Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Pneumococcal carriage among children after four years of routine 10-valent pneumococcal conjugate vaccine use in Brazil: The emergence of multidrug resistant serotype 6C

Felipe P.G. Neves [a,b,*], Nayara T. Cardoso [b], Robert E. Snyder [a], Mariel A. Marlow [a,1], Claudete A.A. Cardoso [c], Lúcia M. Teixeira [d], Lee W. Riley [a]

[a] Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, 530E Li Ka Shing Center, Berkeley, CA 94720, USA
[b] Instituto Biomédico, Universidade Federal Fluminense, Rua Professor Hernani Melo, 101 São Domingos, Niterói, RJ 24210-130, Brazil
[c] Faculdade de Medicina, Universidade Federal Fluminense, Av. Marquês do Paraná, 303 Centro, Niterói, RJ 24033-900, Brazil
[d] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho, 373 – bloco I, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, RJ 21941-902, Brazil

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form 9 April 2017
Accepted 10 April 2017
Available online xxxx

Keywords:
Streptococcus pneumoniae
Nasopharyngeal carriage
Serotypes
Antimicrobial resistance
Pneumococcal conjugate vaccines

ABSTRACT

Background: In 2010, the 10-valent pneumococcal conjugate vaccine (PCV10) was introduced free of charge in Brazil as part of the public immunization program. Here we investigated the carriage prevalence, colonization risk factors, capsular types, and antimicrobial resistance among pneumococcal isolates obtained from children in Brazil four years after routine PCV10 use.

Methods: Between September and December 2014, we conducted a cross-sectional study among children < 6 years old who attended one public and two private clinics in Niterói, RJ, Brazil to evaluate pneumococcal nasopharyngeal carriage. Antimicrobial susceptibility and capsular types were determined for all isolates.

Results: Of 522 children, 118 (22.6%) were pneumococcal carriers. Being ≥ 2 years old, attending childcare center, presenting with any symptoms, having acute or chronic respiratory disease, and residing in a slum were associated with pneumococcal carriage. The most prevalent capsular types were 6C (14.5%), 15B/C (11.5%), 11A/D (9.2%), and 6A (7.6%). PCV10 serotypes represented 2.5%. All isolates were susceptible to levofloxacin, rifampicin, and vancomycin. Penicillin non-susceptible pneumococci (PNSP) comprised 39%, with penicillin and ceftriaxone MICs ranging from 0.12–8.0 μg/ml and 0.012–1.0 μg/ml, respectively. The 33 (28%) erythromycin-resistant isolates (MICs of 1.5 to >256 μg/ml) displayed the $cMLS_B$ (72.7%) or M (27.3%) phenotypes, harboring the *erm*(B) and/or *mef*(A/E) genes. High non-susceptibility rates (>20%) to clindamycin, erythromycin, penicillin, and tetracycline were largely explained by the prevalence of multidrug resistant (MDR) serotype 6C isolates.

Conclusions: Effects of universal childhood PCV10 use on carriage were evident, with the near elimination of PCV10 serotypes. The emergence of MDR serotype 6C isolates, however, is a concern. Ongoing surveillance to monitor serotype 6C increase in invasive diseases is warranted.

Abbreviations: BEC, broth enrichment culture method; BLAST, Basic Local Alignment Search Tool; CCC, childcare center; CI, confidence intervals; CLSI, Clinical and Laboratory Standards Institute; $cMLS_B$, constitutive macrolide, lincosamide and streptogramin B resistance phenotype; ERY-R, erythromycin-resistant; IPD, invasive pneumococcal disease; M, macrolide resistance phenotype; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; NT, non-typeable; UOR, unadjusted odds ratio; PCV, pneumococcal conjugate vaccines; PCR, polymerase chain reaction; PEN-I, intermediate to penicillin; PEN-R, resistant to penicillin; PNSP, penicillin non-susceptible pneumococci; ST, sequence type; STGG, skim milk-tryptone-glucose-glycerin transport medium.

* Corresponding author at: Instituto Biomédico, Universidade Federal Fluminense. Rua Professor Hernani Melo, 101. São Domingos, Niterói, RJ 24210-130, Brazil.
E-mail addresses: fpgneves@vm.uff.br (F.P.G. Neves), nay.torresbio@gmail.com (N.T. Cardoso), robert.snyder@berkeley.edu (R.E. Snyder), klt8@cdc.gov (M.A. Marlow), claudetecardoso@id.uff.br (C.A.A. Cardoso), lmt2@micro.ufrj.br (L.M. Teixeira), lwriley@berkeley.edu (L.W. Riley).
[1] Current address: Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, USA.

http://dx.doi.org/10.1016/j.vaccine.2017.04.019
0264-410X/

1. Introduction

Streptococcus pneumoniae is a major pathogen responsible for community-acquired pneumonia [1]. In 2015, pneumonia killed more than 920,000 children under 5 years old, accounting for 16% of all deaths in this age group [2]. In 2013, lower respiratory tract infections were the fifth most common cause of death in Brazil among individuals aged 0–19 years, with 28.5 deaths per 100,000 population [3]. Additionally, the average incidence of pneumococcal meningitis in Brazil between 2010 and 2014 was 1103 cases/year, with a mortality rate of approximately 30% [4].

Pneumococcal conjugate vaccines (PCV) have been available in Brazil since 2001, when the 7-valent vaccine (PCV7) was offered in both private (out-of-pocket payment) and public (government financed, free of charge) vaccination clinics. However, in public clinics, PCV7 was only available to individuals at high risk for invasive pneumococcal disease (IPD) [5]. As a result, most of the Brazilian population was not vaccinated against *S. pneumoniae* at that time. In 2010, the 10-valent vaccine (PCV10) was progressively implemented free of charge via Brazil's National Immunization Program for routine childhood vaccination [6]. In private vaccination clinics, the PCV7 was replaced by the 13-valent vaccine (PCV13).

In other countries, PCV vaccination has significantly decreased the carriage rate and IPD incidence caused by serotypes included in the vaccine, leading to serotype replacement [7–10]. By assessing the colonization prevalence of pneumococcal serotypes after PCV introduction, we can evaluate the effects of such implementation on pneumococcal carriage, allowing for improved prevention strategies of pneumococcal diseases.

Here we report the effects after four years of routine PCV10 use on pneumococcal carriage among children living in a large metropolitan area in southeastern Brazil, including the capsular type distribution and the prevalence of antimicrobial resistance of the pneumococcal isolates.

2. Material and methods

2.1. Population and study design

For this cross-sectional study, we recruited children < 6 years old who attended two private and one public pediatric clinics for routine check-up or sick visits in Niterói, a city in the greater Rio de Janeiro metropolitan area, Brazil, between September 29 and December 5, 2014. In Brazil, private clinics usually attend to children with private health plans or whose guardians pay out-of-pocket for their healthcare. Brazil's public health system offers free healthcare to all citizens via the country's universal health program [*Sistema Único de Saúde* (SUS)].

We collected a single nasopharyngeal specimen from each child and collected data with questionnaires from legal guardians using the mobile data collection application Magpi (Magpi, Washington, DC). Researchers also reviewed participants' vaccination cards to retrieve information describing their pneumococcal vaccination history.

2.2. Isolation and identification of pneumococcal strains

Nasopharyngeal specimens were collected with nylon fiber mini-tip flocked swabs (Copan, Brescia, Italy). Swabs were immediately placed into a cryotube containing 1.0 mL of skim milk-tryptone-glucose-glycerin (STGG) transport medium, kept on ice until stored at −180 °C on the same day. After three months, pneumococcal strains were isolated by the broth enrichment culture (BEC) method [11], using 2 mL of Todd-Hewitt broth (Difco Laboratories, Detroit, MI, USA), containing 0.5% yeast extract (Difco Laboratories) combined with 0.4 ml of rabbit serum. Alpha-hemolytic colonies suspected to be *S. pneumoniae* were tested for susceptibility to optochin and bile-solubility to confirm the species identification.

2.3. Identification of capsular types

Capsular types were deduced by sequential multiplex PCR [11,12]. Serogroup 6 isolates were subjected to PCR with 6C/D-specific primers targeting the $wciN_{beta}$ gene [13] and Quellung reaction using antisera kindly provided by the Centers for Disease Control and Prevention. Additionally, serogroup 6 isolates and those that amplified only the *wzg* (*cpsA*) pneumococcal identification control gene were further characterized by a capsular sequence typing method described elsewhere [14], with modifications: (i) primer sequences for the partial *wzh* (*cpsB*) gene (Supplementary Table 1), (ii) primers concentration (0.5 μM each), and (iii) using a mix of the three different forward primers to sequence the DNA. The partial *cpsB* gene sequences were queried against GenBank by BLAST (blast.ncbi.nlm.nih.gov) and isolates sharing ≥99.8% identity (maximum divergence in one single nucleotide) were assigned as belonging to the same capsular type.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility of all the isolates was determined by the disk-diffusion method according to the Clinical and Laboratory Standards Institute guidelines [15]. The antimicrobial agents tested were: chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), levofloxacin (5 μg), oxacillin (1 μg), rifampicin (5 μg), sulfamethoxazole/trimethoprim (1.25 μg/23.75 μg), tetracycline (30 μg), and vancomycin (30 μg) (Cecon, São Paulo, SP, Brazil). The E-test® (BioMérieux, Marcy l'Etoile, France) was used to determine the minimum inhibitory concentrations (MICs) to penicillin and ceftriaxone for isolates with reduced susceptibility to penicillin (<20 mm diameter zone of inhibition around the oxacillin disk) as well as the erythromycin MICs for erythromycin non-susceptible isolates (<21 mm diameter zone of inhibition). Macrolide resistance phenotypes were investigated by the double-disk test [15].

Multidrug resistant (MDR) isolates were defined as isolates that were non-susceptible to at least one agent from three or more different classes of antimicrobial agents [16].

2.5. Detection of erythromycin resistance genes

The presence of the *erm*(A), *erm*(B) and *mef*(A/E) genes among erythromycin non-susceptible isolates was evaluated by PCR [17].

2.6. Data analyses

Analyses were done in Stata Version 14.1 (Statacorp, College Station, USA). We estimated unadjusted odds ratio for pneumococcal carriage with bivariate logistic regression. Multivariate generalized linear models were developed to estimate the adjusted risk of colonization in a forward stepwise process with a p-value cutoff of 0.2 for inclusion in the final model. To obtain robust results from the National Immunization Program with the PCV10, we excluded from the analyses children with missing vaccination history and those who were immunized with pneumococcal vaccines other than PCV10. For these analyses, capsular type 9V/A was considered a PCV serotype.

Please cite this article in press as: Neves FPG et al. Pneumococcal carriage among children after four years of routine 10-valent pneumococcal conjugate vaccine use in Brazil: The emergence of multidrug resistant serotype 6C. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.04.019