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BRIEF COMMUNICATION

The effect of immunization with pneumococcal conjugated vaccines on *Streptococcus pneumoniae* resistance patterns in acute otitis media

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Abstract Following the introduction of 7- and 13-pneumococcal conjugate vaccines (PCVs) in Israel, we demonstrated that within *Streptococcus pneumoniae* (Sp) positive middle ear cultures, obtained from young children with severe acute otitis media (AOM) episodes, there were more penicillin-susceptible and less multi-drug resistant Sp isolates in PCV immunized children.

Introduction

Streptococcus pneumoniae (Sp), *Haemophilus influenzae* (Hi), and *Moraxella catarrhalis* (Mc) are the most prevalent bacteria isolated from children with acute otitis media (AOM).[1] To prevent invasive pneumococcal diseases, pneumococcal conjugate vaccines (PCVs) have been introduced worldwide. To some extent, these vaccines have also been shown to reduce AOM burden.[2] The decrease in AOM incidence after 7-valent pneumococcal conjugate vaccine (PCV7) introduction was blunted by the emergence of nonvaccine serotypes of Sp, which eventually led to the introduction of the broader spectrum 13-valent vaccine.

Various national guidelines recommending antibiotic treatment for AOM are continuously updated, based on bacterial data and antibiotic resistance patterns. The American Academy of Pediatrics and the Israeli Task Force

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guidelines for AOM diagnosis and management recommend amoxicillin as the first-line treatment.[3,4] Despite stringent criteria for AOM treatment and efforts to restrain unjustified antibiotic use, prescription rates are still high nationwide (> 85%).[5] In recent years, Sp pathogenicity in children with AOM has been extensively studied. Reported resistance tests to antibiotic agents from different countries are conflicting.[6–8] Knowledge of Sp resistance patterns to antibiotics is essential when choosing appropriate treatment for AOM.

In Israel, PCV7 and 13-valent pneumococcal conjugate vaccine (PCV13) were implemented in a relatively short period in the National Immunization Program (NIP) in 2009 and 2010, respectively. We sought to study the impact of PCVs on pneumococcal isolates obtained from severe forms of AOM episodes, by analyzing the changes in Sp resistance patterns in the pre- and postPCVs era.

Methods

Study design and population

The Institutional Review Board and the Health Information Transfer Committee of the Israeli Ministry of Health approved this study. The study design has been previously described in detail.[9] In brief, we retrospectively identified children < 6 years of age with middle ear fluid (MEF) cultures during 2008–2013 which were obtained from "severe" AOM episodes. "Severe" AOM episodes were defined as those that either required tympanocentesis, due to lack of clinical improvement despite ≥ 48 hours of adequate antibiotic therapy, or if there were any signs of AOM-related complications (mastoiditis and/or subperiosteal abscess and/or sigmoid sinus thrombosis, facial nerve palsy, meningitis, intracranial abscess), or if children presented with spontaneous otorrhea, due to an increased middle ear pressure which caused tympanic membrane perforation. For each eligible child, we retrieved clinical and demographical data from his/her medical records. Concurrently, PCV immunization data were retrieved from the NIP records (permission granted by the Ministry of Health Ethical Committee). Each child was categorized according to his/her PCV status at AOM presentation as "unimmunized", if he had not received any dose of PCV, "PCV7-" or "PCV13-immunized", if he had received ≥ 1 dose(s) of PCV7 or PCV13, respectively. If a child had received PCV7 and PCV13 dose(s), the child was considered as "PCV13-immunized", due to broader coverage of PCV13.[10]

Middle ear fluid culture collection and processing

Tympanocentesis was performed in the anterior–inferior quarter of the tympanic membrane, in the office with local analgesia, or in the operating room under general anesthesia. Middle ear fluid (MEF) specimens were collected using a designated sterile flocked swab (Copan Italia, Brescia, Italy) after cleaning of the external auditory canal with an antiseptic solution, or by sampling of otorrhea through an existing tympanic membrane perforation. Sp, Hi, and Mc were considered as true otopathogens. In bilateral AOM cases, MEF specimens were obtained from both ears, but were considered as one sample.

All specimens were delivered to the microbiology laboratory and processed there within 24–48 hours after sampling. The specimens were plated on blood agar medium with 5% sheep blood and chocolate agar. The plates were incubated at 37°C with 5% CO_2 for 24–48 hours. Sp was identified using morphological characteristics, α-hemolysis, and optochin susceptibility. All other MEF culture results (e.g., *Streptococcus viridians*, *Streptococcus pyogenes* group A, or external ear canal saprophytes) were excluded because they were not regarded as true "classic" otopathogens. Penicillin and amoxicillin susceptibility was examined by the E-test (BioMerieux, Paris, France); the remaining antimicrobials were tested by disc diffusion method (Bio-Rad, Hercules, California, USA). Zone diameter breakpoint and minimal inhibitory concentration (MIC) category interpretations were based on updated standards (European Committee on Antimicrobial Susceptibility Testing, 2013). For the purpose of this study, we focused only on Sp, which is still considered as the major AOM pathogen. Antibiotic susceptibility was determined by Vitek 2. Penicillin-susceptible Sp (PSSP) was defined if MIC was < 0.06 mg/L. Penicillin nonsusceptible Sp (PNSP) was considered if MIC to penicillin was > 0.06 mg/L, and included intermediate-susceptible, if MIC was 0.06–2 mg/L, and resistant, if MIC was ≥ 2 mg/L. Multi-drug resistance (MDR) was defined as nonsusceptibility to β-lactam and resistance to two other antibiotic families, such as cefuroxime, ceftriaxone, erythromycin, and trimethoprim/sulfamethoxazole (TMP/SMX).

Statistical analysis

The unit for analysis was AOM episode with Sp positive MEF culture. Categorical variables are described as *n* (%) and, we used Chi-square test or Fisher's exact test, as appropriate. Statistical significance was defined as $p \leq 0.05$ (2-sided). All analyses were performed using SPSS, version 17.0 (IBM Inc., Chicago, Illinois, USA).

Results

Demographics

A total of 279 children who met the eligibility criteria contributed 295 "severe" AOM episodes. Of those, 106 (36%) MEF cultures from 103 children tested positive for any of the three otopathogens (3 children contributed 2 MEF cultures each. As those MEF cultures were derived from distinct severe AOM episodes, we referred those episodes as independent): Sp, in 59 episodes; Hi, in 39 episodes; Mc, in two episodes; and mixed growth, in six episodes. Therefore, our study population consisted of 65 episodes in which Sp was isolated: as a single bacterium in 59 (91%), and in six (9%) as a mixed growth, with nontypeable Hi. There were more boys (39, 60%), and most of Sp positive MEF cultures were from children < 2 years old (52, 80%). Prior antibiotic therapy was given in 23 (35%) children [the most common antibiotic therapy was amoxicillin, in 14 (61%) episodes]. In 2008, all children were PCV unimmunized, whereas ~90% were PCV-immunized in 2013. The percentage of Sp positive MEF cultures from the sum of

culture-positive MEF cultures in 2008–2013 were 79% (23/29), 47% (7/15), 68% (13/19), 62% (13/21), 44% (4/9), 38% (5/13), respectively. Thirty-one (48%) of the pneumococci were cultured from unimmunized children (23, 6, 1, 0, 0, and 1 in 2008–2013, respectively), 21 (32%) from PCV7-immunized children (0, 1, 12, 6, 1, and 1 in 2008–2013, respectively), and 13 (20%) from PCV13-immunized children (0, 0, 0, 7, 3, and 3 in 2008–2013, respectively).

Resistance patterns

The overall PNSP proportion was 51% (33/65; 31% intermediate, 20% resistant) and was significantly associated with PCV-status ($p = 0.006$). Figure 1 presents PSSP/PNSP partition of isolated Sp in MEF cultures according to PCV status at AOM presentation. PNSP proportion in unimmunized children was higher than in PCV immunized children, 68% (21/31) versus 35% (12/34; $p = 0.009$). The MDR prevalence of pneumococcal strains was 11% (7/65). MDR prevalence in unimmunized children was 23% (7/31) compared with 0% (0/34) in immunized children ($p = 0.004$). Concomitant resistance to TMP/SMX and erythromycin were recorded in 17 (26%) and 15 (23%) cultures, respectively. Sp resistance to erythromycin and TMP/SMX substantially decreased: 40% (12/30) versus 9% (3/35) and 57% (17/30) versus 0% (0/35), 2008–2009 versus 2010–2013,; $p = 0.003$ and $p < 0.0001$, respectively.

Discussion

We retrospectively analyzed Sp resistance patterns in severe AOM episodes in young children who had MEF cultures available for microbiological examination, confirming the role of Sp in positive MEF cultures. In our study population, children presented with severe AOM, despite moderate preadmission antibiotic treatment utilization. In addition, they had low PNSP/PSSP rates and MDR phenotypes. Possible explanations include low compliance (inadequate or faulty intake of antibiotic treatment), insufficient antibiotic dosage (i.e., amoxicillin at 40 mg/kg/d), shorter than recommended duration of therapy (< 7–10 days) or administration of inappropriate antibiotics. In PCV immunized children, there was an increased susceptibility to other common antibiotic treatments (TMP/SMX, erythromycin and cephalosporins), which can be explained by the different antibiotic susceptibilities to antibiotic agents of current Sp strains.

In our study, approximately one third of children received antibiotics for AOM before their admission; most of them were prescribed amoxicillin. Selection of β-lactams as the first line antibiotics in our study population could be considered appropriate. Our results support current recommendation for amoxicillin, because the majority of isolated pneumococci were PSSP.

Our strengths include: (1) analysis of Sp isolated directly from MEF, and not from the nasopharynx, which is regarded by many authors as a proxy to the middle ear; (2) demonstration of 1:1 correlation between PCV status and Sp resistance patterns; and (3) validation and affirmation of the current guidelines recommendation to use amoxicillin for AOM treatment. Limitations include: (1) lack of serotyping (therefore, we could not observe the changes in vaccine and nonvaccine serotypes over time); (2) no multilocus sequence typing analysis of isolates with MDR phenotype; and (3) bias of study population selection from the "advanced" spectrum

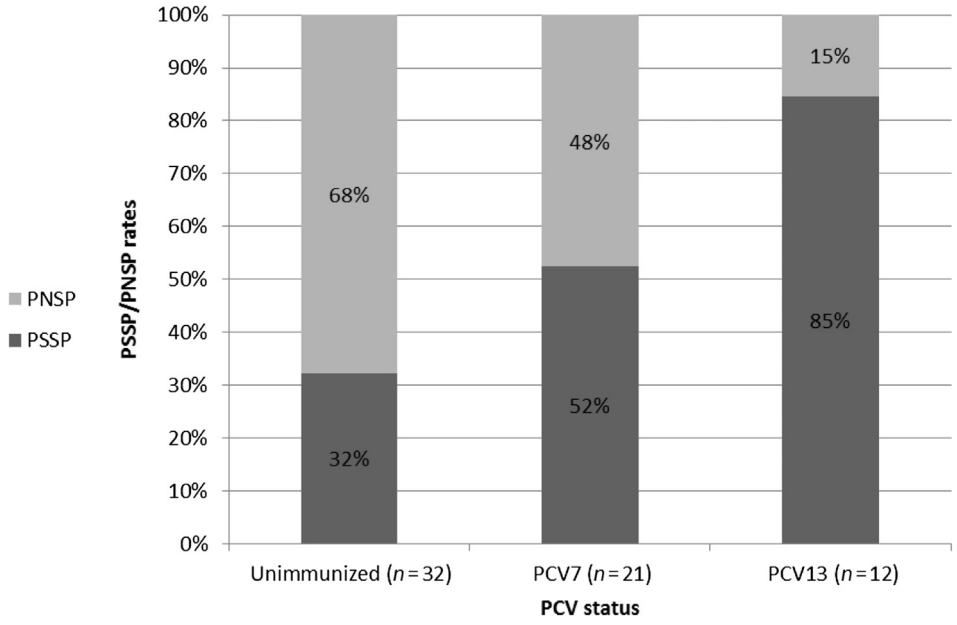

Figure 1. PNSP/PSSP partition in Sp positive MEF cultures from "severe" AOM episodes, according to PCV status. The overall PNSP rate was 51% (31% intermediate, 20% resistant) and demonstrated statistically significant association on PCV-status ($p = 0.006$). Unimmunized children had higher PNSP rate than PCV7 immunized children ($p = 0.15$), and higher PNSP rate than PCV13 immunized children ($p = 0.002$). AOM = acute otitis media; MEF = middle ear fluid; PCV = pneumococcal conjugate vaccine; PCV7 = 7-valent pneumococcal conjugate vaccine; PCV13 = 13-valent pneumococcal conjugate vaccine; PNSP = Penicillin nonsusceptible *Streptococcus pneumoniae*; PSSP = Penicillin susceptible *Streptococcus pneumoniae*; Sp = *Streptococcus pneumoniae*.

of AOM, thus may not represent more common, uncomplicated forms of AOM. However, we believe that our results are an important indirect observation on the circulating Sp serotypes in the postPCV era. Furthermore, uncomplicated AOM cases treated as outpatients, and are usually responsive to antibiotic therapy; therefore, MEF cultures are not obtained from these patients.

In conclusion, we demonstrated a high rate of antibiotic susceptibility of Sp in our study population, despite intermediate use of antibiotics. Undoubtedly, ongoing surveillance of Sp antimicrobial susceptibility, MDR phenotype, and serotype frequency will be critical in order to assess the impact of the broader coverage of PCV13.

Conflicts of interest

All authors have no conflicts of interest to declare.

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