children with communityacquired pneumonia and children attending daycare centers in Fortaleza, Brazil. J. Antimicrob. Chemother., London, v.46, p.757-765, 2000.
27. FERREIRA, L.L.M. et al. Nasopharyngeal colonization and antimicrobial resistance of *Streptococcus pneumoniae* isolated from children with acute rhinopharyngitis. J.Pediatr. (Rio J.), Porto Alegre, v.77, p.227-234, 2001.
28. ROSSI, F. et al. Susceptibility of *S. pneumoniae* to various antibiotics among strains isolated from patients and healthy carries in different regions of Brazil (1999-2000). Braz. J. Infect. Dis., Salvador, v.5, n.6, p.305-312, 2001.
29. LIMA, E.C. Prevalência de portador e sorotipos de *Streptococcus pneumoniae* em nasofaringe de crianças menores de 5 anos de GoiâniaGoiás. 2001. Dissertação (Mestrado), Universidade Federal de Goiás, Goiânia, 2001.
30. LAVAL, C.A. et al. Serotypes of carriage and invasive isolates of *Streptococcus pneumoniae* in Brazilian children in the era of pneumococcal vaccines. Clin. Microbiol. Infect., Paris, v.12, p.50-55, 2006.
31. FRANCO, C.M. et al. Survey of nonsusceptible nasopharyngeal *Streptococcus pneumoniae* isolates in children attending daycare centers in Brazil. Pediatr. Infect. Dis. J., Baltimore, v.29, p.77-79, 2010.
32. O'BRIEN, K.L.; NOHYNEK, H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae.* Pediatr. Infect. Dis. J., Baltimore, v.22, p.133-140, 2003.
33. PAI, R.; GERTZ, R.E.; BEALL, B. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. J. Clin. Microbiol., Washington, DC, v.44, p.124-131, 2006.
34. BRITO, D.A.; RAMIREZ, M.; DE LANCASTRE, H. Serotyping *Streptococcus pneumoniae* by multiplex PCR. J. Clin. Microbiol., Washington, DC, v.41, p.2378-2384, 2003.
35. LAWRENCE, E.R. et al. Evaluation of semiautomated multiplex PCR assay for determination of *Streptococcus pneumoniae* serotypes and serogroups. J. Clin. Microbiol., Washington, DC, v.41, p.601-607, 2003.
36. DIAS, C.A. et al. Sequential multiplex PCR for determining capsular serotypes of pneumococci recovered from Brazilian children. J. Med. Microbiol., London, v.56, p.1185-1188, 2007.
37. MORAIS, L. et al. Seqüencial multiplex PCR for identifying pneumococcal capsular serotypes from South Saharan African isolates. J. Med. Microbiol., London, v.56, p.1181-1184, 2007.
38. LIN, J. et al. Validation of a multiplex pneumococcal serotyping assay with clinical samples. J. Clin. Microbiol., Washington, DC, v.44, p.383-388, 2006.
39. BILLAL, D.S. et al. Determination of pneumococcal serotypes/ genotypes in nasopharyngeal secretions of otitis media children by multiplex PCR. Eur. J. Pediatr., Berlin, v.167, p.401-407, 2008.
40. ANTONIO, M. et al. Evaluation of sequential multiplex PCR for direct detection of multiple serotypes of *Streptococcus pneumoniae* from nasopharyngeal secretions. J. Med. Microbiol., London, v.58, p.296-302, 2009.
41. GRATTEN, M. et al. Upper airway carriage by *Haemophilus influenzae* and *Streptococcus pneumoniae* in Australian aboriginal children hospitalised with acute lower respiratory infection. Southeast Asian J. Trop. Med. Public Health, Bangkok, v.25, p.123-131, 1994.
42. RIVERA OLIVERO, I.A. et al. Multiplex PCR reveals a high rate of nasopharyngeal pneumococcal 7valent conjugate vaccine serotypes colonizing Indigenous Warao children in Venezuela. J. Med. Microbiol., London, v.58, p.584-587, 2009.
43. PNEUMOCOCCAL conjugate vaccine for childhood immunization:WHO position paper. Wkly. Epidemiol. Rec., Geneva, v.82, p.93-104, 2007. Disponível em: <http://www.who.int/wer/2007/wer8212.pdf> Acesso em jun. 2009.
44. CHARALAMBOUS, B.M.; ORIYO, N.M.; GILLESPIE, S.H. How valid is singlecolony isolation for surveillance of *Streptococcus pneumoniae* carriage? J. Clin. Microbiol., Washington, DC, v.46, p.2467-2468, 2008.
45. AURANEN, K. et al. Between strain competition in acquisition and clearance of pneumococcal carriage epidemiologic evidence from a longitudinal study of daycare children. Am. J. Epidemiol., Cary, v.171, p.169-176, 2010.