Seasonal Distribution of Otitis Media Pathogens Among Costa Rican Children

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Background: Otitis media is an important cause of pediatric consultation, and knowledge of yearly pathogen distribution might improve antimicrobial selection.

Objectives: To determine the seasonal pathogen and antimicrobial resistance distribution among Costa Rican children with otitis media.

Methods: Between 1999 and 2004, 952 children with otitis media, aged 3–144 months who participated in various clinical trials, were analyzed. Data obtained from this period were compared against historical data collected between 1992 and 1997.

Results: Five hundred sixteen (52%) children had a baseline middle ear fluid pathogen isolated. The most common pathogens were Streptococcus pneumoniae 252 (49%), Haemophilus influenzae 190 (37%), S. pyogenes 38 (7%), and Moraxella catarrhalis 36 (7%). The overall proportion of H. influenzae (24–37%; P = 0.01) and the production of β-lactamase producing H. influenzae (2.6–7%; P = 0.02) increased from 1992–1997 to 1999–2004. There was a non-statistically significant trend for a higher frequency of S. pneumoniae and H. influenzae isolates detected during the rainy season than during the dry season: S. pneumoniae 58% versus 42% but not significant (P = 0.1) and H. influenzae 68% versus 32% (P = 0.06), respectively. During the rainy season, penicillin-nonsusceptible S. pneumoniae was identified more frequently (38.5%) than during the dry season (18%) (P = 0.003; odds ratio: 2.94; 95% confidence interval: 1.4–6.45). Penicillin-nonsusceptible S. pneumoniae decreased from 46.5% (1999–2001) to 16% (2002–2003) and this was associated with a significant decline of a circulating 19F penicillin-resistant S. pneumoniae serotype (from 89% to 26%), respectively.

Conclusions: S. pneumoniae and H. influenzae are the 2 most common pathogens producing otitis media in Costa Rican children. An increase in the number of H. influenzae and M. catarrhalis was observed in recent years. Penicillin-nonsusceptible S. pneumoniae isolates were more commonly observed during the rainy season, in which increased morbidity with respiratory pathogens is observed.

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Otitis Media in Costa Rican Children

Methods

As part of various antimicrobial efficacy clinical trials conducted between 1999 and 2004 in Costa Rican outpatient children with otitis media, a total of 952 children aged 3–144 months were included in the present analysis. Data from study period (1999–2004) were compared against historical data obtained from Costa Rican children with otitis media from 1992 to 1997. The recommended antimicrobial guidelines for the initial treatment of Costa Rican children with otitis media during both seasons were similar (low dosage amoxicillin).

Patients with the following findings were included in the current analysis: (1) purulent otorrhea of <48 hour duration or (2) at least 2 otoscopic signs of middle ear effusion [decreased or absent tympanic membrane (TM) mobility, yellow or white discoloration of TM, or opacification of TM] and at least 1 indicator of acute inflammation (ear pain, marked redness of TM, or distinct fullness or bulging of TM). Patients were excluded from the analysis if the TM was perforated for ≥48 hours, if tympanic tubes were present, and if they had congenital craniofacial abnormalities or any known immunodeficiency.

All the study protocols were approved by an institutional review board, and informed consent was obtained from the parents of each study participant before enrollment.

Definition of Seasonal Periods. Costa Rica has 2 well-defined seasons: the rainy (cold) season (May through November) and the dry (warm) season (December through April).

MEF Sampling. Diagnostic tympanocentesis was performed according to our standard procedures. When the patient presented with a perforated TM, removal and cleaning of the ear canal material was done and deep aspiration of the MEF material was attempted. MEF samples were immediately transferred to the local research laboratory in a transport medium for processing.

Microbiology. MEF of all patients were inoculated onto blood agar, chocolate agar, McConkey agar, and mannitol salt agar at 37°C in a 5% CO₂ environment for 18–72 hours. If growth was present, identification was performed by standard procedures. For the analysis of this study, S. pneumoniae, H. influenzae, Moraxella catarrhalis, and S. pyogenes were considered otitis media target pathogens. All H. influenzae and M. catarrhalis were tested for beta-lactamase production with the chromogenic nitrocefin test.

Antimicrobial Susceptibility. Susceptibility testing for penicillin, azithromycin, ceftriaxone, and TMP-SMX was done by the Kirby Bauer disk diffusion or by minimal inhibitory concentration by means of the E-test (PDM Epsilometer, AB Biodisk, Solna, Sweden). Interpretation of the results was according to the National Committee for Clinical Laboratory Standards recommendations.

S. pneumoniae Isolates Serotyping. Isolates were stored at −70°C at the Centro de Investigaciones Médicas in Costa Rica using Micro Bank vials (Pro-Laboratory Diagnostics, Austin, TX) and shipped on dry ice or transport medium to the research laboratory at Beer-Sheva, Israel. Serotyping of S. pneumoniae was performed in Beer-Sheva, Israel, by the quellung reaction using antisera from Statens Serum Institute of Copenhagen, Denmark.

Statistical Analysis. The statistical package EPI INFO (version 6.0) was used to test differences on MEF pathogen yearly distribution and antimicrobial susceptibility between the 1992–1997 and 1999–2004 periods (by the Fisher exact test, Yates or square test, as appropriate). A P value <0.05 was considered statistically significant. Odds ratio were used to compare different probabilities, and multivariate analysis (period/age/pathogen) was used to analyze microbiological differences between a study period (1999–2004) and the historical period (1992–1997).

Results

During the study period, 952 children with otitis media were analyzed. Among the study population, 493 children had AOM, 256 children had recurrent otitis media, 158 children were therapeutic failure otitis media, and 45 children had mixed diagnosis (therapeutic failures and recurrent otitis media), and the distribution was similar among the 2 study periods. Study group characteristics are described in Table 1.

Microbiology. Among the 952 children, 516 (52%) had at a baseline MEF target pathogen isolated. The most common pathogen isolated was S. pneumoniae detected in 252 (49%) children including 24 children with mixed infections with H. influenzae (20 children), with M. catarrhalis (3 children), and with H. influenzae and M. catarrhalis (1 child). H. influenzae was the second most common pathogen isolated in 190 children (37%) including 24 children with mixed infections with S. pneumoniae (20 children), with S. pyogenes (3 children), and with S. pneumoniae and M. catarrhalis (1 child). Among 188 H. influenzae isolates typed, 99% were nontypable and 1% were type b or type d (1 isolate each). S. pyogenes and M. catarrhalis were isolated alone or as a mixed infection from 38 (7%) and 36 (7%) children, respectively. Beta-lactamase production among H. influenzae and M. catarrhalis isolates was observed in 7% and 93%, respectively.

The prevalence of S. pneumoniae between 1992 and 1997 and study period from 1999 to 2004 was 50% and 49%,

Table 1. Study Group Characteristics of Costa Rican Children With Otitis Media

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study Periods</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N = 398</td>
<td>N = 952</td>
</tr>
<tr>
<td>Children ≤24 months (%)</td>
<td>165 (41)</td>
<td>544 (57)</td>
</tr>
<tr>
<td>Children with positive middle ear fluid culture (%)</td>
<td>235 (59)</td>
<td>516 (52)</td>
</tr>
<tr>
<td>Mean age months (range)</td>
<td>42 (4–144)</td>
<td>26 (2–150)</td>
</tr>
<tr>
<td>Number of children ≤24 months with positive middle ear fluid culture (%)</td>
<td>75 (44)</td>
<td>323 (62.5)</td>
</tr>
</tbody>
</table>

*Not done.
respectively. The prevalence of *H. influenzae* (24–37%; *P* < 0.001) and *M. catarrhalis* (2.5–7%; *P* = 0.02) increased from 1999–2004 and 1992–1997, respectively. These increases remained significant after correction for age and previous antimicrobial use differences between the 2 study periods.

**Seasonal Distribution.** There were 307 (50.5%) and 209 (56%) children with a positive tympanocentesis during the rainy season and during dry season. Although the values did not achieve statistical significance, there was a trend for more *S. pneumoniae* and *H. influenzae* detected during the rainy season than during the dry season. The corresponding number of isolates observed during both seasons were 146 (58%) and 106 (42%) for *S. pneumoniae* (*P* = 0.1), and 130 (68%) and 60 (32%) for *H. influenzae* (*P* = 0.06), respectively (Table 2). The majority *M. catarrhalis* (58%) and *S. pyogenes* (58%) were detected during the dry season (Table 2).

Among all the MEF *S. pneumoniae* strains isolated from children during 1999–2004 and for which susceptibility testing was done, the percentage of susceptible, intermediate, and resistant strains were: penicillin (196 strains)—69.5%, 27.5%, and 3%, respectively; azithromycin (141 strains)—90%, 1%, and 9%, respectively; TMP-SMX (212 strains) 41%, 16%, and 43%, respectively; and ceftriaxone (144 strains)—98%, 2%, and 0%, respectively.

The antimicrobial susceptibility (susceptible or nonsusceptible) *S. pneumoniae* strains isolated during study period from children ≤24 months and from children >24 months were similar for penicillin, TMP-SMX, and ceftriaxone; however, there were more *M. catarrhalis*-nonsusceptible isolates among children ≤24 months of age (19% versus 4%, respectively; *P* = 0.03) (Table 3).

### TABLE 2. Seasonal Middle Ear Fluid Pathogen Distribution in Costa Rican Children During 1999–2004

<table>
<thead>
<tr>
<th></th>
<th>Dry Season</th>
<th>Rainy Season</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em></td>
<td>106 (42%)</td>
<td>146 (58%)</td>
<td>0.01</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>60 (31.6%)</td>
<td>130 (68.4%)</td>
<td>0.06</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>21 (58.3%)</td>
<td>15 (41.7%)</td>
<td>0.01</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>22 (57.9%)</td>
<td>16 (42.1%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*P* = 0.1, OR: 1.28; 95% CI: 0.95–1.73.

*P* = 0.06, OR: 0.72; 95% CI: 0.5–1.02.

*P* = 0.01, OR: 2.38; 95% CI: 1.18–4.82.

*P* = 0.01, OR: 2.39; 95% CI: 1.16–4.45.

*P* = 0.01, OR: 0.72; 95% CI: 0.5–1.02.

Penicillin susceptibility distribution among *S. pneumoniae* strains isolated during the rainy season versus those isolated during the dry season was susceptible strains 61% and 82%, respectively (*P* = 0.003); intermediate strains—34% and 8%, respectively (*P* = <0.01); and resistant strains—5% and 10%, respectively (*P* = 0.2). Penicillin-nonsusceptible strains (intermediate and resistant) were more commonly observed during the rainy season (39%) than during the dry season (18%) (*P* = 0.003) (Table 4), and clearly this resistance pattern was driven mainly by the number of penicillin intermediate strains isolated during the rainy season (34%) than during the dry season (8%) (*P* = 0.0001) (Table 4).

Although the numbers did not achieve statistical significance, the amount of azithromycin-nonsusceptible *S. pneumoniae* isolates were higher during the rainy season (23%) than during the dry season (10%) (*P* = 0.83), and the number of fully azithromycin-resistant strains increased from 1992–1997 to 1999–2004 (4–13% respectively, *P* = 0.31). In the case of TMP-SMX, most of the isolates were consistently resistant.

### TABLE 3. *Streptococcus pneumoniae* Antimicrobial Resistance Among Middle Ear Isolates Obtained From Costa Rican Children ≤24 Months With Otitis Media During 12-Year Period

<table>
<thead>
<tr>
<th></th>
<th>Penicillin*</th>
<th>Azithromycin†</th>
<th>TMP-SMX‡</th>
<th>Ceftriaxone§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermediate</td>
<td>4 (17%)</td>
<td>5 (20%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Resistant</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1999–2004</td>
<td>N = 121</td>
<td>N = 121</td>
<td>N = 125</td>
<td>N = 88</td>
</tr>
<tr>
<td>Intermediate</td>
<td>23 (19%)</td>
<td>7 (6%)</td>
<td>19 (15%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Resistant</td>
<td>9 (7.5%)</td>
<td>16 (13%)</td>
<td>41 (32%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*Penicillin nonsusceptible isolates: rainy season versus dry season P = 0.03 (OR: 2.38; 95% CI: 1.18–4.82) and penicillin intermediate isolates: rainy season versus dry season P = 0.003 (OR: 1.18–4.82).

†Azithromycin nonsusceptible isolates: rainy season versus dry season P = 0.01 (OR: 2.39; 95% CI: 1.16–4.45).

‡TMP-SMX nonsusceptible isolates: rainy season versus dry season P = 0.003 (OR: 1.18–4.82).

§Ceftriaxone nonsusceptible isolates: rainy season versus dry season P = 0.003 (OR: 1.18–4.82).

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During both seasons (67% and 53%, respectively), and for ceftriaxone, resistant was uncommon (Table 4).

Among MEF *S. pneumoniae* isolates obtained from children with AOM, recurrent otitis media, therapeutic failure and from children with recurrent and therapeutic failure the corresponding penicillin-nonsusceptible isolates values were: 20%, 38% 47% and 11%, respectively. During the study period the percentage of penicillin-nonsusceptible *S. pneumoniae* strains from children of all age group, decreased from 70% (1999–2001) to 16% (2002–2003) (*P* = 0.006).

Among the *H. influenzae* strains isolated during the study period of 1999–2004 and for which susceptibility testing was done, the prevalence of susceptible, intermediate, and resistant strains as was follows: ampicillin (104 strains) – 89%, 1%, and 10%, respectively; TMP-SMX (121 strains) – 48%, 10%, and 42%, respectively. All isolates were susceptible to azithromycin (127 strains) and ceftriaxone (116 strains). Although the susceptibility pattern for ampicillin, azithromycin, and ceftriaxone remained the similar when compared with the historical period, a decrease in the number of TMP-SMX-susceptible strains was observed from 1992–1997 to 1999–2004 (85–48%, respectively; *P* = 0.008).

### DISCUSSION

This analysis conducted on isolates obtained from prospective studies of the seasonal distribution of MEF isolates confirms that *S. pneumoniae* is the most common pathogen isolated from Costa Rican children with otitis media followed by *H. influenzae*.1,3,4

In the past 7 years the prevalence of *H. influenzae* and *M. catarrhalis*, and the proportion of β-lactamase producing *H. influenzae* strains have increased (2.3–7%, respectively).13 Similar to previous reports from other geographic regions, the increase in the prevalence MEF *H. influenzae* has not been associated with the introduction of the heptavalent conjugated *S. pneumoniae* vaccine, which is not yet available as part the national immunization program (available in approximately 15% of the population).11–13 Based on a multivariate analysis, this finding was not driven by differences in the age of the study groups analyzed by the historical period (1992–1997) and those patients evaluated between 1999 and 2004 (*P* = 0.81). It was interesting to observe an important decline in the incidence of type b *H. influenzae* from the 33.9% observed between 1992 and 1997 to only 1 case (0.5%) observed between 1999 and 2004 (*P* < 0.05). This observation is probably related to the introduction of universal type b *H. influenzae* in the Costa Rican national immunization program in 1996.

There was a nonsignificant trend toward the detection of more *S. pneumoniae* and *H. influenzae* strains isolated during the cool rainy season than during the dry season. Also, similar to previous reports, AOM episodes produced by penicillin-nonsusceptible *S. pneumoniae* strains were more frequently observed during the rainy season (39%) than during the dry season (18%) (*P* = 0.003).14

During the study period the reduction of penicillin-nonsusceptible strains (70% in 1999–2001 to 16% in 2002–2004) was clearly related to a high prevalence of a penicillin-nonsusceptible 19F *S. pneumoniae* clone prevalent during both seasons in the first years of the analysis.15 This observation highlights the importance of incorporating serotype surveillance into the microbiologic follow-up of patients with otitis media. Similar to previous reports, we observed an increase in the number of macrolide/azalide-resistant *S. pneumoniae* from 1992 to 2004, probably a result of use of new macrolides in the treatment of children with respiratory tract infections.

The results of our study are in agreement with those previously reported, in which *S. pneumoniae* and *H. influenzae* were more frequently isolated during the rainy season, and most importantly, antimicrobial-resistant isolates were also more often observed during the rainy season.14 Although we were unable to correlate the higher resistance rates observed during the rainy season with the number of local antimicrobial prescriptions, previous studies have demonstrated that this occurrence is strongly associated with the increase in the number of antimicrobial prescriptions observed during winter promoting selection of resistance among pathogens colonizing the nasopharynx and oropharynx.15–24

Factors associated with an increased antimicrobial consumption, such as during the influenza season, are present in Costa Rica during the rainy season. Additionally, previous studies in Costa Rican children admitted to the National Children’s Hospital with *S. pneumoniae* invasive diseases have shown a trend toward more hospitalizations during the rainy season than during the dry season, suggesting that more antimicrobials are prescribed during this time of the year.25,26

These data provide information regarding the seasonal distribution of resistant MEF pathogens among Costa Rican children with otitis media and suggest that clinicians should consider the use of high dosage amoxicillin or another alternative antimicrobial during the rainy season, particularly if another high risk factor is present.

### REFERENCES


