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Original Article

Recent epidemiology of *Streptococcus pneumoniae* in nasopharynxes of Korean children with acute otitis media

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ABSTRACT

Background: This prospective study was performed to evaluate serotype distribution, multilocus sequence typing, and antibiotic susceptibility of *Streptococcus pneumoniae* identified in Korean children with acute otitis media (AOM) after the introduction of a 7-valent pneumococcal conjugate vaccine (PCV7).

Methods: Nasopharyngeal aspirates were collected from children diagnosed with AOM in seven hospitals in Korea. The bacteria identified in these samples and the serotypes, sequence types (STs), and antibiotic susceptibilities of *S. pneumoniae* isolates were evaluated.

Results: A total of 390 children were enrolled, and bacteria were identified in 376 (96.4%) children. *S. pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* were identified in 155 (39.7%), 127 (32.6%) and 86 (22.1%) children, respectively. Serotype 19A (22.4%) was the most common *S. pneumoniae* serotype, with serogroups 11 (14.7%) and 15 (13.5%) following. ST320 (23.5%) was the most common ST; ST166 (17.0%) and ST83 (8.5%) followed. The overall susceptibility rates of *S. pneumoniae* to oral penicillin V and amoxicillin/clavulanate were 2.6% and 53.2%, respectively. The susceptibility rate to cefditoren was 91.0%; however, the rates for other cephalosporins were less than 10.0%. Compared with other serogroups, *S. pneumoniae* serogroups 19, 11, and 15 showed significantly lower susceptibility rates to all the antibiotics tested.

Conclusion: *S. pneumoniae* serotype 19A, serogroups 11 and 15 were the major nasopharyngeal-colonizing bacteria in Korean children with AOM after the introduction of PCV7. These relatively prevalent serotype/serogroups showed lower antibiotic susceptibility rates.

1. Introduction

Acute otitis media (AOM) is the most common disease for which antibiotics are prescribed in children [1]. *Streptococcus pneumoniae* (Sp), *Haemophilus influenzae* (Hi), and *Moraxella catarrhalis* (Mc) are the most common causes of AOM [1], and continuous surveillance of the distribution of these otopathogens and their antibiotic susceptibilities is necessary to establish appropriate treatment strategies for AOM. In particular, AOM treatment is primarily based on the antibiotic susceptibility of Sp because children with AOM caused by Hi and Mc tend to recover without antibiotic therapy and the illness in these children is less severe than that caused by Sp [1]. A change in the serotype distribution and antibiotic susceptibility of otopathogenic Sp was observed after the introduction of a

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pneumococcal conjugate vaccine (PCV) [2,3]. Such a change occurred globally; however, regional differences according to the vaccine coverage rate of PCV and antibiotic use in each country were also observed [3]. Although regional information on changes in the serotype distribution and antibiotic susceptibility of Sp is necessary, there has been no recent report on Korea. Even in East Asia, where PCV was introduced later than western countries, only a few studies on changes in the serotype distribution and antibiotic susceptibility of Sp in childhood AOM after the introduction of PCV have been reported [4].

Middle ear fluid (MEF) should be obtained by tympanocentesis to determine the causative microorganisms of AOM. However, because AOM occurs in young children with a mean age of 3–4 years [1], children and their parents are often concerned about the invasiveness of tympanocentesis. Fortunately, the nasopharyngeal (NP)-colonizing bacteria and otopathogens identified in MEF are closely related [5,6]. NP flora sampled during AOM or just before the development of AOM, rather than when a child was healthy, was representative of the otopathogens [5,7,8]. Therefore, several studies have used NP rather than MEF specimens from AOM patients, based on the assumption that NP-colonizing bacteria may be potential otopathogens [9–12].

In this study, the distribution of NP-colonizing bacteria in children with AOM during the early 2010s was determined using specimens collected by nasopharyngeal washing. Among the bacteria identified, the serotype distribution and antibiotic susceptibilities of Sp were evaluated. In Korea, the 7-valent PCV (PCV7) was introduced in the fall of 2000, and individual vaccinations began. The 10-valent PCV (PCV10) and 13-valent PCV (PCV13) replaced PCV7 in the fall of 2010, and PCV was included in the National Immunization Program for universal vaccination in May 2014.

2. Patients and methods

2.1. Patients and study design

This prospective study was performed in seven hospitals located in six regions of Korea (Seoul St. Mary's Hospital and Ewha Womans University Mokdong Hospital in Seoul, St. Vincent Hospital in Gyeonggi province, Wonju Severance Christian Hospital in Gangwon province, Chungnam National University Hospital in Chungcheongnam-do province, Chonnam National University Hospital in Gwangju, Keimyung University Dongsan Medical Center in Daegu, Changwon Fatima Hospital in Gyeongsangnam-do province). Children diagnosed with AOM in the outpatient department of the participating hospitals between March 2011 and May 2012 were enrolled in this study. AOM was diagnosed by pediatricians of the participating hospitals based on the 2004 AOM guidelines from the American Association of Pediatrics and American Academy of Family Physicians [13]. The following three criteria were required for diagnosis of AOM: 1) a history of acute onset of symptoms and signs, 2) the presence of MEF, and 3) symptoms and signs of middle-ear inflammation. Children who had presumable or confirmed immune deficiencies were excluded. Children who had received immune suppressants or immune-modulating agents in the 6 months before AOM diagnosis and those with congenital anomalies or significant chronic illnesses that were prone to AOM were also excluded.

For the enrolled children, NP washes were performed on diagnosis of AOM, and demographic factors including sex, age, and PCV history were gathered. The distribution of bacteria identified from NP washes was investigated and compared to PCV histories. On the colonizing bacteria, serotyping and multilocus sequence typing (MLST) of Sp were performed, and the results were compared to PCV histories. In addition, antibiotic susceptibility rates of the Sp isolates to commonly used oral antibiotics were determined.

2.2. Collection of specimens and microbiological assays

Upon diagnosis of AOM, specimens were collected by NP washing using a mucus extractor. In brief, 0.5–1.0 mL of normal saline was inserted into the nasal cavity through the nares. After 1–2 min, nasal aspiration was performed with 1.0 mL of saline added. The collected fluid was mixed well by shaking and then transported to the central laboratory (Neodin Reference Lab., Seoul, Republic of Korea) within 24 h at 2–8 °C.

In the central laboratory, the NP aspirates were cultured on blood agar and chocolate agar plates for 48 h at 35–37 °C. Each specimen was inoculated onto all quadrants of the agar plate to determine the dominant bacterium using a semi-quantitative method. Heavy, moderate, light, and rare growths were defined by a bacterium growing on four, three, two, and one of the quadrants, respectively. The presence of Sp in cultured colonies showing α-hemolysis was confirmed based on optochin susceptibility and a negative catalase result. Hi was confirmed using an API® NH strip (bioMérieux, Hazelwood, MO, USA). Mc was confirmed by positive oxidase and catalase results of colonies presumed to be Mc based on colony morphology. Confirmed Sp colonies were stored at −70 °C until antibiotic susceptibility testing.

Minimal inhibitory concentrations of oral penicillin V (non-meningitis), amoxicillin/clavulanate, cefuroxime, cefaclor, cefpodoxime, cefdinir, cefditoren, clarithromycin, and trimethoprim against the identified Sp were determined using a broth dilution method, and antibiotic susceptibilities were determined based on the 2010 guidelines of the Clinical and Laboratory Standards Institute (CLSI) [14]. Because no breakpoint for cefditoren was recommended by the CLSI, susceptibility to cefditoren was determined based on the breakpoint recommended by the Spanish Agency for Medicine and Medical Devices [15]. *S. pneumoniae* ATCC 49619 was used as a standard.

2.3. Serotyping and MLST of Streptococcus pneumoniae

Serotyping of Sp was performed at the Center for Vaccine Evaluation and Study, Medical Research Institute, Ewha Womans University School of Medicine, Seoul, Republic of Korea, which is World Health Organization pneumococcal serology reference laboratory, using an automated and multiplexed pneumococcus serotyping method [16].

MLST was performed according to a previously reported method of sequencing seven housekeeping genes (*aroE, gdh, gki, recP, spi, xpt,* and *ddl*) [17]. Sequence types (STs) were determined by comparing the sequences of the seven loci with sequences previously reported on the MLST website (http://pubmlst.org).

2.4. Statistical analysis

According to PCV history, the distribution of colonizing bacteria and the serotype distribution and antibiotic susceptibility rates of Sp were compared using a chi-square test. Antibiotic susceptibility rates according to Sp serotypes were also compared using a chi-square test. SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses, and statistical significance was defined by a two-tailed P-value <0.05.

2.5. Ethical approval

This study was approved by the institutional review boards of each participating hospital, and informed consent was obtained