Etiology of Acute Otitis Media in Children Less Than 5 Years of Age

A Pooled Analysis of 10 Similarly Designed Observational Studies

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Background: Acute otitis media (AOM) is an important cause of childhood morbidity and antibiotic prescriptions. However, the relative importance of the well-known otopathogens, *Streptococcus pneumoniae* (Spn) and *Haemophilus influenzae* (Hflu), remains unclear because of a limited number of tympanocentesis-based studies that vary significantly in populations sampled, case definitions and heptavalent pneumococcal conjugate vaccine use.

Methods: We conducted a pooled analysis of results from 10 AOM etiology studies of similar design, the protocols of which were derived from a common protocol and conducted in children 3 months to 5 years of age in different countries. Generalized estimating equations were used to account for within-study correlations.

Results: The majority, 55.5% (95% confidence interval: 47.0%–65.7%) of 1124 AOM episodes, were bacterial pathogen positive: 29.1% (24.8%–34.1%) yielded Hflu and 23.6% (19.0%–29.2%) Spn. Proportions of Hflu and Spn were higher and lower, respectively, in heptavalent pneumococcal conjugate vaccine–vaccinated children. Hflu and Spn were each isolated from 20% to 35% of children in every 1-year age range. Hflu was less likely to be isolated from first (vs. subsequent) episodes [relative risk (RR): 0.71 (0.60–0.84)]. Spn was more often isolated from sporadic (vs. recurrent) cases [RR: 0.76 (0.61–0.97)]; the opposite was true for Hflu [RR: 1.4 (1.00–1.96)]. Spn cases were more likely to present with severe (vs. mild) symptoms [RR: 1.42 (1.01–2.01)] and Hflu cases with severe tympanic membrane inflammation [RR: 1.35 (1.06–1.71)].

Conclusions: Spn and Hflu remain the leading otopathogens in all populations examined. While associated with overlapping symptoms and severity, they exhibit some differences in their likelihood to cause disease in specific subpopulations.

Key Words: otitis media, etiology, pediatric

Acute otitis media (AOM) is a leading cause of global childhood morbidity and empiric antibiotic prescriptions despite guidelines recommending judicious antimicrobial use.[1–3] Prevention of AOM is an important public health objective, and pediatric pneumococcal conjugate vaccines (PCVs), which are licensed to prevent AOM caused by *Streptococcus pneumoniae* (Spn), are an important tool to reduce costs associated with medical care and reduce antibiotic resistance.[4]

Double-blind, randomized control trials with different PCVs in infants have reported reductions in overall AOM episodes ranging from −1% to 34% and in antibiotic prescriptions from 5% to 8%.[5–10] These reported differences in vaccine effect have been variously attributed to differences in study design (eg, case definition), access to care, local distribution of otopathogens and the specific vaccine formulation tested.[11]

Spn and nontypable *Haemophilus influenzae* (NTHi) have historically been the leading causes of AOM, with the former generally believed to be more associated with acute disease and the latter with recurrent cases as well as otitis media with effusion.[12,13] Pneumococcal serotypes represented in the heptavalent PCV (PCV7-CRM) covered approximately 60%–70% of pneumococcal isolates collected from PCV-naive children 6–59 months of age with AOM.[14] The efficacy studies noted above revealed that decreases in overall clinical AOM are driven primarily by reductions in vaccine-type pneumococcal AOM. However, there is some evidence that PCVs may also differ in their impacts on nonvaccine type pneumococcal and *Haemophilus influenzae* (Hflu) AOM.[6,10,15] Thus, understanding the relative importance of Spn and Hflu in different age groups and populations may have important implications for the magnitude of protection expected from currently available PCVs (10-valent pneumococcal Hflu protein D-containing conjugate and 13-valent CRM conjugate).

Until recently, AOM etiology data have primarily come from studies in the United States, Europe, Israel and Costa Rica, but information is needed more broadly as PCVs are now used in the routine immunization program of at least 125 countries globally.[16] It has been difficult to draw general conclusions from those studies

because of differences in study populations, PCV use and case definitions. Instead of performing a meta-analysis in which a range of studies may be considered for inclusion according to defined criteria, we sought to provide a more uniform assessment by conducting a pooled analysis of 10 similarly designed cross-sectional, observational studies that assessed AOM etiology in children in various countries,[17–25] as each study's protocol derived from a common protocol.[26] This article presents the results of this pooled analysis.

METHODS

Study Design

This pooled analysis included 10 observational studies with similar protocols (eg, same case definitions, same age grouping, similar sample handling and laboratory procedures and identical plans for data collection to limit heterogeneity between studies). However, some modification was allowed to account for potential differences in local acceptability of performing tympanocentesis in different AOM subpopulations. These studies were conducted between 2007 and 2011 in children of 3–59 months of age. In each study, inclusion criteria required a bulging, diffused or localized inflamed tympanic membrane or spontaneous otorrhea of <24 hours since symptom onset, accompanied by one of the functional or general signs of otalgia/irritability, conjunctivitis or fever. The history of AOM cases enrolled, either sporadic or recurrent, could vary by country, depending on local clinical practice for management and treatment of disease. Middle ear fluid (MEF) samples were collected before antibiotic receipt at that visit by tympanocentesis or careful sampling of spontaneous otorrhea. Children receiving antibiotics before enrollment in the 72 hours before the visit as (1) systemic therapy for other conditions or (2) prophylactically for recurrent AOM were excluded, but AOM treatment failures were included. Further details about inclusion/exclusion criteria have been published previously.[17–25]

At the time of enrollment, a clinical examination was performed and medical history and general symptoms were collected by questionnaire. Severity of tympanic membrane inflammation was classified using the otoscopy scale-8 (described previously[17–25]). An episode of AOM was classified as new if there was a ≥30-day AOM symptom-free period before onset. AOM cases were classified as treatment failures if symptoms persisted despite initiation of physician-prescribed antibiotics in the 48–72 hours before study enrollment. Recurrent AOM was defined as a reported history of ≥3 episodes within the past 6 months or ≥4 episodes within the past 12 months. A child was classified as vaccinated if he/she received ≥2 PCV doses before age 1 year or ≥1 PCV dose after age 1 year. Cases were classified as severe if a child had ear pain or an axillary temperature ≥38.5°C and/or if his/her tympanic membranes were scored as OS-6 (indicative of hyperemia—bulging rounded doughnut appearance of tympanic membrane) or OS-7 (indicative of hyperemia with bulla formation).

Laboratory Methods

MEF samples were first inoculated in Amies transport medium (with or without charcoal) and then cultured within 48 hours on blood agar, MacConkey and/or Sabouraud media supplemented with the appropriate antimicrobial agent to select the pathogens of interest.[17–25] Only Spn, Hflu, *Moraxella catarrhalis* (Mcat) and *Streptococcus pyogenes* (Spyo) isolated by bacterial culture were considered to be true otopathogens for further evaluation and characterization. Spn isolates were serotyped by Quellung reaction or polymerase chain reaction, and PCV7-CRM types include serotypes 4, 6B, 9V, 14, 18C, 19F and 23F as well as 6A.[27,28] Hflu isolates were serotyped (a, b, c, d, e, f or nontypable) by monovalent antisera and/or real-time polymerase chain reaction.[29] Antibiotic susceptibility testing was performed using E-tests, agar dilution or microdilution, and nonsusceptibility

was defined according to Clinical and Laboratory Standards Institute 2009 cut-offs.[30] Antibiotic nonsusceptibility was defined to encompass both intermediate and resistant results. Beta-lactamase production was evaluated for Hflu and Mcat isolates using a nitrocefin test.[31]

Statistical Analysis

Because the studies were conducted in different countries, potential heterogeneity was tested using Cochran Q test based on inverse variance weights on the proportion of culture-positive samples to determine the need for a random effects model to account for within- and between-study heterogeneity in the pooled analysis, and reflected in the estimated variance used to build the confidence intervals (CIs). Generalized estimating equations were used to account for correlation between subjects from each study/country.[32,33] All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). The generalized estimating equation models were fitted using the GENMOD procedure assuming an exchangeable correlation matrix. Finally, the analysis plan of the pooled analysis was defined when all the study databases were already frozen.

Individual patient data were pooled and analyzed according to episode status (first-reported episode or not), treatment status (untreated or treatment failure), sample collection type (otorrhea or tympanocentesis), PCV status (vaccinated or not), recurrent AOM (or not) and age group. For subgroup comparisons, pooled relative risks (RRs) with their 95% CIs were estimated. Each study RR was weighted by the inverse of the within-study variance plus the between-study variance. The basis of the pooled analysis is to combine the values derived from each intrastudy comparison. It is not a combination of all individual-level data from all studies. In some individual studies, no such comparisons were possible (eg, Saudi Arabia and PCV vaccination), so those data are not included. Therefore, subgroups should be and are compared using RR estimates as the proportions in each subgroup are not comparable from a statistical perspective. For analyses of etiology by serotype, serotype 6A was considered a PCV7-CRM type.

Ethical Considerations

All studies were conducted according to Good Clinical Practice, the Declaration of Helsinki and local rules and regulations of the country. All studies were approved by local institutional review committees and a parent/guardian provided written informed consent. Pooled analyses included only deidentified individual patient data, so additional consent was not required.

RESULTS

The pooled analysis included records from 1124 AOM episodes (Table 1). For each study, the median age of children enrolled was between 20 and 36 months and 46%–69% were male; additionally, 44%–80% of samples collected were culture positive, and 24%–95% of samples were obtained by tympanocentesis. The most predominant otopathogen reported was Hflu, closely followed by Spn. The proportion of children classified as PCV7-CRM vaccinated ranged widely from 0% to 83%, and the contribution of recurrent AOM varied from 0% to 98%. Because heterogeneity among studies in the proportions of culture-positive samples was statistically significant ($P < 0.0001$), the random effects pooling method was used to explore the relative contribution of each pathogen, adjusting for potential confounders such as age, vaccination status, AOM history, severity and history of antibiotic use. Figure 1 (left) presents the individual and pooled study proportions of Spn, Hflu, Spyo and Mcat.

Etiology by Age and Vaccination Status

Among all studies, 55.5% (95% CI: 47.0%–65.7%) of the 1124 AOM episodes were culture positive: 29.1% (24.8%–34.1%)

yielded Hflu, 23.6% (19.0%–29.2%) Spn, 3.7% (2.0%–6.8%) Spyo and 2.8% (1.4%–5.4%) Mcat (Fig. 1). These figures were not mutually exclusive, but only 2.7% (1.4–5.1%) reported any coinfection. Accordingly, Hflu, Spn, Spyo and Mcat represented 52.4%, 42.5%, 6.7% and 5.0%, respectively, of all culture-positive isolates. Of Spn episodes, 42.8% (29.0%–63.2%) were caused by PCV7-CRM serotypes, and 86.1% (71.7%–100%) of Hflu episodes were nontypable.

TABLE 1. Overview of Included Studies

Country (GSK Study ID)	Data Collection	N	Median Age, mo (Range)	Male (%)	PCV7-CRM Vaccinated (%)	Treatment Failure (%)	Recurrent Otitis Media (%)	Otorrhea (%)	Culture Positive (%)
Chile (submitted) (112671)	September 2009 to September 2010	164	24 (4–59)	56	2	5	5	11	80
Colombia[18] (111397)	February 2008 to January 2009	99	29 (5–55)	55	18	0	10	16	62
France[21] (109369)	May 2007 to April 2009	143	14 (4–47)	53	83	73	27	18	61
Germany[17] (111640)	November 2008 to April 2010	100	36 (3–59)	56	72	3	23	76	44
Mexico[24] (110796)	March 2008 to April 2009	111	23 (4–59)	69	42	3	30	20	64
Saudi Arabia[22] (111337)	June 2009 to May 2011	66	20 (3–58)	59	17	6	0	32	21
South Africa[25] (112135)	May 2009 to May 2010	182	15 (4–53)	55	9	0	1	5	45
Spain* [19] (110513)	June 2008 to March 2010	56	20 (5–35)	64	63	18	98	25	61
Thailand[23] (110646)	April 2008 to August 2009	112	36 (5–59)	46	0	8	7	10	50
Venezuela[20] (111828)	December 2008 to December 2009	91	26 (5–59)	48	74	1	34	10	66

*In order to keep consistency with regard to the diverse case definitions, only a selection of the entire cohort from the study conducted in Spain was included in the analyses.
N indicates number of AOM episodes; GSK, GlaxoSmithKline.
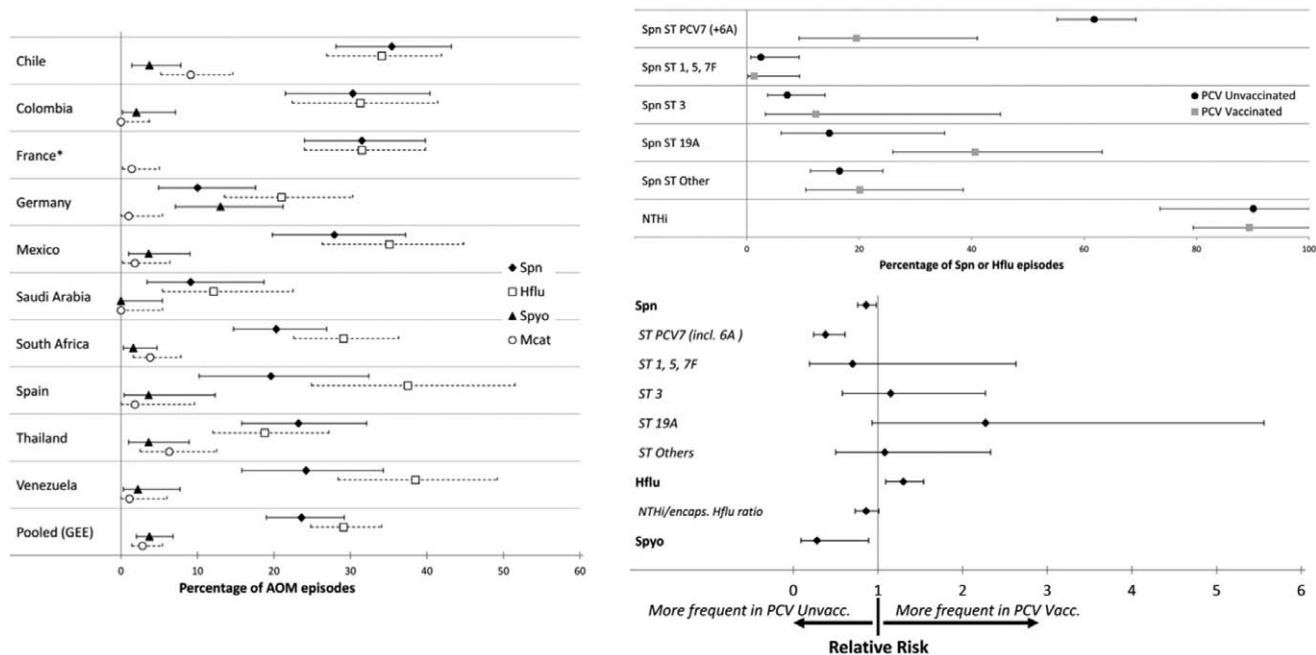
FIGURE 1. Bacterial etiology of AOM episodes. Left, etiology by individual study and pooled study proportions, expressed as percentages of all AOM episodes. Right, Spn and Hflu serotype distribution of AOM episodes according to PCV7-CRM vaccination status, expressed as percentages of Spn and Hflu episodes (upper), and as relative risk (lower). Error bars represent 95% CI, and 55.5% (47.0–65.7%) of all AOM episodes were culture positive (not including contaminants); the sum of individual pathogen pooled (GEE) proportions does not add up to 55.5% because of coinfections. *Spyo results were not available for France. Proportions of Spn (ST) serotypes are represented as percentage of all Spn cases (N = 170 in PCV unvaccinated and N = 83 in PCV vaccinated categories), and proportions of NTHi are represented as percentage of Hflu (N = 183 in PCV unvaccinated and N = 133 in PCV vaccinated categories). See statistical methods section for details related to RR calculation: Briefly, an RR <1 for a pathogen indicates that it comprises a smaller percentage of all pathogens isolated in PCV7-CRM–vaccinated compared with unvaccinated individuals; an RR <1 for a specific pneumococcal serotype or NTHi indicates that it comprises a smaller percentage of all pneumococci or of all *Haemophilus* isolated in PCV7-CRM–vaccinated than in unvaccinated individuals. 6A was included in PCV7 ST category.

The age distributions of Hflu and Spn were similar (Fig. 2), with Spn isolated from 20% to 29% and Hflu from 20% to 35% of AOM cases in each age group examined. The percentages were slightly lower (Spn) or higher (Hflu) in PCV7-CRM–vaccinated children compared with unvaccinated (Table 2 and Fig. 1, right), while Spyo appeared less likely to be identified in PCV7-CRM-vaccinees (RR: 0.28; 95% CI: 0.09–0.89; lower right Fig. 1).

Nonetheless, certain observations were consistent in both PCV7-CRM–vaccinated and unvaccinated children, and were significant in pooled analysis. Neither pathogen was more or less likely to be associated with the youngest age group (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612) than with older ages. Hflu was less likely to be identified in the first episode (RR: 0.71; 0.60–0.84) than in subsequent episodes and more likely to be associated with recurrent rather than sporadic disease (RR: 1.40; 1.00–1.96). In contrast, Spn was less likely to be associated with recurrent disease (RR: 0.76; 0.61–0.97).

Clinical Severity by Etiology

Encapsulated Hflu isolates were more frequently associated with high severity of tympanic membrane bulging and inflammation (73% were OS-6/7), compared with Spn or NTHi or Mcat (30%–35% OS-6/7), whereas Spyo cases were only infrequently severe (8%–9%, Table, Supplemental Digital Content 2, http://links.lww.com/INF/C613). Conjunctivitis was more commonly identified in NTHi cases, but otherwise reported symptoms were similar between Spn and NTHi cases. Ear tugging and trouble sleeping were less frequently observed in cases caused by encapsulated Hflu than NTHi.

Both major otopathogens were thus associated with a range of disease severity (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612): Hflu was more likely to be identified in cases with an OS-6 or 7 than in less severe cases (RR: 1.35; 1.06–1.71), and Spn was more likely to be identified in children with reported ear pain or a temperature ≥38.5°C (RR: 1.42; 1.01–2.01). Spyo was more likely (RR: 8.61; 4.74–15.63) and Mcat less likely (RR: 0.17; 0.04–0.69) to be found in MEF samples collected from otorrhea than by tympanocentesis.

Observations According to Vaccination Status

Some observations were statistically significant only in vaccinated or only in unvaccinated children, with little indication of a similar trend in the other group. For example, in PCV7-CRM–vaccinated children, Spn was more likely to be found in otorrhea rather than in tympanocentesis samples (RR: 1.66; 1.23–2.25) and in cases with severe tympanic membrane inflammation (RR: 3.58; 1.34–9.61). It was also more likely to be isolated from a treatment failure (RR: 1.63; 1.32–2.01) in vaccinated but not in unvaccinated children, and less likely to be identified in a first-reported episode in the youngest children (RR: 0.58; 0.37–0.91) (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612).

In vaccinated children, Hflu was less likely to be isolated by otorrhea than by tympanocentesis (RR: 0.63; 0.47–0.85). In unvaccinated children, Hflu was less likely to be found in a child with symptoms such as ear pain or high fever (RR: 0.63; 0.43–0.91) than the one with milder symptoms (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612). However, in none of the above examples was a similar association apparent in the other group.

In unvaccinated children, the proportions of Spn and Hflu cases presenting with severe symptoms were 37.5% (30.6%–46.0%)
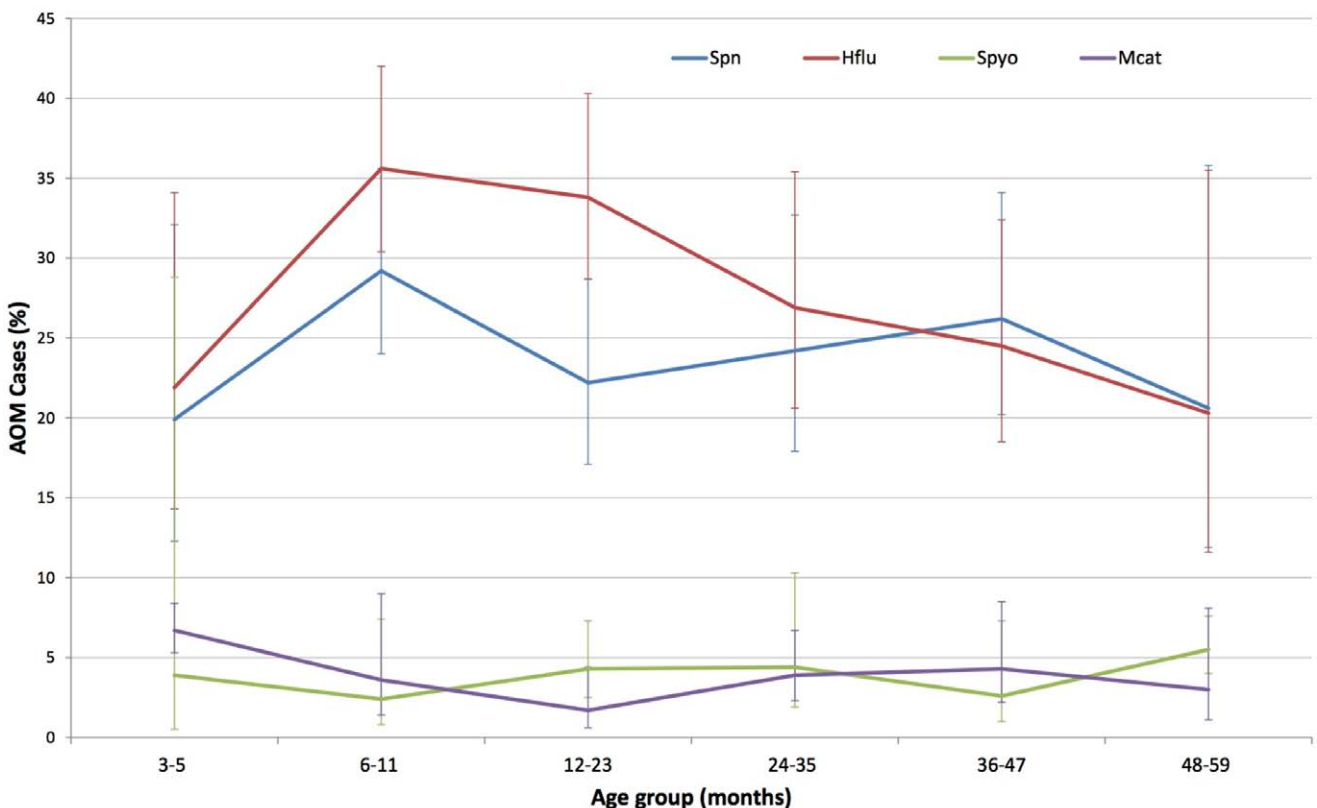
FIGURE 2. Distribution of AOM otopathogens by age. Proportion of all AOM cases within specific age groups that were caused by Spn, Hflu, Spyo or Mcat, pooled study proportions. Error bars represent 95% CI. full color online

TABLE 2. Acute Otitis Media Etiology According to PCV7-CRM Vaccination Status

	Overall					Unvaccinated					Vaccinated				
	N	Spn % (95% CI)	Spn RR (95% CI)	Hflu % (95% CI)	Hflu RR (95% CI)	N	Spn % (95% CI)	Spn RR (95% CI)	Hflu % (95% CI)	Hflu RR (95% CI)	N	Spn % (95% CI)	Spn RR (95% CI)	Hflu % (95% CI)	Hflu RR (95% CI)
Overall	1124	23.6 (19.0–29.2)	NA	29.1 (24.8–34.1)	NA	669	25.1 (20.4–30.9)	NA	27.0 (22.8–32.0)	NA	388	20.8 (15.7–27.5)	NA	35 (30.0–40.8)	NA
3–11 mo of age	241	27.6 (22.2–34.4)	1.17 (0.97–1.42)	33.6 (30.9–36.4)	1.19 (0.99–1.43)	145	31.2 (25.2–38.6)	1.31 (0.99–1.74)	32.8 (26.8–40.1)	1.21 (0.88–1.67)	86	26.7 (23.4–30.5)	0.85 (0.72–1.01)	38.9 (35.1–43.1)	1.09 (0.86–1.39)
1st reported episode	464	23.1 (17.5–30.4)	1.18 (0.87–1.59)	23.6 (18.8–29.6)	0.71 (0.60–0.84)	308	26 (20.3–33.2)	1.29 (0.94–1.77)	22.4 (17.3–29.1)	0.71 (0.63–0.80)	105	17.8 (12.7–24.8)	0.89 (0.55–1.45)	30.8 (25.7–36.9)	0.84 (0.68–1.04)
1st reported episode in 3–11 mo of age	111	24.9 (21.0–29.6)	1.14 (0.78–1.67)	32 (30.0–34.2)	1.07 (0.83–1.38)	76	30.5 (29.3–31.7)	1.32 (0.91–1.92)	20.2 (9.7–41.8)	1.07 (0.71–1.61)	27	-	0.58 (0.37–0.91)	36.8 (20.9–64.7)	0.95 (0.51–1.78)
Recurrent AOM	208	19.7 (15.3–25.4)	0.76 (0.61–0.97)	26.3 (21.7–31.9)	1.4 (1.00–1.96)	77	20.3 (15.0–27.4)	0.86 (0.59–1.26)	37.2 (30.1–46.1)	1.56 (1.19–2.05)	129	19.7 (14.8–26.2)	0.77 (0.61–0.98)	36.6 (26.3–50.9)	1.15 (0.75–1.76)
Otorrhea	223	29.9 (22.1–40.3)	1.34 (0.94–1.92)	30.2 (21.9–41.5)	0.97 (0.69–1.38)	100	25.8 (17.0–39.2)	0.98 (0.59–1.62)	33.1 (23.3–47.1)	1.28 (0.92–1.78)	105	28.8 (17.8–46.4)	1.66 (1.23–2.25)	26.1 (18.4–37.1)	0.63 (0.47–0.85)
Severe symptoms	273	32.9 (25.7–42.1)	1.42 (1.01–2.01)	25.2 (17.9–35.4)	0.8 (0.58–1.10)	125	37.5 (30.6–46.0)	1.47 (0.96–2.27)	17 (10.1–28.6)	0.63 (0.43–0.91)	135	28.3 (20.1–39.9)	1.51 (0.92–2.47)	33.8 (22.4–50.9)	0.91 (0.63–1.32)
Severe OS status	372	25.7 (21.4–30.7)	1.5 (0.95–2.36)	33.3 (27.4–40.5)	1.35 (1.06–1.71)	217	27.9 (23.4–33.1)	1.31 (0.81–2.11)	30 (23.2–38.8)	1.41 (1.11–1.81)	134	24.1 (21.0–27.8)	3.58 (1.34–9.61)	42.2 (40.5–43.9)	1.46 (0.93–2.29)
History of previous antibiotics	310	-	1.18 (0.97–1.43)	32.4 (28.7–36.5)	1.15 (0.94–1.40)	129	31.6 (26.1–38.3)	1.21 (0.94–1.56)	27.5 (19.1–39.7)	-	170	-	-	-	1.06 (0.82–1.37)
Treatment failure	143	-	1.22 (1.04–1.42)	26.8 (20.0–35.9)	0.81 (0.47–1.40)	42	31.7 (27.0–37.2)	1.07 (0.80–1.45)	22.1 (10.0–48.5)	0.69 (0.35–1.39)	93	27.1 (25.5–28.9)	1.63 (1.32–2.01)	27.1	0.75 (0.51–1.11)

Values in shaded cells are statistically significant. Pooled study proportions (%) and RR were computed using GEE model.
GEE indicates generalized estimating equations; N, number of episodes; NA, not applicable.

TABLE 3. Percentages of Spn and Hflu Isolates Nonsusceptible to a Specific Antibiotic

Antibiotic	Spn		Hflu	
	Overall, % (95% CI)	RR (Vaccinated vs. Unvaccinated) (95% CI)	Overall, % (95% CI)	RR (Vaccinated vs. Unvaccinated) (95% CI)
Aminopenicillin	18.5 (10.3–33.2)	1.27 (0.85–1.92)	20.0 (11.8–33.7)	1.39 (0.99–1.92)
Cefotaxime	9.8 (4.6–20.7)	-	0.9 (0.3–2.9)	-
Azithromycin	69.9 (45.2–100.0)	0.88 (0.76–1.02)	6.2 (2.7–14.2)	-
Tetracycline	37.2 (24.2–57.0)	0.99 (0.53–1.85)	7.5 (5.1–10.8)	0.31 (0.11–0.92)
Trimethoprim/sulfamethoxazole	53.7 (36.6–78.6)	0.93 (0.57–1.49)	47.8 (33.2–68.8)	1.37 (1.08–1.75)
Amoxicillin/clavulanate	0.5 (0.1–3.2)	-	5.4 (2.6–11.3)	0.95 (0.25–3.57)

Values in shaded cells are statistically significant. Pooled study proportions (%) and RR were computed using GEE model.
GEE indicates generalized estimating equations.

and 17.0% (10.1%–28.6%), respectively, but in vaccinated children 28.3% (20.1%–39.9%) of the Spn cases and 33.8% (22.4%–50.9%) of the Hflu cases were severe.

Not surprisingly, serotype distribution also differed according to vaccination status (Fig. 1, right). PCV7-CRM types (including 6A) were less likely to be identified in PCV7-CRM–vaccinated children compared with unvaccinated [RR: 0.38 (0.24–0.61)]. Serotype 19A appeared to be more frequently identified in children who were PCV7-CRM vaccinated, although this did not achieve statistical significance (RR: 2.27; 0.93–5.56). In contrast, there was no obvious association of vaccination status with the proportion of serotype 3 (RR: 1.15; 0.58–2.27), that of the group of 1, 5 and 7F (RR: 0.70; 0.19–2.63), or the proportion of other non-PCV7-CRM types (RR: 1.08; 0.50–2.33).

Antibiotic Nonsusceptibility

Nonsusceptibility (Table 3) to first-line therapies such as aminopenicillin was 18.5% (10.3%–33.2%) among Spn isolates and 20.0% (11.8%–33.7%) for Hflu isolates. Nonsusceptibility to alternate first-line therapies differed: Spn isolates were rarely nonsusceptible to cefotaxime (9.8%; 4.6%–20.7%) but were frequently nonsusceptible to azithromycin (69.9%; 45.2%–100.0%). Small proportions of Hflu isolates were nonsusceptible to cefotaxime (0.9%; 0.3%–2.9%) and azithromycin (6.2%; 2.7%–14.2%). Spn and Hflu were also frequently nonsusceptible to second-line agents such as tetracycline and co-trimoxazole but were largely susceptible to amoxicillin/clavulanate. The percentage of Hflu isolates that were beta-lactamase negative was 74.6% (57.9%–96.0%), and 3.7% (1.7%–8.0%) were both beta-lactamase negative and ampicillin resistant. There were generally no significant differences in nonsusceptibility according to vaccination status, except for tetracycline nonsusceptible Hflu isolates, which were less likely to be found in vaccinated children [RR: 0.31 (0.11–0.92)], and trimethoprim/sulfamethoxazole nonsusceptible isolates, which were more likely to be observed in vaccinated children [RR: 1.37 (1.08–1.75)].

DISCUSSION

This analysis focused on 10 recent studies that had been implemented with similar study designs and characteristics so that a pooled analysis could assess the relative importance of different pathogens worldwide. It, thus, complements and extends previous reviews of pneumococcal serotype data from contemporaneous pediatric AOM studies,[14,34] as well as other reviews of bacterial AOM etiology.[13] The finding that Spn and Hflu were the 2 major pathogens in the pooled analysis is consistent with results from each of the included studies and provides a more definitive confirmation of what has been reported in most other published single-country studies.[35,36] The increased proportion of Hflu and moderately decreased proportion of Spn among cases who were PCV7-CRM vaccinated were

also consistent with previous reports.[37,38] Comparable results were observed within an eleventh individual study sharing the same design and conducted in Costa Rica; the results of that study were unfortunately not available at the time the pooling analysis was performed.[39]

Because individual patient information was included in this pooled analysis, it was possible to explore more completely than usually possible in single studies the role that other covariates play in etiology. For example, Hflu is sometimes thought to cause only mild AOM but in this study, approximately 25% of Hflu AOM cases presented with more severe symptoms and almost one-third presented with severe inflammation of tympanic membranes. In addition, there appeared to be differences associated with vaccination status. While in unvaccinated children the percentage of Hflu AOM cases presenting with severe symptoms (17%; 10.1%–28.6%) appeared lower than that of Spn cases (37.5%; 30.6%–46.0%), in vaccinated children the percentages were more similar [33.8% (22.4%–50.9%) and 28.3% (20.1%–39.9%), respectively], a finding for which we have no straightforward explanation. As previously reported, conjunctivitis was also particularly associated with NTHi AOM.[13]

A noteworthy observation is that although Hflu was less likely to be reported as the cause of a first AOM episode than of later episodes and more likely to be identified as recurrent, it nonetheless still comprised 20.2% (9.7%–41.8%) of first-reported AOM episodes in unvaccinated children of <12 months of age, a proportion not much different from that seen for Spn (30.5%; 29.3%–31.7%). This finding, in addition to >20% of isolates being nonsusceptible to first-line therapy with aminopenicillin, suggests that Hflu is an important target for primary prevention of AOM. Similar observations regarding NTHi were made in a study assessing AOM cases in children with single otopathogen in their nasopharyngeal carriage.[40] Conversely, the observation that Spn (along with Hflu) remains a prominent AOM pathogen even in children 3 or 4 years of age implies that duration of protection would be an important attribute of any PCV in the prevention of childhood AOM.

The shift in pneumococcal serotype distribution toward non-vaccine types in AOM cases who were PCV7-CRM vaccinated was expected and similar to results seen in US studies.[37,38] It is tempting to speculate that the predominantly non-PCV7-CRM types causing pneumococcal AOM in PCV7-CRM–vaccinated children, especially 19A and 3, are responsible for some of the other findings observed here—specifically, that only in PCV-CRM-vaccinated children was Spn more often found in otorrhea than in tympanocentesis samples, more often associated with severe tympanic membrane inflammation than mild, and more often from treatment failures than other cases.

The observation that Spyo is more likely and Mcat is less likely to be associated with spontaneous otorrhea rather than tympanocentesis is consistent with reports from other investigators.[41–43] Spyo appeared less likely to be isolated in PCV7-CRM–vaccinated

than in unvaccinated children, but unless prior infection with the serotypes contained in PCV7-CRM selectively predisposes the child to a subsequent Spyo infection, this appears a chance finding.

This pooled analysis is subject to limitations. First, there was statistically significant heterogeneity among the studies included, despite the use of similar protocols. For example, heterogeneity of PCV7-CRM use across the included studies limits broad interpretation of potential serotype and pathogen replacement. In an attempt to control for this, subgroup analyses were performed, the results were weighted, a random effect model was used and vaccination status was accounted for whenever possible when formulating the presented conclusions. Nonetheless, some countries had experienced a broad uptake of PCV7-CRM before the conduct of the study, and thus the contribution of herd effects was impossible to factor in. Evaluation after vaccination for a longer duration would be important.

As noted earlier, the pooling methodology relies solely on intrastudy comparisons, meaning that some pooled RR or proportion estimations did not include data from individual studies where such a comparison was not possible. It could, therefore, be argued that this introduces additional bias. However, the strength of the pooling methodology lies precisely in its focus on the consistency (or not) of the comparisons of the values obtained within each study. This should minimize variability that could otherwise arise from inclusion of values from only 1 group lacking a comparison set of value.

Second, despite efforts to standardize the design across studies, there were important differences in local guidelines for MEF collection. This is a recognized limitation of such prospectively planned pooled studies.[26] One country required MEF to be collected only in cases that were considered to be recurrent. There are likely differences in care-seeking practices for AOM in other countries that could have affected the severity of cases enrolled. Nonetheless, a significant finding of this study is that despite these differences, no evidence suggested major setting-related differences in etiology.

Third, bacterial etiology was not determined with molecular techniques and the true contribution of these bacteria to AOM is likely higher than reported here (as previously described).[19] It is also possible that culture-negative but polymerase chain reaction–positive samples may show different clinical severity or age tropisms compared with those described here.

Finally, although this analysis included 10 countries, most countries and regions are naturally not represented, including North America, most of Asia and sub-Saharan Africa, as well as native high-risk populations. Consequently, the generalizability of some of our findings may be limited to the health-care–seeking pediatric population in the countries included. However, as already noted, the identity of the most prominent etiologic pathogens as determined in the pooled analysis was largely consistent between the studies included, as well with other published reports, so those results may be considered to be more broadly applicable.

Despite differences in PCV7-CRM use and clinical management of AOM, this pooled analysis indicates that Spn and Hflu remain the leading causes of AOM in the different regions investigated. Both pathogens cause disease in the youngest and the oldest children, and it is known that early AOM cases are associated with more AOM cases/recurrence. Infants, thus, remain important targets for vaccination for either pathogen. Both pathogens are responsible for both mild and more severe AOM, as assessed by different measures and so are clinically significant. Finally, both pathogens are associated with antibiotic resistance. Although most serotypes in PCV7-CRM-vaccinated children now could be covered by 1 or both expanded serotype PCVs, one can note that already >20% of isolates were not covered by any currently licensed PCV, and

this proportion will certainly increase as higher valency vaccines are fully implemented. The availability of next-generation PCVs should address some residual disease burden, but replacement and antibiotic resistance make elimination an elusive target. Continued evaluation of otopathogens is needed to support up-to-date treatment guidelines and inform decision making for new prevention strategies.

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