J Infect Chemother 20 (2014) 423–428

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Impact of the pneumococcal conjugate vaccine on serotype distribution and susceptibility trends of pediatric non-invasive *Streptococcus pneumoniae* isolates in Tokai, Japan over a 5-year period

Hayato Okade [a,b,*], Tori Funatsu [b], Maki Eto [b], Yuri Furuya [a,b], Shingo Mizunaga [b], Nobuhiko Nomura [b], Junichi Mitsuyama [c], Yuka Yamagishi [d], Hiroshige Mikamo [a,d]

[a] Department of Clinical Infectious Diseases, Aichi Medical University Graduate School of Medicine, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan
[b] Research Laboratories, Toyama Chemical Co., Ltd., 2-4-1 Shimookui, Toyama 930-8508, Japan
[c] Development Division, Toyama Chemical Co., Ltd., 3-2-5 Nishishinjyuku, Shinjyuku-ku, Tokyo 160-0023, Japan
[d] Department of Clinical Infectious Diseases, Aichi Medical University Hospital, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan

ARTICLE INFO

Article history:
Received 15 October 2013
Received in revised form
8 January 2014
Accepted 6 March 2014

Keywords:
PCV7
Streptococcus pneumoniae
Non-invasive pneumococcal disease

ABSTRACT

Introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in February 2010 markedly reduced the burden of invasive pneumococcal disease (IPD) and changed serotype distribution in Japan. We investigated the serotype distribution and susceptibility trends of non-invasive *Streptococcus pneumoniae* isolates collected from pediatric patients. A total of 564 pneumococcal isolates were collected over a 5-year period between 2008 and 2012. The coverage of PCV7 significantly decreased throughout the study period, from 49.3% in period 1 (between June 2008 and April 2009) to 23.4% in period 4 (between October 2011 and March 2012). This change was mainly due to a large decrease in the frequency of 19F (from 20.6% to 9.9%) and 6B (from 10.3% to 2.7%) and an increase in serotype 3 (from 5.1% to 13.5%) and serogroup 15 (from 4.4% to 9.0%). According to serotype replacement, the susceptible ratios of *S. pneumoniae* to β-lactams increased slightly while macrolide resistance remained high. The high frequency of macrolide-resistant pneumococcal isolates may continue because of the high frequency of *erm(B)* in replace serotypes such as serotype 3 and serogroup 15. The continuous surveillance study is essential following the introduction of a second generation 13-valent pneumococcal conjugate vaccine (PCV13).

1. Introduction

Streptococcus pneumoniae is one of the most important pathogens causing pneumonia, bacteremia, meningitis, and acute otitis media (AOM), especially among young children and older adults [1]. A 7-valent pneumococcal conjugate vaccine (PCV7), which was first introduced in the United States in 2000, has significantly reduced the burden of pneumococcal disease in many populations around the world [2,3]. PCV7 was shown to contain conjugate capsular polysaccharides directed at 7 (4, 6B, 9V, 14, 18C, 19F, and 23F) of the 93-plus pneumococcal serotypes [2,3].

The incidence of invasive pneumococcal disease (IPD) markedly decreased in countries in which the PCV7 vaccine was introduced [4,5]. In contrast, the epidemiology of *S. pneumoniae* has been changing in many countries [3,4,6]. One of the most prominent changes is the emergence of non-vaccine serotypes such as multidrug-resistant serotype 19A [2,7,8]. In addition to IPD, recent studies on the effect of PCV7 on non-IPD such as AOM have shown a significant reduction in the incidence of non-IPD and alternations in its microbiology [9]. A second generation 13-valent pneumococcal conjugate vaccine (PCV13), which covers the 7 serotypes in PCV7 as well as serotypes 1, 3, 5, 6A, 7F, and 19A, was introduced in the United States in 2010, and was subsequently introduced in many other countries [4].

In Japan, PCV7 was introduced in February 2010, and the vaccination rate was estimated to be approximately 10% in 2010 [10]. The PCV7 vaccination rate was estimated to reach 50%–60% in 2011 because it was encouraged by the official program in November 2010 [10]. Although several surveillances have been reported on serotype distribution in Japan before the introduction

* Corresponding author. Research Laboratories, Toyama Chemical Co., Ltd., 2-4-1 Shimookui, Toyama 930-8508, Japan. Tel.: +81 76 431 8268; fax: +81 76 431 8208.
E-mail address: hayato_okade@toyama-chemical.co.jp (H. Okade).

http://dx.doi.org/10.1016/j.jiac.2014.03.010
1341-321X/

of PCV7, only a few reports are known about serotype distribution of non-IPD in post-PCV7 periods [11,12].

In this study, we collected 564 non-invasive pneumococcal isolates from pediatric patients during the period between 2008 and 2012, and investigated serotype distribution as well as susceptibility trends.

2. Materials and methods

2.1. Pneumococcal isolates

A total of 564 nonduplicate *S. pneumoniae* isolates were collected from pediatric patients aged 0–15 years at 10 hospitals in Gifu or Aichi, Japan between June 2008 and March 2012: divided into Period 1 (between June 2008 and April 2009, 136 isolates), Period 2 (between September 2009 and April 2010, 191 isolates), Period 3 (between September 2010 and March 2011, 126 isolates), and Period 4 (between October 2011 and March 2012, 111 isolates). Pneumococcal isolates were collected from nasal discharge, throat swab, ear discharge, and sputum specimens. Pneumococcal isolates were identified using the VITEK 2 system, and stored in Microbank vials at ultralow temperature (−125 °C).

2.2. Serotyping

Serogroups were determined using the Pneumococcal antisera kit Seiken (Denka Seiken Co., Ltd., Tokyo, Japan) in accordance with the manufacturer's instructions. Serotypes were subsequently determined by the capsular swelling reaction using serotype-specific antisera (Statens Serum Institut, Copenhagen, Denmark).

2.3. Detection of mutations in penicillin binding protein (pbp) and macrolide resistance genes

Mutations in *pbp* (*pbp1a*, *pbp2x*, and *pbp2b*) were detected by PCR using the Penicillin Kit (Wakunaga Pharmaceuticals Co., Hiroshima, Japan). Similarly, macrolide resistance gene *mef(A)* and *erm(B)* were detected by PCR using the Penicillin Kit. Isolates were classified as gPSSP, gPISP, or gPRSP, as previously reported [11].

2.4. Susceptibility testing

Susceptibility testing was performed by the agar dilution method in Period 1 and Period 2, or by the microbroth dilution method using a Dry Plate (Eiken Chemical, Tokyo, Japan) in Period 3 and Period 4, with reference to the Clinical and Laboratory Standards Institute (CLSI) [13–15]. A total of 19 antimicrobial agents were tested: penicillin G (PCG), amoxicillin (AMPC), piperacillin (PIPC), clavulanic acid/amoxicillin (CVA/AMPC), tazobactam/piperacillin (TAZ/PIPC), cefdinir (CFDN), cefcapene (CFPN), cefditoren (CDTR), cefteram (CFTM), imipenem (IPM), panipenem (PAPM), meropenem (MEPM), clarithromycin (CAM), azithromycin (AZM), levofloxacin (LVFX), tosufloxacin (TFLX), garenoxacin (GRNX), pazufloxacin (PZFX), and minocycline (MINO).

2.5. Statistical analysis

Statistical analysis was performed by the chi-square test using SAS release 8.1.

3. Results

3.1. Collection of pneumococcal isolates

A total of 564 non-invasive pneumococcal isolates were collected from pediatric patients. A total of 285 samples were nasal discharges (69 in Period 1, 62 in Period 2, 88 in Period 3, and 66 in Period 4), 241 were throat swabs (63 in Period 1, 113 in Period 2, 32 in Period 3, and 33 in Period 4), 24 were ear discharges (1 in Period 1, 13 in Period 2, 3 in Period 3, and 7 in Period 4), and 14 were sputum (3 in Period 1, 3 in Period 2, 3 in Period 3, and 5 in Period 4). The most common types of specimens were nasal discharge and throat swab.

3.2. Serotype distribution

Changes in serotype distribution by study period are shown in Fig. 1. The most frequent serotype overall was 19F (16.5%), followed by 23F (10.6%), serogroup 15 (8.2%), and 6B (7.8%). Serogroup 15 included 18 serotype 15A, 13 serotype 15B, and 15 serotype 15C isolates. The coverage of PCV7/PCV13 decreased throughout the study period, from 49.3%/70.6% in Period 1 to 23.4%/47.7% in Period 4 ($P < 0.0001$/$P = 0.0003$). These changes were mainly due to a large decrease in the frequency of 19F (from 20.6% to 9.9%, $P = 0.0221$) and 6B (from 10.3% to 2.7%, $P = 0.0191$). On the other hand, serotype 3, one of the PCV13 serotypes, increased throughout the study periods (from 5.1% to 13.5%, $P = 0.0217$). The ratio of non-vaccine serotypes increased (from 29.4% in period 1 to 52.3% in period 4, $P = 0.0003$). Serogroup 15 such as 15A, 15B, and 15C, in particular, inclined to increase (from 4.4% in period 1 to 9.0% in period 4, $P = 0.1442$).

3.3. Distribution of mutations in pbp and macrolide resistance genes

The distribution of the *pbp* mutations and macrolide resistance genes by study period is shown in Table 1. gPRSP isolates, having mutations in *pbp1a*, *pbp2x*, and *pbp2b*, decreased gradually throughout the study period, from 56.6% in Period 1 to 36.9% in Period 4. In contrast, gPISP (*pbp2x* mutation)/gPISP (*pbp1a* and *pbp2x* mutations) increased, from 23.5%/11.0% in Period 1 to 34.2%/20.7% in Period 4, respectively.

The *erm(B)* gene, encoding rRNA adenine N-6-methyltransferase, significantly increased (58.8%–72.1%), while the *mef(A)* gene, encoding an efflux pump, decreased (51.5%–37.8%) throughout the study period. The frequencies of *pbp* mutations and macrolide resistance genes differed markedly between serotypes (Table 2). The rate of gPRSP was the most frequent in serotype 19F (96.7%) and 23F (96.6%), followed by 6A (90.7%). The frequency of *erm(B)* was high in serogroup 15 (97.8%), serotype 6A (97.7%), 23F (96.7%), and 3 (92.9%), while that of *mef(A)* was high in serotype 19F (89.2%), 23F (68.3%), and 19A (64.7%). The frequency of dual *mef(A)* and *erm(B)* resistance was high in serotype 23F (65.0%).

3.4. Susceptibility trend of S. pneumoniae

Changes in the MIC_{50}, MIC_{90}, and susceptible ratios according to the CLSI breakpoints of tested antimicrobial agents are shown in Table 3. According to the CLSI breakpoint for parenteral PCG (non-meningeal), the susceptible ratio of PCG was high throughout the study periods (98.5%–99.2%). The susceptible ratios were 43.2% for CFDN, 94.6% for IPM, and 93.7% for MEPM in Period 4. No differences greater than ±1 dilutions in the MIC_{90} of tested β-lactams were observed between any of the study periods. The susceptible ratios of β-lactams increased slightly, especially in carbapenems.