

Bacterial Etiology and Pneumococcal Serotypes in Turkish Children with Acute Otitis Media

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Abstract

Background: Acute otitis media (AOM) is one of the most common childhood diseases requiring antimicrobial prescription drugs in pre-school children. In this study, we sought to describe the bacterial etiology of pediatric cases of AOM in Turkey.

Materials and Methods: This prospective, multi-center, tympanocentesis-based epidemiological study was performed during 2010-2012 in children aged between 3 months and 6 years. All isolates were cultured and serogrouped by the Quellung reaction.

Results: During 2010-2012, 38 cases fulfilled the study inclusion criteria. Overall, 45% of samples were culture positive for bacterial pathogens. *Streptococcus pneumoniae* (13.1%) was the leading cause of bacterial AOM followed by *Streptococcus pyogenes* (10.5%) and *H. influenzae* (7.9%). Serotype-3 was detected in two of the samples, and serotypes 9V, 19, and 19A were isolated from one patient each. *S. pneumoniae* was detected in 36% (4/11) of otorrhea samples. All *H. influenzae*-positive samples were collected by tympanocentesis. All *H. influenzae* isolates were identified as non-typeable. The pneumococcal serotype coverage rates for PCV-7, PVC-10 and PCV-13 were 20% (1/5), 20% (1/5), and 80% (4/5), respectively. PHiD-CV (PCV-7 types plus 1, 5, and 7F) targets non-typeable *H. influenzae*, and 4 of 38 (11%) of the pathogens causing episodes of AOM were also covered.

Conclusion and Recommendation: In Turkey, *S. pneumoniae* remains the most common pathogen in children with AOM. Both *S. pneumoniae* and non-typeable *H. influenzae* represent important targets for vaccination strategies to reduce AOM in children. Based on our results, conjugated pneumococcal vaccines may have potential impact to decrease the burden of AOM.

Keywords: Acute otitis media; *Streptococcus pneumoniae*; Children; Vaccine

Introduction

Acute otitis media (AOM) is one of the most commonly diagnosed childhood infections, and this is true not only in the USA where it accounts for more than 20 million visits to pediatricians every year. The disease is most prevalent in children younger than 2 years of age [1-3]. The etiology of AOM varies with age, the most frequently implicated agents being viruses such as rhinoviruses, influenza viruses, or respiratory syncytial viruses and bacteria, such as non-encapsulated *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* [4]. *Streptococcus pneumoniae* and *Haemophilus influenzae* have been consistently reported to be the two major bacterial pathogens responsible for AOM, mainly by studies in the USA and European countries [5-7]. Three pneumococcal conjugate vaccines (PCVs) are currently licensed for use in children and they have showed modest efficacy against AOM overall [8]. Randomized clinical trials with a 7-valent pneumococcal conjugate vaccine (PCV-7) in the USA and Finland showed reductions in the incidence of AOM of 6-9% [9-12]. Another pneumococcal vaccine that has been used the outer membrane protein D carrier derived from *H. influenzae* as a carrier showed 35% efficacy against clinical AOM, with statistically significant protection against both *S. pneumoniae* and *H. influenzae*-related AOM [3]. In children, PCV-13 provides serotype coverage of 90.2% for AOM [13].

Understanding the pathogens involved in the epidemiology of AOM is important not only for treatment options but also because pneumococcal conjugate vaccines have shown promise in preventing the disease. The potential impact of the vaccines cannot be estimated without comprehensive data on AOM incidence and etiology in the specific area. Because the incidence of the bacteria and serotype distribution of etiologic agents differ from a region to others. However, data on bacterial pathogens causing AOM in Turkey are limited. In this study, we aimed to characterize the bacterial etiology, and serotypes of *S. pneumoniae* AOM cases in Turkey.

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Materials and Methods

Study population and definitions

This was a prospective, multi-center, epidemiological study conducted within a routine clinical setting in five pediatric centers in Turkey. Turkish children between 3 and 59 months of age with otitis media from whom a middle ear fluid (MEF) sample was obtained with an indication for tympanocentesis by a near, nose, and throat (ENT) specialist or whom with otorrhea were included. Demographics, medical history, and general symptoms were collected at baseline, and a clinical examination was performed. Children were excluded if they suffered from a severe underlying disease. This study mainly included recurrent or treatment failure AOM. Recurrent AOM was defined as at least three episodes in the past 6 months or at least four episodes in the past 12 months. Patients identified for recruitment were subjects with a new episode of AOM (within <72 h of onset) who had not yet received antibiotics for the episode, and subjects who had a diagnosis of AOM within 48-72 h prior to study enrollment and who received antibiotic therapy from a physician but remained symptomatic at the time of study entry (treatment failures). To be considered a new episode, there had to be a symptom-free interval of at least 30 days since resolution of the previous episode. AOM was diagnosed after otoscopic examination of the ear and tympanic membrane by a pediatrician, and was classified according to the otoscopy score (eight grades) (OS-8), which measures the severity of tympanic-membrane inflammation. The diagnosis was then verified upon referral to and examination by an ENT specialist.

Middle ear fluid samples

A MEF sample was collected by tympanocentesis by needle insertion or otorrhea sample was obtained in the perforated tympanic membrane. In the case of a perforated tympanic membrane, the sample was cultured if it was taken within 24 h of the perforation. Middle ear fluid was collected by an ENT specialist by tympanocentesis or by sampling of otorrhea after confirmation of AOM. Children were excluded from the analysis if their tympanic membranes had been perforated for more than 48 h.

Microbiology and serotypes

Because the pathogen distribution of tympanocentesis isolates can be different than otorrhea isolates [14], number of otorrhea samples was limited up to 30% of total subjects. Samples were analyzed at Microbiology Laboratory of Istanbul University, Faculty of Medicine to isolate bacterial pathogens and determine the serotypes of *S. pneumoniae*. Serotyping was performed by the Quellung reaction using serotype-specific antisera (Statens Serum Institut, Copenhagen, Denmark).

Informed consent was obtained from each parent/guardian prior to perform all study-specific procedures. The study was reviewed and approved by the Hacettepe University Institutional Ethical Committee (Approval number: EPI-STREP-111339).

Results

Between December 2010 and January 2012, 38 patients were eligible for inclusion criteria. Eleven of the 38 (29%) episodes were classified as treatment failures, and the remaining 27 (71%) of episodes were classified as recurrent cases. All of the AOM episodes were unilateral infections for which a single sample was collected. Of the 38 samples, 27 (71%) were collected by tympanocentesis, and 11 (29%) were collected by otorrhea.

The age of participants vary from 6 months to 5.8 years in all AOM cases. Twenty three of children were male, and 15 were female. In total, 13 patients (34%) had received at least one dose of the PCV-7, and only one patient had received at least one dose of influenza vaccine. Antibiotic use within the past 1 month was reported for 13 (34%) of the cases. Irritability was the most frequently reported symptom, followed by ear pain, and tugging.

Overall, 45% (17/38) of the samples from the 38 episodes yielded cultures with one of the bacteria considered potential pathogens (*S. pneumoniae*, *S. aureus*, *S. pyogenes*, *H. influenzae* or α -hemolytic *Streptococcus*). The most commonly recovered bacteria were *S. pneumoniae*, which were detected in 13.2% (5/38) of samples, followed by *S. pyogenes* and *H. influenzae*, which were detected in 10.5% (4/38), and 7.9% (3/38), respectively (Table 1). The pneumococcal serotypes isolated from the *S. pneumoniae* positive samples were 3 (2/5), 9V (1/5), 19A (1/5), and 19 (1/5). *S. pneumoniae* serotype-3 was isolated from one of the patients who were vaccinated with PCV-7. The remaining pneumococcal serotypes were isolated from unvaccinated patients. *S. pneumoniae* was detected in 36% (4/11) of samples collected by otorrhea. All of the samples where *H. influenzae* was detected were collected by tympanocentesis (Figure 1). All *H. influenzae* isolates were identified as non-typeable.

Discussion

Acute otitis media (AOM) is one of the most frequent bacterial infections in children and the primary reason for the prescription of antibiotics by pediatricians [15,16]. *S. pneumoniae* is a major etiologic agent causing various infectious diseases ranging from non-invasive diseases such as AOM, rhinosinusitis and pneumonia to invasive disease such as sepsis and meningitis. Children, especially those below

*n:patient number, % percent

Table 1: Etiology of bacteria identified from samples cultured from middle ear fluid of children experiencing episodes of AOM.

Bacteria	n (%)*
<i>S. pneumoniae</i>	5 (13.2)
<i>S. pyogenes</i>	4 (10.5)
<i>H. influenzae</i> (non-typable)	3 (7.9)
α -hemolytic streptococcus	2 (5.3)
<i>Staf. aureus</i>	1 (2.6)
<i>Staf. auricularis</i>	1 (2.6)
<i>Pseud. aeruginosa</i>	1 (2.6)
The total of bacteria positive samples	17 (44.7)
Total	38 (100)

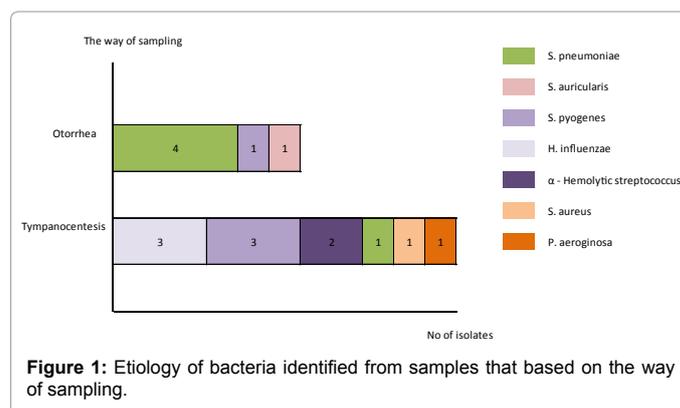


Figure 1: Etiology of bacteria identified from samples that based on the way of sampling.

the age of 5, are particularly at risk from invasive infections [17,18]. *Streptococcus pneumoniae* is the most commonly reported bacterial cause of acute otitis media, accounting for 28-55% of cases [19,20]. In this study, 45% (17/38) of samples cultured positive for one of the pathogens under study, which was lower than expected, perhaps due to high antibiotic use before sampling which has been reported as 53 to 58% [21,22]. *S. pneumoniae* was the leading cause of bacterial AOM in this study, consistent with other reports from Turkey [23,24], followed by *S. pyogenes* (4/38) and *Haemophilus influenzae* (3/38). *S. pneumoniae* and *H. influenzae* were the most prevalent species in pediatric cases with AOM in the literature [25,26], as consistently our findings. A higher proportion of *S. pyogenes* was observed in this study compared with others.

Following licensing of PCV-7 in 2000 for use in several countries worldwide, a decrease in invasive pneumococcal disease (IPD), pneumonia, and otitis media of any etiology was observed [9,10]. Recent reports from countries where PCV-7 has been implemented into national immunization program (NIP) have shown a minor to moderate decline in the incidence of otitis media visits and antimicrobial consumption to treat this condition [27,28], an increase in non-vaccine *S. pneumoniae* serotypes, especially of serotype 19A [25], and a proportional increase in the number of *H. influenzae* cases [29] as seen in the present study. Marta Alonso et al. [25] reported that most prevalent pneumococcal serotypes in pediatric cases with AOM were 3 and 19A. Reijtman et al. [26] reported that frequent pneumococcal serotypes were 19A, 9V, and 3 in AOM. Serotypes 3, 9V and 19A were detected in the present study as consistently with those studies. PCV-7 was implemented in the NIP in 2008 in Turkey, and it was then replaced with 13-valent vaccine (PCV-13) in 2011. Of the children enrolled in the study, 34% had been vaccinated with PCV-7, whereas none had been vaccinated with PCV-13 because of unavailability in the study period.

Recent studies have introduced two conjugate vaccines: PCV-10 and PCV-13. Data suggested that after changing from PCV-7 to PCV-10, the proportion of serotypes covered would increase to varying degrees in USA, Europe, Africa, and Asia. Changing from PCV-10 to PCV-13 would further improve the coverage of serotypes by 4% to 7% globally [30]. Too little information about the incidence and serotype distribution of *S. pneumoniae* in IPD or otitis media during the prepneumococcal conjugate vaccine era in Turkey is available. Based on limited data about serotype distribution, *S. pneumoniae* seems to be the most common etiologic AOM agent in Turkey [24,31]. Based on the pathogen distribution seen in this study, pneumococcal conjugate vaccines could be an important tool in reducing the burden of AOM in this setting. The pneumococcal serotypes targeted by PCV-7 and PCV-13 comprise 20% (1/5) and 80% (4/5), respectively of the *S. pneumoniae*-positive AOM episodes in this study, and coverage was 3% (1/38) for PCV-7 and 11% (4/38) for PCV-13, respectively, in all AOM episodes. The pneumococcal serotypes targeted by PHiD-CV (PCV-7 types plus 1, 5, and 7F) comprised 20% (1/5) of the *S. pneumoniae*-positive AOM episodes in this study. Additionally, PHiD-CV targets *H. influenzae*, and thus 11% (4/38) of the pathogens causing episodes of AOM in this study.

S. pneumoniae was found among otorrhea samples in this study, consistent with some other studies, where *S. pneumoniae* was reported to be more often isolated from otorrhea samples [32,33]. In contrast to Parra et al. [16], in this study, all of *H. influenzae* samples were found in tympanocentesis samples.

Moraxella catarrhalis could not be found in any AOM episode in this study. This finding was not inconsistent with other studies that have reported low percentages of *Moraxella* [7,16].

This study has some limitations. First, it was performed in a specific setting, and only a relatively small number of children who were admitted to hospital were enrolled. All patients who had acute AOM episodes were excluded. The AOM cases in this study may not accurately represent all AOM cases in Turkey due to potential differences between the enrolled and screened patients. This prevents further generalization of the findings. There may also have bias in the selection of patients. Second, the number of children was small. Thus, there may also be an assessment bias that may affect the reliability of the data.

In conclusion, the bacteriology of otitis media has been studied in several parts of the world; however, there are few current data from Turkey. This assessment of AOM etiology in Turkish children aged 3 months to less than 6 years, who visited pediatric clinics for AOM, showed that 47% (8/17) of all culture-positive samples were positive for *S. pneumoniae* or *H. influenzae*. Both *S. pneumoniae* and non-typeable *H. influenzae* represent important targets for vaccination strategies to reduce AOM in Turkish children. A continuous surveillance program is needed to detect bacteriological and/or serotype modifications that may occur over time and following vaccine interventions.

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