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Etiology and Antimicrobial Susceptibility of Middle Ear Fluid Pathogens in Costa Rican Children With Otitis Media Before and After the Introduction of the 7-Valent Pneumococcal Conjugate Vaccine in the National Immunization Program

Acute otitis media microbiology in Costa Rican children

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Abstract: Acute otitis media (AOM) microbiology was evaluated in children after 7-valent pneumococcal conjugate vaccine (PCV7) introduction in Costa Rica (private sector, 2004; National Immunization Program, 2009).

This was a combined prospective and retrospective study conducted in a routine clinical setting in San José, Costa Rica. In the prospective part of the study, which was conducted post-PCV7 introduction (2010–2012), standard bacteriological procedures were used to evaluate the etiology and serotype distribution of middle ear fluid samples collected by tympanocentesis or otorrhea from children aged 3–59 months diagnosed with AOM. E-tests were used to evaluate antimicrobial susceptibility in culture-positive samples. Retrospective data recorded between 1999 and 2004 were used for comparison of bacterial etiology and serotype distribution before and after PCV7 introduction. Statistical significance was evaluated in bivariate analyses at the P-value < 0.05 level (without multiplicity correction).

Post-PCV7 introduction, *Haemophilus influenzae* was detected in 118/456 and *Streptococcus pneumoniae* in 87/456 AOM episodes. Most *H. influenzae* isolates (113/118) were non-typeable. *H. influenzae* was more (27.4% vs 20.8%) and *S. pneumoniae* less (17.1% vs 25.5%) frequently observed in vaccinated ($\geq$2 PCV7 doses or $\geq$1 PCV7 dose at $>$1 year of age) versus unvaccinated children. *S. pneumoniae* non-susceptibility rates were 1.1%, 34.5%, 31.7%, and 50.6% for penicillin, erythromycin, azithromycin, and trimethoprim/sulfamethoxazole (TMP-SMX), respectively. *H. influenzae* non-susceptibility rate was 66.9% for TMP-SMX. Between pre- and post-PCV7 introduction, *H. influenzae* became more (20.5% vs 25.9%; P-value < 0.001) and *S. pneumoniae* less (27.7% vs 19.1%; P-value $= 0.002$) prevalent, and PCV7 serotype proportions decreased among pneumococcal isolates (65.8% vs 43.7%; P-value $= 0.0005$). Frequently identified pneumococcal serotypes were 19F (34.2%), 3 (9.7%), 6B (9.7%), and 14 (9.7%) pre-PCV7 introduction, and 19F (27.6%), 14 (8.0%), and 35B (8.0%) post-PCV7 introduction.

Following PCV7 introduction, a change in the distribution of AOM episodes caused by *H. influenzae* and pneumococcal serotypes included in PCV7 was observed in Costa Rican children. Pneumococcal vaccines

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impact should be further evaluated following broader vaccination coverage.

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Abbreviations: AOM = acute otitis media, CI = confidence interval, MEF = middle ear fluid, NIP = national immunization program, NTHi = non-typeable *Haemophilus influenzae*, PCV7 = 7-valent pneumococcal conjugate vaccine, PCV13 = 13-valent pneumococcal conjugate vaccine, TMP-SMX = trimethoprim/sulfamethoxazole.

INTRODUCTION

Acute otitis media (AOM) is one of the most frequent pediatric bacterial infections and one of the primary reasons for antibiotic use in children.[1] Between 1992 and 2008, *Streptococcus pneumoniae* was identified as the most common pathogen in children with AOM or recurrent otitis media, and non-typeable *Haemophilus influenzae* (NTHi) in children with therapeutic failure otitis media in Costa Rica.[2–8]

During the last decade, a 7-valent pneumococcal conjugate vaccine (*Prevnar/Prevenar*™, Pfizer Inc., New York, NY; PCV7) has been used in various countries and was effective in reducing the burden of pneumococcal disease, including AOM.[9,10] In the United States, PCV7 introduction has been associated with an important decline in AOM incidence, as well as changes in etiology and antimicrobial non-susceptibility patterns of otopathogens; *H. influenzae* and serotypes not included in PCV7 became more prevalent, and PCV7-serotypes and penicillin non-susceptible isolates less prevalent in middle ear fluid (MEF) samples.[10–14]

In Costa Rica, PCV7 was introduced in the private sector in 2004 (<5% of the birth cohort), in the national immunization program (NIP) in 2009, and was replaced by the 13-valent pneumococcal conjugate vaccine (*Prevnar13/Prevenar13*™, Pfizer Inc., New York, NY; PCV13) in August 2011. Vaccination coverage rates reached 97% in 2010 (infants who received 3 primary doses), decreased to 68% due to vaccine shortage in 2011, and increased again to 83% during the first trimester of 2012 (infants aged <6 months who received 2 primary doses).[8]

This study aimed to characterize the etiology and serotype distribution of bacterial otopathogens in Costa Rican children in the first few years following PCV7 introduction in the NIP (2010–2012), and to compare these results with those obtained before PCV7 introduction (1999–2004). Antimicrobial susceptibility patterns of identified otopathogens and AOM management were also evaluated post-PCV7 introduction.

MATERIALS AND METHODS

Study Design and Population

This was a combined prospective and retrospective epidemiological study conducted in a routine clinical setting (Instituto de Atención Pediátrica) in San José, Costa Rica. Children aged 3 months to 5 years, diagnosed with AOM, and from whom a MEF sample was available, were recruited prospectively between March 2010 and April 2012. Symptomatic children were identified through a pediatric network, which was mainly private, and then referred to study investigators for diagnosis confirmation. Signs and symptoms of AOM that were verified by the investigators included one of the functional or general signs of otalgia, conjunctivitis and fever, and either Paradise's criteria (bulging, diffused or localized inflamed tympanic membranes) or spontaneous otorrhea of less than 1 day.

AOM episodes were classified as untreated if signs and symptoms were of onset within 72 hours and children had not received antibiotic therapy; as recurrent if children had reported ≥3 AOM episodes in the previous 6 months or ≥4 AOM episodes in the previous 12 months; or as treatment failures if children had received antibiotic therapy within 3 days after onset of signs and symptoms of AOM but remained symptomatic at study entry (between 2–3 days after initiation of this treatment).

Children were excluded if they were hospitalized during AOM diagnosis or treatment; had otitis externa or otitis media with effusion; had a transtympanic aerator (tympanic tubes); had received systemic antibiotic therapy in the past 3 days for a separate illness, antimicrobial prophylaxis for recurrent AOM, or antibiotics for otitis media in the 24 hours prior to enrollment (except for treatment failures); or were children in care. Children could be enrolled for more than 1 AOM episode if there was a symptom-free interval of ≥30 days between episodes. Children were classified as vaccinated if they had received ≥2 doses of PCV7 during the first year of life or ≥1 dose of PCV7 when they were ≥1 year of age.

Informed consent was obtained from each parent/guardian prior to performing any study-specific procedure. The protocol was reviewed and approved by local Ethics Committees, and the study was conducted according to Good Clinical Practice and the Declaration of Helsinki.

Retrospective, anonymized information from the Instituto de Atención Pediátrica (1999–2004) database was available for comparison of bacterial etiology and serotype distribution before and after PCV7 introduction.

Study Procedures

All children with AOM presenting to study physicians were anonymously recorded in screening logbooks and assessed for eligibility. Demographic characteristics, medical history, vaccination status, and general symptoms of eligible children were collected, and clinical examinations, including assessments of tympanic membranes, were performed at baseline. Children received standard antimicrobial therapy as per AOM recommendations in Costa Rica. MEF samples were collected by tympanocentesis (Channel Directed Tympanocentesis speculum [Walls Precision Instruments LLC, USA]) or by otorrhea in children with tympanic membrane perforation occurring less than 24 hours prior to the visit (via deep aspiration of MEF through needle insertion after ear canal cleaning). Tympanocentesis is not performed routinely in Costa Rica and was done specifically for the study purpose. In children with bilateral infections, MEF samples from both ears were analyzed.

To assess treatment, enrolled children were asked to return for a planned follow-up visit 12 to 14 days after their initial visit, regardless of symptoms. Unplanned visits for AOM were recorded during 30 days after the initial visits. If children had signs and symptoms consistent with AOM at planned or unplanned follow-up visits, a second tympanocentesis was performed (with a limit of two tympanocenteses per episode).

Laboratory Procedures

MEF samples were inoculated in chocolate and blood agar (with and without gentamycin). Bacterial identification was made using standard bacteriological procedures at the

Laboratorio Centro de Investigaciones Médicas in San José, Costa Rica: *S. pneumoniae*, by Optochin test; *H. influenzae*, on the basis of Gram staining, growth on chocolate agar, failure to grow in trypticase agar with added sheep blood, and nutritional requirement of hemin and nicotine adenine dinucleotide; *Moraxella catarrhalis*, on the basis of Gram staining, positive oxidase reaction, and characteristic biochemical profiling; and *Streptococcus pyogenes*, on the basis of the presence of β-hemolysis and susceptibility to bacitracin. Pneumococcal serotypes were identified by Quellung reaction at the laboratory of the Pediatric Infectious Disease Unit of the Soroka University Medical Center in Beer-Sheva, Israel. *H. influenzae* serotypes were identified using monovalent antisera at the Instituto de Atención Pediátrica in San José, Costa Rica.

Antibiotic susceptibility to amoxicillin/clavulanate, ceftriaxone, erythromycin, azithromycin, levofloxacin, and trimethoprim/sulfamethoxazole (TMP-SMX) was evaluated in culture-positive samples, and to penicillin in *S. pneumoniae*- and *S. pyogenes*-positive samples, using E-test. In *H. influenzae*- and *M. catarrhalis*-positive samples, antibiotic susceptibility to ampicillin was evaluated and a β-lactamase test (nitrocefin) was performed. Interpretation of the results was based upon the Clinical and Laboratory Standards Institute guidelines published in 2009.[15] Otopathogens were defined as multidrug resistant if they were resistant to ≥3 different antimicrobial classes.[16]

Statistical Analysis

To detect differences in proportions of *S. pneumoniae*- and *H. influenzae*-positive samples before and after PCV7 introduction with minimum 80% of power, at least 387 MEF samples had to be collected. All statistical analyses were performed using SAS version 9.1 or later, and Microsoft Excel 2002 SP3 or later.

Most analyses in this study were descriptive. Proportions of culture-positive episodes were calculated by group of children with exact 95% confidence intervals (CIs). Categorical variables were described with frequency tables, and exploratory statistical comparisons were performed using Pearson's Chi-squared test. Statistical significance for comparisons in proportions was evaluated globally in bivariate analyses at the *P*-value <0.05 level (without multiplicity correction).

RESULTS

Study Participants

Between March 2010 and April 2012, 504 episodes of AOM were screened and 387 children with 485 episodes were enrolled in the total cohort. Of these, 29 episodes were excluded, largely due to symptoms beginning outside the protocol-defined period, and the final study analysis included 366 children with 456 episodes. Multiple enrolments were reported for 57/366 (15.6%) children: 40, 9, and 8 children had 2, 3, and ≥4 episodes, respectively.

There were bilateral infections in 156/456 (34.2%) episodes. MEF samples were collected from otorrhea in 45/456 (9.9%) and by tympanocentesis in 411/456 (90.1%) episodes. Among the 456 AOM episodes, 332 (72.8%) were untreated, 92 (20.2%) recurrent, 22 (4.8%) treatment failure, and 10 (2.2%) both recurrent and treatment failure episodes.

The mean age of children ± standard deviation was 22 ± 13 months (Table 1). Among the 456 episodes, 296 (64.9%) were reported in children aged <2 years, 183 (40.1%) in girls, 298 (65.4%) in children who had received ≥1 dose of influenza vaccine, 350 (76.8%) in children who had (received ≥2 pneumococcal conjugate vaccine doses at any age or ≥1 dose at >1 year of age (most of these episodes were isolated in children having received PCV7), and 34 (7.5%) in children who reported antibiotic use within the month before the initial visit.

Etiology and Antimicrobial Susceptibility at Initial Visit

Post-PCV7 introduction, 204/456 (44.7%) episodes were culture-positive: *H. influenzae* was detected in 118 (25.9%), *S. pneumoniae* in 87 (19.1%), *M. catarrhalis* in 23 (5.0%), and *S. pyogenes* in 9 (2.0%) episodes (Table 2). At the initial visit, 37/45 (82.2%) otorrhea and 167/411 (40.6%) tympanocentesis samples were culture-positive. Co-infections were detected in 28/456 (6.1%) episodes; the most frequent was *S. pneumoniae* and *H. influenzae* (n = 19).

The observed pathogen distributions were similar among children from different age groups (data not shown). The proportions of culture-positive episodes were 133/296

TABLE 1. Demographic Characteristics and Vaccination Status (Final Study Analysis)

	Untreated (N = 332)	Recurrent (N = 92)	Treatment failure (N = 22)	Recurrent and treatment failure (N = 10)	Total (N = 456)
Age, months					
Mean ± SD	21 ± 14	24 ± 13	17 ± 8	23 ± 10	22 ± 13
Median	17	19.5	14.5	21	18
Range	3–59	7–57	4–32	15–48	3–59
Gender, n (%)					
Female	135 (40.7)	31 (33.7)	11 (50.0)	6 (60.0)	183 (40.1)
Male	197 (59.3)	61 (66.3)	11 (50.0)	4 (40.0)	273 (59.9)
Child received ≥2 PCV7 doses at any age or ≥1 PCV7 dose at >1 year of age, n (%)	254 (76.5)	67 (72.8)	20 (90.9)	9 (90.0)	350 (76.8)
Child received ≥1 dose of an influenza vaccine, n (%)	245 (73.8)	35 (38.0)	16 (72.7)	2 (20.0)	298 (65.3)

N = number of episodes, n (%) = number (percentage) of episodes in a given category, SD = standard deviation.

TABLE 2. Bacterial Etiology and Serotype Distribution of AOM Episodes at Initial Visit by Vaccination Status (Final Study Analysis)

Serogroup and serotype	Vaccinated* (N = 350) n (%)	Unvaccinated (N = 106) n (%)	Total (N = 456) n (%)
Streptococcus pneumoniae	**60 (17.1)**	**27 (25.5)**	**87 (19.1)**
19F†, 14, 6B, 23F	28 (46.7)	10 (37.0)	38 (43.7)
19A, 6A, 3, 7F	9 (15.0)	8 (29.6)	17 (19.5)
35B, 15B, 6C, 11A, 15A, 10F, 15C, 18A, 21, 34, 22F, 23A	23 (38.3)	9 (33.3)	32 (36.8)
Haemophilus influenzae	**96 (27.4)**	**22 (20.8)**	**118 (25.9)**
NTHi	92 (95.8)	21 (95.5)	113 (95.8)
a–f	4 (4.2)	1 (4.5)	5 (4.2)
Streptococcus pyogenes	**3 (0.9)**	**6 (5.7)**	**9 (2.0)**
Moraxella catarrhalis	**17 (4.9)**	**6 (5.7)**	**23 (5.0)**

Bold values refer to the total number of bacterial pathogens and total number of pathogens for each bacterial type (eg *Streptococcus pneumonia; Haemophilus influenzae; Streptococcus pyogens; Moraxella catharralis*).

The pneumococcal serotypes are listed in descending order of predominance (for total episodes). N = number of episodes, n (%) = number (percentage) of episodes in a given category (number and percentage of AOM episodes positive for the 4 otopathogens are shown compared to the total number of AOM episodes, while the serotype distribution is shown compared to otopathogen-positive episodes).

* Children were classified as vaccinated against pneumococcal diseases if they had received ≥2 PCV7 doses at any age or ≥1 PCV7 dose at >1 year of age.

† includes 1 bilateral episode of 6C/19F.

(44.9%) in children aged <2 years and 71/160 (44.4%) in children aged ≥2 years.

The culture-positivity rates were 146/332 (44.0%) for untreated, 43/92 (46.7%) for recurrent, 8/22 (36.4%) for treatment failure, and 7/10 (70.0%) for both recurrent and treatment failure episodes (Figure 1). *H. influenzae* was more frequently detected than *S. pneumoniae* in treatment failure (22.7% vs 9.1%) and recurrent (32.6% vs 17.4%) episodes, while the proportion of both pathogens was similar in untreated episodes (23.8% vs 20.5%).

In vaccinated and unvaccinated children, 153/350 (43.7%) and 51/106 (48.1%) episodes were culture-positive, respectively (Figure 2). Although not statistically significant, slightly higher proportions of *H. influenzae* (27.4% vs 20.8%) were observed in vaccinated versus unvaccinated children; the opposite was observed for *S. pneumoniae* (17.1% vs 25.5%).

The most frequent pneumococcal serotypes were 19F (24/87 [27.6 %]), followed by 14 and 35B (7/87 [8.0%] for each) (Table 2). The most common serotypes were 19F (22/68 [32.4%]) and 35B (6/68 [8.8%]) for untreated, 6A, 6B and 11A

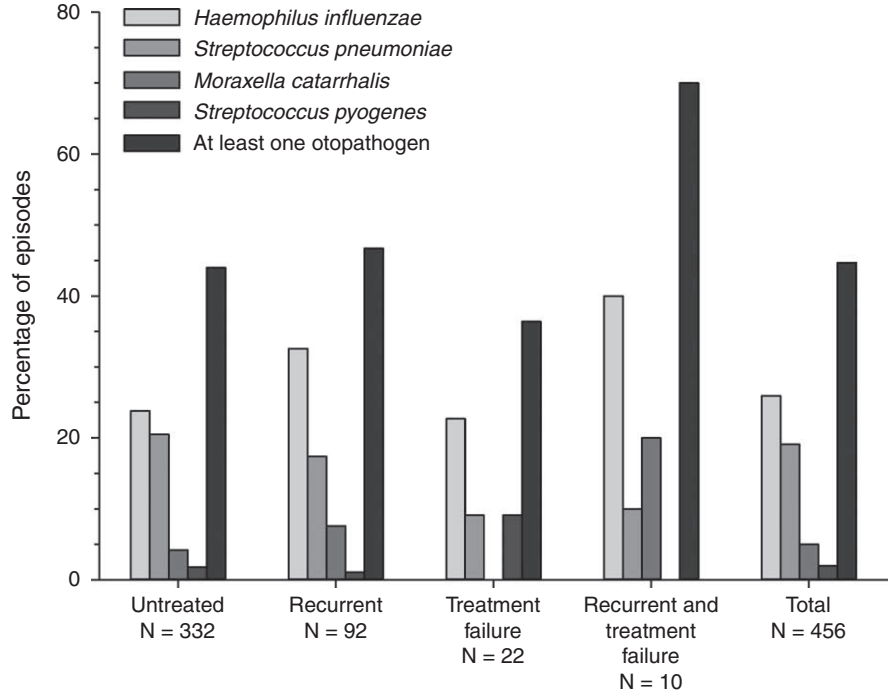

FIGURE 1. Bacterial etiology by type of episode (final study analysis). N = number of episodes; Where co-infections occurred, both bacteria were reported independently in the relevant category.

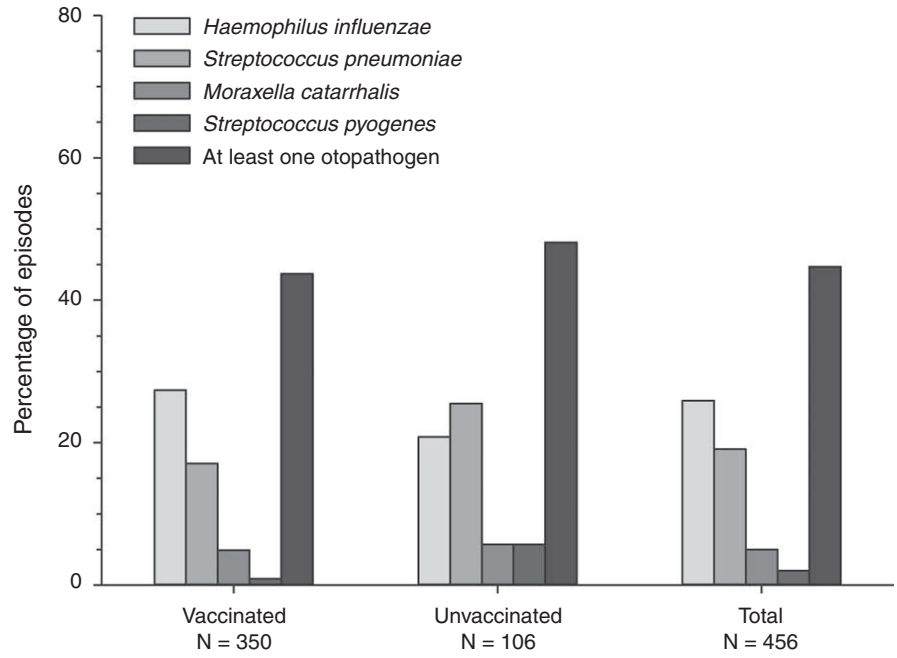

FIGURE 2. Bacterial etiology by vaccination status (final study analysis). N = number of episodes; Where co-infections occurred, both bacteria were reported independently in the relevant category; Children were classified as vaccinated against pneumococcal diseases if they had received ≥2 PCV7 doses at any age or ≥1 PCV7 dose at >1 year of age.

(3/16 [18.7%] for each) for recurrent, 14 and 19F (1/2 [50.0%] for each) for treatment failure, and 19F (1/1 [100%]) for both recurrent and treatment failure episodes (data not shown). Although not statistically significant, slightly higher (28/60 [46.7%] vs 10/27 [37.0%]) proportions of PCV7-serotypes (19F, 14, 6B, and 23F) were identified in pneumococcal isolates from vaccinated versus unvaccinated children; the opposite (9/60 [15.0%] vs 8/27 [29.6%]) was observed for serotypes included in new generation vaccines (19A, 6A, 3, and 7F). Among the 24 serotype 19F isolates, 1 (4.2%), 4 (16.7%), 3 (12.5%), 8 (33.3%), and 8 (33.3%) were in children who had received 0, 1, 2, 3, and 4 pneumococcal vaccine doses, respectively. The majority of *H. influenzae* isolates (113/118 [95.8%]) were NTHi.

In pneumococcal isolates, the observed antibiotic non-susceptibility rates were low for penicillin (1/87 [1.1%]), but high for erythromycin (30/87 [34.5%]), azithromycin (13/41 [31.7%]), and TMP-SMX (44/87 [50.6%]) (Table 3). In *S. pneumoniae*-positive episodes with available results, strains non-susceptible to erythromycin were isolated in 22/68 untreated, 7/16 recurrent, and 1/2 treatment failure episodes; to azithromycin in 7/26 untreated, 5/12 recurrent, and 1/2 treatment failure episodes; and to TMP-SMX in 34/68 untreated, 7/16 recurrent, 2/2 treatment failure, and 1/1 both recurrent and treatment failure episodes. Among the serotype 19F isolates, non-susceptibility rates were 8/24 (33.3%) for erythromycin, 20/24 (83.3%) for TMP-SMX, and 1/9 (11.1%) for azithromycin.

In *H. influenzae* isolates, high antibiotic non-susceptibility rates were observed for TMP-SMX (79/118 [66.9%]). In *H. influenzae*-positive episodes with available results, strains non-susceptible to TMP-SMX were isolated in 52/79 untreated, 20/30 recurrent, 4/5 treatment failure, and 3/4 both recurrent and treatment failure episodes. Among *H. influenzae* isolates, 1/118 (0.8%) was β-lactamase-negative ampicillin-resistant and 23/118 (19.5%) was β-lactamase-positive.

Multidrug resistance was observed in 20/87 (23.0%) *S. pneumoniae*-positive and in 8/118 (6.8%) *H. influenzae*-positive episodes. All *M. catarrhalis*-positive episodes (n = 23) were β-lactamase-positive.

Follow-Up Visits

Among the 456 episodes included in the final analysis, 445 (97.6%), 45 (9.9%), and 1 (0.2%) were followed by the planned, 1 unplanned, and 2 unplanned follow-up visits, respectively. The planned and unplanned follow-up visits took place 2–31 days after the initial visit.

Antibiotics were taken between the initial and follow-up visits in 438/445 (98.4%) episodes. The prescribed antibiotics were amoxicillin (294/456 [64.5%]), ceftriaxone (149/456 [32.7%]), azithromycin (11/456 [2.4%]), and amoxicillin and clavulanate potassium (2/456 [0.4%]) during the initial visits, and ceftriaxone (14/445 [3.1%]), amoxicillin and clavulanate potassium (4/445 [0.9%]), and azithromycin (1/445 [0.2%]) during the follow-up visits.

During the follow-up visits, 38 samples were collected from children with signs and symptoms consistent with AOM (12 and 26 samples during planned and unplanned visits, respectively). Of these, 17/38 (44.7%) samples were culture-positive: *H. influenzae* was detected in 14/17 (82.3%; all NTHi), *S. pneumoniae* in 6/17 (35.3%; serotypes 3 [2/6], 6A [2/6], 14 [1/6], and 16F [1/6]), and *M. catarrhalis* in 1/17 samples (5.9%; data not shown).

During the follow-up visits, high antibiotic non-susceptibility rates were observed in *S. pneumoniae* isolates for erythromycin (3/6) and azithromycin (2/3), and in *H. influenzae* isolates for TMP-SMX (11/14). Multidrug resistance was

TABLE 3. Antibiotic Non-Susceptibility of *S. pneumoniae, H. influenzae,* and *M. catarrhalis* Isolates at the Initial Visit (Final Study Analysis)

Antibiotic/test*	*S. pneumoniae* (N = 87) n (%)	*H. influenzae* (N = 118) n (%)	*M. catarrhalis* (N = 23) n (%)
Penicillin (MIC >2 μg/mL)	1 (1)	–	–
Ampicillin (MIC >1 μg/mL)	–	20 (18)‡	17 (74)
Amoxicillin/Clavulanate (MIC >2/1 or >4/2 μg/mL)†	1 (1)	1 (1)	0 (0)
Ceftriaxone (MIC >1 μg/mL)	1 (1)	1 (1)	0 (0)
Erythromycin (MIC >0.25 μg/mL)	30 (34)	–	–
Azithromycin (MIC >0.5 μg/mL)§	13 (32)	1 (1)	0 (0)
Levofloxacin (MIC >2 μg/mL)	0 (0)	0 (0)	0 (0)
TMP-SMX (MIC >0.5/9.5 μg/mL)	44 (51)	79 (67)	2 (9)
Nitrocefin (positive)	–	23 (19)	23 (100)
Multi-drug resistance (positive)	20 (23)	8 (7)	0 (0)

MIC = minimum inhibitory concentration, n (%) = number (percentage) of intermediate/resistant episodes, N = number of culture-positive episodes.

* Interpretation of the results was based upon the Clinical and Laboratory Standards Institute guidelines published in 2009.[15]

† MIC = 2/1 μg/mL for *S. pneumoniae* and 4/2 μg/mL for *H. influenzae* and *M.* catarrhalis.

‡ Results were missing for 5 samples.

§ Results were missing for 46 *S. pneumoniae* isolates, 47 *H. influenzae* isolates, and 9 *M. catarrhalis* isolates.

observed in 2/6 *S. pneumoniae*-positive and 1/14 *H. influenzae*-positive episodes.

In 9/38 samples collected during the planned and unplanned follow-up visits, the same pathogens were detected as those identified in the same children during the initial visit. These samples were collected in children who had received a prescription for amoxicillin (6/9), ceftriaxone (2/9), or azithromycin (1/9) at the initial visit.

Comparison Between Pre- and Post-PCV7 Introduction

Before PCV7 introduction, 471/884 episodes were culture-positive; this proportion decreased significantly after PCV7 introduction (53.3% vs 44.7%; *P*-value = 0.003) (Table 4).

The proportions of *S. pneumoniae*-positive episodes were significantly higher (27.7% vs 19.1%; *P*-value = 0.002) and of

TABLE 4. Bacterial Etiology of AOM Episodes for the Pathogens Under Study Before (1999–2004) and After (2009–2010) PCV7 Introduction (Final Study Analysis)

	Before PCV7 introduction (N = 884) n (%)	After PCV7 introduction (N = 456) n (%)	*P*-value
Any pathogen under study	**471 (53.3)**	**204 (44.7)**	**0.003**
Streptococcus pneumoniae	**245**‡ **(27.7)**	**87 (19.1)**	**0.002**
19F, 14, 6B, 23F, 9V*, 4*	129 (65.8)	38|| (43.7)	
3, 6A, 7F, 1*, 19A†	26 (13.3)	17 (19.5)	
11A, 16F*, 18A, 18C*, 35B, 15B, 10A*, 15A, 6C, 2*, 15C, 17F*, 10F, 18B*, 13*, 9L*, 21†, 22F†, 23A†, 34†	41 (20.9)	32 (36.8)	
Haemophilus influenzae	**181**§**(20.5)**	**118 (25.9)**	**0.0007**
NTHi	167 (100)	113 (95.8)	
a–f	0 (0)	5 (4.2)	
Streptococcus pyogenes	**34 (3.8)**	**9 (2.0)**	**0.639**
Moraxella catarrhalis	**34 (3.8)**	**23 (5.0)**	**0.090**

Bold values refer to the total number of bacterial pathogens and total number of pathogens for each bacterial type (eg *Streptococcus pneumonia; Haemophilus influenzae; Streptococcus pyogens; Moraxella catharralis*).

Serotypes are listed in descending order of predominance (for pre-PCV7). N = number of episodes, n (%) = number (percentage) of episodes in a given category (number and percentage of AOM episodes positive for the 4 otopathogens are shown compared to the total number of AOM episodes, while the serotype distribution is shown compared to otopathogen-positive episodes).

* serotypes identified pre-PCV7 only.

† serotypes identified post-PCV7 only.

‡ information regarding serotype distribution missing for 49 episodes.

§ Information regarding serotype distribution missing for 14 episodes.

|| includes 1 bilateral episode of 6C/19F.

H. influenzae-positive episodes significantly lower (20.5 % vs 25.9%; *P*-value <0.001) before than after PCV7 introduction. No significant differences were observed for *S. pyogenes* and *M. catarrhalis*.

Among pneumococcal isolates with available results, PCV7-serotypes tended to be more frequently identified before than after PCV7 introduction (129/196 [65.8%] vs 38/87 [43.7%]; *P*-value = 0.0005). Following PCV7 introduction, proportions of serotypes 6A (4/196 [2.0%] vs 5/87 [5.7%]) and 19A (0/196 [0.0%] vs 6/87 [6.9%]) increased and proportions of serotype 3 decreased (19/196 [9.7%] vs 5/87 [5.7%]). All 167 *H. influenzae*-positive episodes with available results before PCV7 introduction were NTHi.

DISCUSSION

Continuous monitoring of etiology and antimicrobial susceptibility of otopathogens is important to detect changes, and to evaluate the potential impact of vaccines and antimicrobial therapies. During the first few years following PCV7 introduction in the NIP in Costa Rica, *H. influenzae* (25.9%, mainly NTHi) followed by *S. pneumoniae* (19.1%) were the most frequently identified otopathogens in children with AOM, while *M. catarrhalis* (5.0%) and *S. pyogenes* (2.0%) were less common. As expected, the most frequent co-infection was *S. pneumoniae* and *H. influenzae* (4.2% of episodes). Bacterial distribution patterns were similar for children aged <2 years and older children.

In line with studies conducted in Costa Rica before PCV7 introduction, *H. influenzae* was more frequent than *S. pneumoniae* in treatment failure AOM episodes.[3,6] This could be explained by the difficulty to treat *H. influenzae* and the common relapses with this pathogen.[17–21] The observations that *H. influenzae* was more frequent than *S. pneumoniae* in recurrent episodes and that both pathogens were found in similar proportions of untreated episodes were made for the first time in Costa Rican children with AOM.[3,6] As expected, *H. influenzae* was slightly more frequent in vaccinated than unvaccinated children, while the opposite was observed for *S. pneumoniae*. Among participating children, 76.8% were considered, per protocol, vaccinated with PCV7, which is in line with reported vaccination coverage rates in Costa Rica at the study time, but is probably lower than the current vaccination coverage since older participants were not age-eligible for vaccination in the NIP.[8]

Only 38/445 children who attended the follow-up visits were symptomatic and had an additional MEF sample collected, suggesting that most children's symptoms had resolved. Of the 17 otopathogens identified during the follow-up visits, 9 (53%) were identical to those of the initial visit. Post-PCV7 introduction, pneumococcal isolates were largely non-susceptible to macrolides and TMP-SMX, and *H. influenzae* isolates to TMP-SMX. Penicillin non-susceptibility rates in pneumococcal isolates were lower than expected; this may be explained by the fact that the new guidelines of the Clinical and Laboratory Standards Institute were used in this study.[15]

While NTHi was the most common pathogen post-PCV7 introduction, *S. pneumoniae* was more frequently identified than *H. influenzae* (27.7% vs 20.5%) before PCV7 introduction (1999–2004). This expected change is consistent with observations from other countries.[10–13,22] The most common pneumococcal serotypes were 19F (34.2%), 3 (9.7%), 6B (9.7%), and 14 (9.7%) pre-PVC7 introduction, and 19F (26.4%), 14 (8.0%), and 35B (8.0%) post-PCV7 introduction. Although the impact of PCV7 introduction in the NIP was reflected by the decline in proportions of PCV7-serotypes (65.8% in 1999–2004 vs 43.7% in 2010–2012), serotype 19F remained the most frequently identified serotype and 16/24 serotype 19F isolates were in children who had received 3 or 4 PCV7 doses. Although these trends were not statistically significant, the proportions of AOM cases caused by additional serotypes included in the new generation vaccines did not decrease post-PCV7 introduction, unlike PCV7-serotypes, similarly to a study conducted in United States,[12] with the exception of serotype 3 (9.7% vs 5.7%). We observed statistically non-significant increases in proportion of episodes caused by serotypes 6A (2.0% vs 5.7%) and 19A (0.0% vs 6.9%) following PCV7 introduction. Even though a likewise trend was noted in one previous study,[23] these results remain descriptive and no formal conclusion can be drawn regarding the proportion of AOM cases caused by PCV7 related serotypes 6A and 19A in Costa Rica post-PCV7 introduction. Another important finding of this study was the potential emergence of pneumococcal serotypes that are not included in any licensed vaccine, such as serotype 35B, in line with previously observed changes in serotype distribution following pneumococcal conjugate vaccination.[24–27] It was interesting to observe that although after several years of PCV7 in the NIP and a reduction of the prevalence of PCV7-serotypes was observed, the persistence of PCV7-serotypes (43.7%) was mostly due to a lesser reduction in the prevalence of serotype 19F between the pre-vaccination period (34.2%) and the post-vaccination period (27.6%). This was not unexpected as similar results of lower MEF protection with PCV7 were observed in the OM pivotal clinical trial.[28]

This study describes important early changes following PCV7 introduction in Costa Rica, but has several limitations: no information on incidence rates was collected, the study power was <80%, and most results were descriptive and should be interpreted cautiously. Moreover, although PCV13 was licensed and replaced PCV7 in the NIP in August 2011 in Costa Rica, 2012 was not considered as a transitional year, and no specific information was collected for children vaccinated with other pneumococcal conjugate vaccines (as reflected in our study, most episodes in vaccinees were isolated from children having received PCV7, even though enrolment ended in April 2012). Further evaluation of the impact of pneumococcal conjugate vaccines should be evaluated following broader vaccination coverage, since older children, who were not age-eligible for vaccination, would now be vaccinated.

This study showed that in the first few years following PCV7 introduction into the NIP in Costa Rica, *H. influenzae* followed by *S. pneumoniae* were the most common otopathogens in children with AOM, and the observed prevalence of *S. pneumoniae* and PCV7-serotypes tended to decrease, while that of *H. influenzae* tended to increase. These results are preliminary and the full impact of PCV7 and new-generation vaccines on AOM etiology and antibiotic resistance should be further evaluated following broader vaccination coverage in Costa Rican children.

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