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Surveillance

Surveillance on susceptibility of strains isolated from pediatric infections☆

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ABSTRACT

During the period from January to December 2015, 104 *Streptococcus pneumoniae strains*, 129 *Haemophilus influenzae* strains and 54 *Moraxella catarrhalis* strains isolated from clinical specimens of pediatric infections in the national 16 institutions, studied susceptibilities of total 28 antibiotics, the capsular serotype for *S. pneumoniae*, the capsular b type and β-lactamase production capability for *H. influenzae*, and the β-lactamase production capability for *M. catarrhalis* were measured.

In *S. pneumoniae*, the results showed that 68 strains (65.4%) were PSSP, 32 (30.8%) were PISP, and 4 (3.8%) were PRSP. The susceptibilities of TBPM and GRNX among oral antibiotics, and PAPM among injectable antibiotics demonstrated the lowest value with MIC90 $\leq$ 0.06 μg/mL. The most frequent distribution of *S. pneumoniae* serotypes was seen in 15B, followed by 19A, and 35B. Serotype strains contained in 13-valent pneumococcal conjugate vaccine (PCV13) were 19 strains (18.3%).

In *H. influenzae*, the results showed that BLNAS accounted for 40 strains (31.0%), BLNAI for 28 strains (21.7%), BLNAR for 47 strains (36.4%), β-lactamase producing for 14 strains (10.8%). The susceptibilities of quinolones demonstrated the lowest outcome among oral antibiotics with MIC90 $\leq$ 0.06 μg/mL, and CTRX and TAZ/PIPC (TAZ4 fixed) among injectable antibiotics with MIC of 0.25 μg/mL. There was no detection of capsular type b strains.

In *M. catarrhalis*, all the isolates were β-lactamase producing strains. The susceptibilities of TBPM, CPFX, TFLX and GRNX among oral antibiotics, and TAZ/PIPC (TAZ4 fixed), PAPM, MEPM and DRPM among injectable antibiotics demonstrated the lowest outcome with MIC of $\leq$0.06 μg/mL.

1. Introduction

The 4-year reexamination period has concluded for pediatric quinolones that were approved as the second line therapy for infections with resistant bacteria in December 2009 in Japan. With the conclusion of the reexamination period, the marketing of the generic product became possible and, currently, a few companies are selling them. To maintain pediatric quinolones as an effective treatment option for as long as possible, not only it is necessary to expand the use of clinical practice guidelines such as through the activities of academic societies, but also it is necessary for companies to thoroughly deliver information about proper use and to assess the status of the resistance to the drugs continuously.

For that purpose, all companies that are selling pediatric quinolones, including the generic companies that will be entering the market in the future, are expected to conduct activities such as the promotion of proper use for medical institutions, regular assessment of the status of resistance to the drugs, collection of the information on adverse drug reactions, and appropriate and prompt provision of information. Therefore, the Japanese Society of Chemotherapy, with the cooperation of the Japanese Association for Infectious Diseases and the Japanese Society for Pediatric Infectious Diseases, took the lead and started activities to launch the

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"Promotion Committee for Proper Use of Pediatric Quinolones." This reports the results of the antibacterial resistance surveillance in the area of pediatrics, the main activity of the committee.

2. Objectives

The frequency of the emergence of antibiotic-resistant bacteria is affected greatly by the use of antibacterial agents. With the increase in the usage of quinolone, a high frequency of bacterial resistance to the quinolones is expected. In the area of pediatrics, the pediatric resistant bacteria study group has been conducting the susceptibility surveillance on *Streptococcus pneumoniae* and *Haemophilus influenzae* every three years since 2001 and conducted the susceptibility surveillance on *Moraxella catarrhalis* in 2012, which collected isolates from pediatric institutions nationwide. By comparing the results of the above-mentioned surveillances [1–8] with those conducted most recently in this surveillance, we evaluated whether the quinolones are contributing to the increase in bacterial resistance.

3. Subjects and methodology

During the period from January to December 2015, in cooperation with the 16 pediatric institutions from across the country (Supplement 1), *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* strains were isolated from clinical specimens of pediatric infections and were accumulated at the Infection Control Center, Kitasato Institute for Life Science, Kitasato University, where the drug susceptibility and the frequency of resistant bacterial isolates for the 3 strains, the capsular serotype for *S. pneumoniae*, the capsular b type and β-lactamase production capability for *H. influenzae*, and the β-lactamase production capability for *M. catarrhalis* were measured.

This study was conducted with the examination in the ethical review board in each institution.

3.1. The measurement methods for minimum inhibitory concentration (MIC)

The measurement was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) (broth microdilution method). The measuring culture media included Cation adjusted Mueller-Hinton broth (CAMHB) with 5% lysed horse blood for *S. pneumonia*e, Haemophilus Test Medium broth for *H. influenzae*, and CAMHB for *M. catarrhalis*. Twenty eight test antibiotics were measured (Supplemental 2). The test antibiotics were prepared in a 12-point two-fold dilution series ranging from 128 to 0.063 μg/mL. However, amoxicillin (AMPC), clavulanic acid/amoxicillin (CVA/AMPC), cefditoren (CDTR), cefdinir (CFDN), cefteram (CFTM), doripenem (DRPM), azithromycin (AZM) and clarithromycin (CAM) were prepared in 11 points from 64 to 0.063 μg/mL and tosufloxacin (TFLX) in 9 points from 16 to 0.063 μg/mL.

The inoculation amount was set for the final concentration to be approximately 10^4 CFU/well (10^5 CFU/mL), and they were cultured at 35 ± 2 °C for 20–24 h.

3.2. Confirmation of H. influenzae type b by PCR method

For primers (127bp), HibFP, 5′-CAAGATACCTTTGGTCGTCTGCTA-3' (positions 5481 to 5504) HibRP and 5′-TAGGCTCGAAGAATGAGAAGTTTTG-3' (positions 5631 to 5607) were used.

PCR conditions were one cycle at 98°C for 30 s, followed by 30 cycles of 10 s at 98°C, 5 s at 57°C and 5 s at 72°C and then followed by one cycle of 2 minutes at 72°C [9].

3.3. β-lactamase production test

β-lactamase production was assessed by nitrocefin method using Cefinase™ (BD BBL™).

3.4. Capsular serotype test for S. pneumoniae

Capsular swelling (quelling) reaction was performed by factor or type serum (Statens Serum Institute) based on the results of Mutiplex PCR that was implemented in accordance with CDC Streptococcus Laboratory protocol (USA set Reaction 1 to 8).

3.5. Criteria of resistance

Microorganisms were assessed and classified into S (Susceptible), I (Intermediately susceptible) or R (Resistant) using the break points stipulated in CLSI M100-S26 for *S. pneumoniae* and *H. influenzae* and CLSI M45 3rd edition for *M. catarrhalis*. *S. pneumoniae* other than meningitis was classified only for PCG in accordance with CLSI M100-S17 and *S. pneumoniae* with penicillin (PCG) MIC ≤0.06 μg/mL was classified as penicillin susceptible *S. pneumoniae* (PSSP), that with MIC ≥ 0.125 μg/mL; ≤1.0 μg/mL as penicillin intermediate resistant *S. pneumoniae* (PISP) and that with MIC ≥ 2 μg/mL as penicillin resistant *S. pneumoniae* (PRSP). One strain was derived from spinal fluid; however, we considered it as non-meningitis and summarized the results as a whole for descriptive purposes. Regarding *H. influenzae*, non-β-lactamase producing strains are classified as β-lactamase negative ampicillin (ABPC) susceptible (BLNAS) when ABPC MIC was ≤1 μg/mL, strains with ABPC MIC of 2 μg/mL as β-lactamase negative ABPC intermediate resistant (BLNAI), and strains with ABPC MIC ≥ 4 μg/mL as β-lactamase negative ABPC resistant (BLNAR). Strains that are producing β-lactamase were classified as β-lactamase producing ABPC resistant (BLPAR) when CVA/AMPC MIC was ≤4 μg/mL and strains with CVA/AMPC MIC ≥ 8 μg/mL as β-lactamase producing CVA/AMPC resistant (BLPACR).

3.6. Analysis of background factors

Patients' background was analyzed regarding age, presence/absence of sibling, presence/absence of group nursing, and presence/absence of antibacterial agent use within one month. Statistical significance was assessed by χ [2] test, and $p < 0.05$ was considered as statistically significant. Statistical analyses were made with SAS v.9.2 (SAS Institute, Cary, NC).

4. Results

4.1. S. pneumoniae

A total of 104 isolates were analyzed for *S. pneumoniae*. Isolates included 73 isolates of mucous membrane of the upper pharynx, 24 sputum isolates, 6 blood isolates, and 1 spinal fluid isolate. The distribution of ages of patients was 31 patients aged <1 year, 30 patients aged 1 year, 12 patients aged 2 years, 8 patients aged 3 years, 9 patients aged 4 years, 3 patients aged 5 years, 3 patients aged 6 years, and 8 patients aged 7 years and older. Fig. 1 shows the frequency of the resistant strains together with the past surveillance results. The results showed that there were more susceptible strains with 68 PSSP strains (65.4%), 32 PISP strains (30.8%) and 4 PRSP strains (3.8%). Table 1 shows the results of the drug susceptibility test. When comparing the MIC_{90}, tebipenem (TBPM) among oral beta-lactam antibiotics and panipenem (PAPM) and carbapenems among injectable beta-lactam antibiotics demonstrated the best outcome with MIC_{90} ≤0.06 μg/mL. Among quinolones,

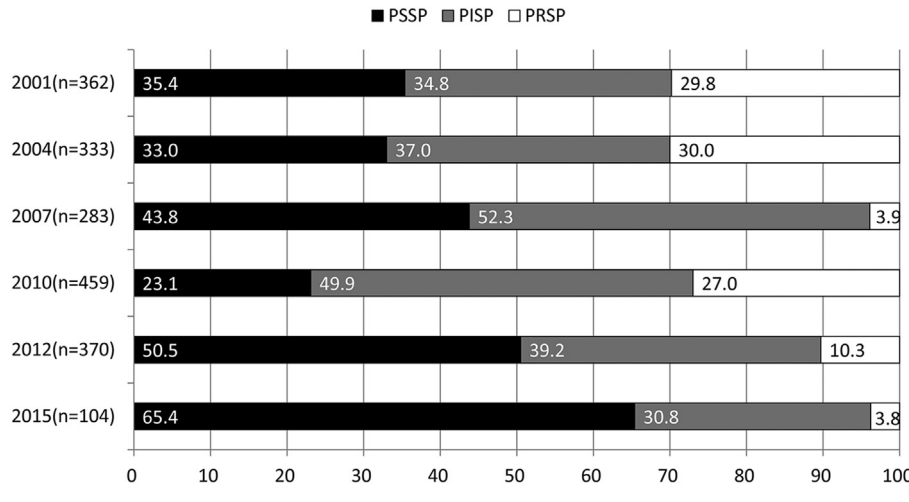

Fig. 1. Changes of *S. pneumoniae* strains classified by penicillin G resistance. PSSP: penicillin-susceptible *S. pneumoniae*, PISP: penicillin-intermediate *S. pneumoniae*, PRSP: penicillin-resistant *S. pneumoniae*. The results of 2001–2012 derived from references 2), 4), and 7).

Table 1
Minimum inhibitory concentrations of *S. pneumoniae*.

Drug	Total (n = 104)			PSSP (n = 68)			PISP (n = 32)			PRSP (n = 4)
	MIC50	MIC90	range	MIC50	MIC90	range	MIC50	MIC90	range	range (μg/mL)
PCG	≤0.06	1	≤0.06 ~ 2	≤0.06	≤0.06	≤0.06	0.5	1	0.125 ~ 1	2
ABPC	≤0.06	2	≤0.06 ~ 4	≤0.06	≤0.06	≤0.06 ~ 0.25	0.5	2	≤0.06 ~ 2	2 ~ 4
AMPC	≤0.06	1	≤0.06 ~ 2	≤0.06	≤0.06	≤0.06 ~ 0.125	0.5	1	≤0.06 ~ 1	2
PIPC	≤0.06	2	≤0.06 ~ 4	≤0.06	≤0.06	≤0.06 ~ 0.125	1	2	≤0.06 ~ 4	2
CDTR	0.125	0.25	≤0.06 ~ 2	0.125	0.25	≤0.06 ~ 0.5	0.25	0.5	≤0.06 ~ 2	0.25 ~ 0.5
CFPN	0.25	0.5	≤0.06 ~ 8	0.25	0.5	≤0.06 ~ 1	0.5	1	≤0.06 ~ 8	0.5 ~ 1
CFDN	0.25	2	≤0.06 ~ 4	0.25	0.5	≤0.06 ~ 2	1	4	0.125 ~ 4	2 ~ 4
CTM	0.25	2	≤0.06 ~ 8	0.25	0.25	≤0.06 ~ 2	1	4	0.25 ~ 8	4 ~ 8
CTRX	0.25	0.5	≤0.06 ~ 4	0.25	0.5	≤0.06 ~ 1	0.5	1	0.125 ~ 4	0.5 ~ 1
CTX	0.25	0.5	≤0.06 ~ 8	0.25	0.5	≤0.06 ~ 1	0.5	1	≤0.06 ~ 8	0.5 ~ 1
CFTM	0.25	0.5	≤0.06 ~ 8	0.25	0.5	≤0.06 ~ 1	0.5	1	0.125 ~ 8	0.5 ~ 1
PAPM	≤0.06	≤0.06	≤0.06 ~ 0.25	≤0.06	≤0.06	≤0.06 ~ 0.125	≤0.06	0.125	≤0.06 ~ 0.125	0.125 ~ 0.25
MEPM	≤0.06	0.5	≤0.06 ~ 0.5	≤0.06	≤0.06	≤0.06	0.125	0.5	≤0.06 ~ 0.5	0.25 ~ 0.5
DRPM	≤0.06	0.25	≤0.06 ~ 0.5	≤0.06	≤0.06	≤0.06	0.125	0.25	≤0.06 ~ 0.25	0.25 ~ 0.5
TBPM	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
FRPM	≤0.06	0.5	≤0.06 ~ 1	≤0.06	≤0.06	≤0.06 ~ 0.125	0.125	0.5	≤0.06 ~ 1	0.5
NFLX	4	8	4 ~ 64	4	16	4 ~ 32	4	8	4 ~ 64	8 ~ 16
CPFX	1	2	0.5 ~ 32	1	2	0.5 ~ 4	1	2	0.5 ~ 32	1 ~ 2
TFLX	0.25	0.25	0.125 ~ 16	0.25	0.25	0.125 ~ 0.5	0.25	0.25	0.125 ~ 16	0.125 ~ 0.25
LVFX	1	2	1 ~ 16	1	2	1 ~ 2	1	2	1 ~ 16	1 ~ 2
GRNX	≤0.06	≤0.06	≤0.06 ~ 0.5	≤0.06	≤0.06	≤0.06 ~ 0.125	≤0.06	≤0.06	≤0.06 ~ 0.5	≤0.06 ~ 0.125
AZM	≧128	≧128	≤0.06 ~ ≧128	≧128	≧128	≤0.06 ~ ≧128	8	≧128	≤0.06 ~ ≧128	8 ~ ≧128
CAM	≧128	≧128	≤0.06 ~ ≧128	≧128	≧128	≤0.06 ~ ≧128	8	≧128	≤0.06 ~ ≧128	8 ~ ≧128
VCM	0.25	0.5	≦0.06 ~ 0.5	0.25	0.5	≦0.06 ~ 0.5	0.25	0.5	0.125 ~ 0.5	0.25 ~ 0.5

garenoxacin (GRNX) had MIC ≤0.06 μg/mL and TFLX had MIC of 0.25 μg/mL. There was one strain that showed an MIC as high as 16 μg/mL to TFLX.

Fig. 2 shows the distribution of serotypes. The most frequent distribution was seen in 15B, followed by 19A, and 35B. Serotype strains contained in 13-valent pneumococcal conjugate vaccine (PCV13) were 19 strains (18.3%). The relationship between serotypes and resistant strains was investigated. The 19 strains that were contained in PCV13 presented 6 types, including 3, 6A, 6B, 7F, 19A, and 23F, and among them 12 strains (63.2%) were PSSP, 7 strains (36.8%) were PISP, and no PRSP were observed. Strains that are not contained in PCV13 were 85 strains, among which 56 strains (65.9%) were PSSP, 25 strains (29.4%) were PISP, and 4 strains (4.7%) were PRSP.

Regarding the background, the percentage of PSSP in every isolate was used for the assessment. However, the results showed no significant difference in any comparison: patients aged <3 years vs. those aged ≥3 years was 71.2% vs. 51.6%, no sibling vs. sibling was 65.3% vs. 66.7%, no group nursing vs. group nursing was 75.0% vs. 60.0%, and no pretreatment with antibiotics vs. pretreatment with antibiotics was 60.4% vs. 69.4%.

4.2. *H. influenzae*

A total of 129 isolates were analyzed for *H. influenzae*. Isolate materials included 90 isolates of mucous membrane of the upper pharynx, 37 sputum isolates, 1 otorrhea isolate, and 1 eye discharge isolate. The distribution of the ages of patients was 34 patients aged <1 year, 47 patients aged 1 year, 11 patients aged 2 years, 15 patients aged 3 years, 7 patients aged 4 years, 3 patients aged 5 years, 5 patients aged 6 years, and 7 patients aged 7 years and older. Fig. 3 shows the frequency of the resistant strains together with the past surveillance results. The results showed that BLNAS accounted for 40 strains (31.0%),

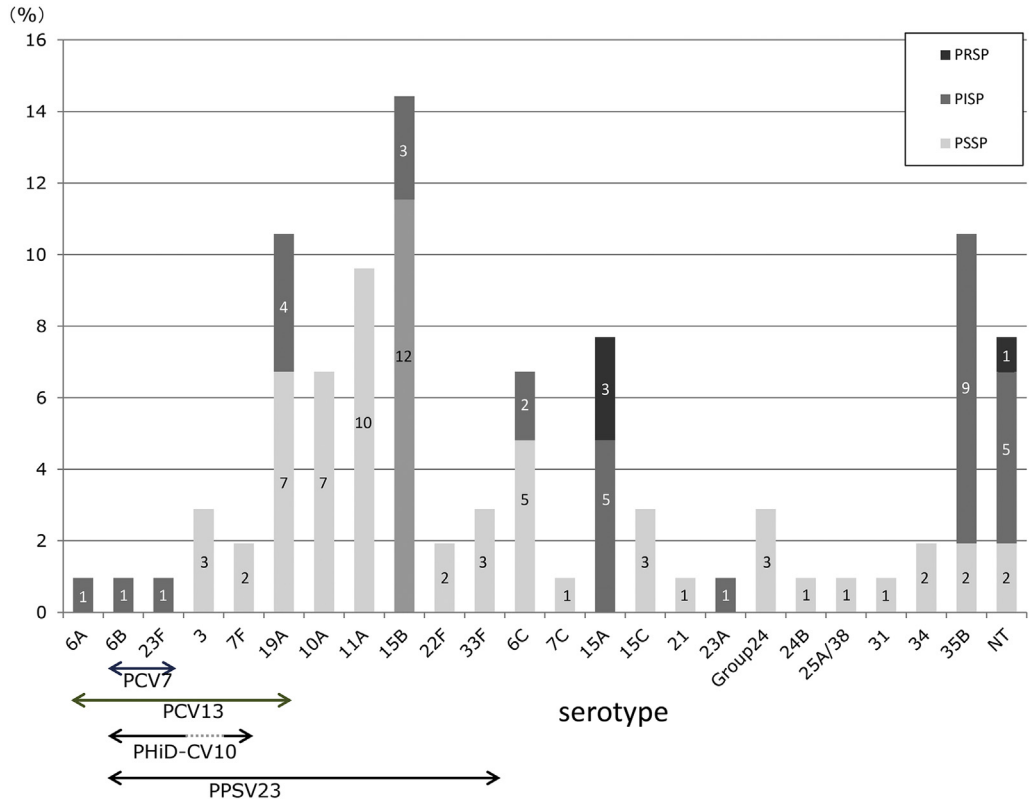

Fig. 2. Distribution of serotypes of *S. pneumoniae*. PSSP: penicillin-susceptible *S. pneumoniae*, PISP: penicillin-intermediate *S. pneumoniae*, PRSP: penicillin-resistant *S. pneumoniae*. PCV: pneumococcal conjugate vaccine, PHiD-CV: pneumococcal non-typeable *H. influenzae* protein D conjugate vaccine. PPSV: pneumococcal polysaccharide vaccine.

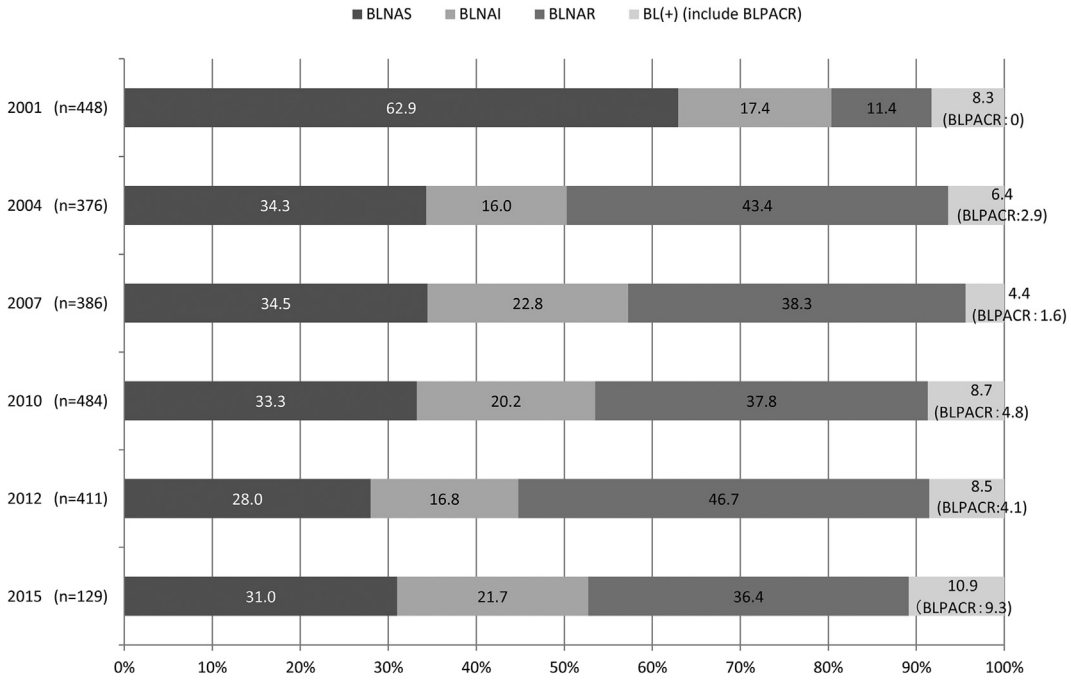

Fig. 3. Changes of *H. influenzae* strains classified by ampicillin (ABPC) or clavulanic acid/amoxicillin (CVA/AMPC) resistance. BLNAS: β-lactamase negative ampicillin susceptible, BLNAI: β-lactamase negative ampicillin intermediate resistant. BLNAR: β-lactamase negative ampicillin resistant, βL: β-lactamase, BLPACR: β-lactamase producing clavulanic acid/amoxicillin resistant. The results of 2001–2012 derived from references 1), 3), and 5).

BLNAI for 28 strains (21.7%), BLNAR for 47 strains (36.4%), BL producing for 14 strains (10.8%), among which BLPACR accounted for 12 strains (9.3%).

Table 2 shows the results of the drug susceptibility test. When comparing the MIC_{90}, CDTR demonstrated the best outcome among oral beta-lactam antibiotics with MIC of 0.5 μg/mL, and ceftriaxone

(CTRX) and tazobactam/piperacillin (TAZ/PIPC) (TAZ4 fixed) among injectable beta-lactam antibiotics with MIC of 0.25 μg/mL. Among quinolones, ciprofloxacin (CPFX), TFLX, levofloxacin (LVFX), GRNX had MIC ≤0.06 μg/mL. There was one strain that showed an MIC as high as 4 μg/mL to TFLX. There was no detection of capsular type b strains.

Regarding the background, the percentage of BLNAS in every isolate was used for assessment. However, the results showed no significant difference in any comparison: patients aged <3 years vs. those aged ≥3 years was 28.3% vs. 37.8%, no sibling vs. sibling was 27.5% vs. 31.9%, no group nursing vs. group nursing was 35.5% vs. 30.6%, and no pretreatment with antibiotics vs. pretreatment with antibiotics was 28.6% vs. 34.4%.

4.3. M. catarrhalis

A total of 54 isolates were analyzed for *M. catarrhalis*. Isolate materials included 42 isolates of mucous membrane of the upper pharynx and 12 sputum isolates. The distribution of the ages of the patients was 19 patients aged <1 year, 17 patients aged 1 year, 7 patients aged 2 years, 7 patients aged 3 years, 2 patients aged 4 years, and 2 patients aged 7 years and older.

All the isolates were β-lactamase producing strains. Table 3 shows the results of the drug susceptibility test. When comparing the MIC_{90}, TBPM demonstrated the best outcome among oral β-lactam antibiotics with MIC ≤0.06 μg/mL, and TAZ/PIPC (TAZ4 fixed), PAPM, meropenem (MEPM) and DRPM demonstrated the best outcome among injectable β-lactam antibiotics with MIC of ≤0.06 μg/mL. Among quinolones, CPFX, TFLX and GRNX had MIC ≤0.06 μg/mL.

Table 3
Minimum inhibitory concentrations of *M.catarrhalis*.

Drug	Total (n = 54)		
	MIC50	MIC90	range (μg/mL)
ABPC	8	16	1 ~ 16
AMPC	8	16	1 ~ 32
CVA/AMPC(1:2)	0.25	0.25	≦0.06 ~ 0.5
CVA/AMPC(1:14)	0.25	0.5	≦0.06 ~ 0.5
PIPC	2	8	0.25 ~ 32
TAZ/PIPC(TAZ4)	≦0.06	≦0.06	≦0.06
TAZ/PIPC(1:8)	≦0.06	0.125	≦0.06 ~ 0.125
CDTR	0.5	2	≦0.06 ~ 4
CFPN	0.5	1	≦0.06 ~ 2
CFDN	0.5	1	0.125 ~ 2
CTM	2	2	0.5 ~ 8
CTRX	1	4	≦0.06 ~ 8
CTX	1	2	0.125 ~ 4
CFTM	1	4	0.25 ~ 8
PAPM	≦0.06	≦0.06	≦0.06
MEPM	≦0.06	≦0.06	≦0.06
DRPM	≦0.06	≦0.06	≦0.06
TBPM	≦0.06	≦0.06	≦0.06
FRPM	0.5	1	≦0.06 ~ 1
NFLX	0.25	0.25	0.125 ~ 16
CPFX	≦0.06	≦0.06	≦0.06 ~ 8
TFLX	≦0.06	≦0.06	≦0.06 ~ 2
LVFX	≦0.06	0.125	≦0.06 ~ 4
GRNX	≦0.06	≦0.06	≦0.06 ~ 1
AZM	≦0.06	≦0.06	≦0.06 ~ 0.25
CAM	0.125	0.25	≦0.06 ~ 1

5. Discussion

This surveillance in 2015 was carried out in the same medical institution in the past. The strains number of shares was small in comparison with the other years.

In 2010, PRSP accounted for 27.0% of *S. pneumoniae*, which greatly decreased to 10.3% in 2012 and to 3.8% in this surveillance. Along with the change, PSSP increased from 23.1% in 2010 to 65.4% in this surveillance. One of the major factors to explain this change is an introduction of PCVs. In Japan, PCV7 became commercially available as non-mandatory vaccination in March 2010. In November 2010, the national government started the

Table 2
Minimum inhibitory concentrations of *H. influenzae*.

Drug	Total (n = 129)			BLNAS (n = 40)			BLNAI (n = 28)			BLNAR (n = 47)			β-lactamase (+) (n = 14)		
	MIC50	MIC90	range	MIC50	MIC90	range	MIC50	MIC90	range	MIC50	MIC90	range	MIC50	MIC90	range (μg/mL)
ABPC	2	8	0.125 ~ ≧256	0.25	1	0.125 ~ 1	2	2	2 ~ 2	4	8	4 ~ 8	≧256	≧256	4 ~ ≧256
AMPC	4	16	0.25 ~ ≧128	0.5	2	0.25 ~ 2	2	8	1 ~ 8	8	8	2 ~ 16	≧128	≧128	4 ~ ≧128
CVA/AMPC(1:2)	4	16	0.25 ~ 32	0.5	2	0.25 ~ 4	4	8	2 ~ 8	8	16	2 ~ 16	16	32	2 ~ 32
CVA/AMPC(1:14)	4	16	0.25 ~ 64	0.5	2	0.25 ~ 4	4	8	1 ~ 8	8	16	2 ~ 16	16	32	2 ~ 64
PIPC	≦0.06	0.5	≦0.06 ~ ≧256	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	0.25	≦0.06 ~ 0.5	0.125	0.25	≦0.06 ~ 0.5	128	≧256	0.25 ~ ≧256
TAZ/PIPC(TAZ4)	≦0.06	0.25	≦0.06 ~ 0.5	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	0.25	≦0.06 ~ 0.5	0.125	0.25	≦0.06 ~ 0.5	0.125	0.25	≦0.06 ~ 0.5
TAZ/PIPC(1:8)	≦0.06	0.5	≦0.06 ~ 8	≦0.06	≦0.06	≦0.06 ~ 0.25	0.125	0.25	≦0.06 ~ 0.5	0.125	0.25	≦0.06 ~ 0.5	1	8	0.125 ~ 8
CDTR	0.25	0.5	≦0.06 ~ 1	≦0.06	0.125	≦0.06 ~ 0.5	0.25	0.5	≦0.06 ~ 0.5	0.25	0.5	0.125 ~ 1	0.25	0.25	≦0.06 ~ 0.25
CFPN	1	4	≦0.06 ~ 8	≦0.06	1	≦0.06 ~ 2	1	4	0.125 ~ 4	2	4	0.5 ~ 8	2	4	≦0.06 ~ 4
CFDN	2	8	≦0.06 ~ 16	0.5	2	≦0.06 ~ 8	2	8	0.5 ~ 8	4	8	1 ~ 16	4	8	0.5 ~ 8
CTM	8	64	0.25 ~ 128	2	8	≦0.06 ~ 64	8	64	2 ~ 64	32	64	2 ~ 128	32	64	2 ~ 128
CTRX	0.25	0.25	≦0.06 ~ 0.5	≦0.06	0.125	≦0.06 ~ 0.5	0.25	0.25	≦0.06 ~ 0.25	0.25	0.5	0.125 ~ 0.5	0.25	0.25	≦0.06 ~ 0.5
CTX	0.5	1	≦0.06 ~ 4	≦0.06	0.5	≦0.06 ~ 1	0.5	1	≦0.06 ~ 2	1	2	0.5 ~ 4	1	1	≦0.06 ~ 2
CFTM	0.5	1	≦0.06 ~ 2	≦0.06	0.5	≦0.06 ~ 1	0.5	1	≦0.06 ~ 2	1	1	0.5 ~ 1	1	1	≦0.06 ~ 1
PAPM	1	2	0.125 ~ 4	0.5	1	0.125 ~ 2	1	2	0.25 ~ 2	1	2	0.25 ~ 4	1	4	0.5 ~ 4
MEPM	0.25	0.5	≦0.06 ~ 1	≦0.06	0.125	≦0.06 ~ 0.25	0.25	0.25	≦0.06 ~ 0.5	0.25	1	≦0.06 ~ 1	0.25	0.5	≦0.06 ~ 1
DRPM	0.5	2	≦0.06 ~ 2	0.125	0.25	≦0.06 ~ 1	0.5	1	≦0.06 ~ 2	1	2	0.125 ~ 2	1	2	0.125 ~ 2
TBPM	0.25	1	≦0.06 ~ 2	≦0.06	0.25	≦0.06 ~ 0.5	0.25	0.5	≦0.06 ~ 0.5	0.5	1	≦0.06 ~ 2	0.5	1	0.125 ~ 1
FRPM	2	4	0.25 ~ 4	0.5	2	0.25 ~ 2	2	4	0.5 ~ 4	2	4	1 ~ 4	2	4	0.5 ~ 4
NFLX	≦0.06	0.125	≦0.06 ~ 8	≦0.06	≦0.06	≦0.06 ~ 0.5	≦0.06	0.5	≦0.06 ~ 4	≦0.06	0.125	≦0.06 ~ 0.5	≦0.06	0.125	≦0.06 ~ 8
CPFX	≦0.06	≦0.06	≦0.06 ~ 4	≦0.06	≦0.06	≦0.06 ~ 0.125	≦0.06	0.125	≦0.06 ~ 1	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	≦0.06	≦0.06 ~ 4
TFLX	≦0.06	≦0.06	≦0.06 ~ 4	≦0.06	≦0.06	≦0.06 ~ 0.125	≦0.06	0.125	≦0.06 ~ 1	≦0.06	≦0.06	≦0.06 ~ 0.125	≦0.06	≦0.06	≦0.06 ~ 4
LVFX	≦0.06	≦0.06	≦0.06 ~ 2	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	0.125	≦0.06 ~ 1	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	≦0.06	≦0.06 ~ 2
GRNX	≦0.06	≦0.06	≦0.06 ~ 4	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	0.125	≦0.06 ~ 2	≦0.06	≦0.06	≦0.06 ~ 0.25	≦0.06	≦0.06	≦0.06 ~ 4
AZM	1	2	≦0.06 ~ 4	1	2	≦0.06 ~ 2	1	2	0.5 ~ 4	1	2	0.25 ~ 4	8	8	0.5 ~ 4
CAM	4	8	0.5 ~ 16	4	8	0.5 ~ 16	4	8	2 ~ 8	8	16	2 ~ 16	8	8	2 ~ 8

Urgent Vaccination Promotion Project, with which temporary special subsidies were provided to support the necessary cost for vaccination not only by the municipality government that is the responsible organization for vaccination, but also from the Ministry of Health, Labour and Welfare. The vaccination rate increased greatly since the self-pay was reduced for vaccination. PCV7 was included in the routine immunization schedule in April 2013 and was switched to PCV13 in October 2013. Since it was supported by public expenditure, the immunization rate increased greatly and is assumed to be over 90% after it was included in the routine immunization schedule. Immediately after the start of immunization in 2010, the most common serotype was 19F with 79 strains (17.3%), followed by 6B with 77 strains (16.8%) [8]. In 2012, 6C was the most common with 37 strains (10.0%), followed by 19F with 36 strains (9.7%), showing a considerable change [8]. The percentage of the serotypes that were contained in PCV13 was 77.7% in 2010, which decreased to 39.5% in 2012 and further down to 18.3% in this surveillance [8]. The frequency of PRSP was high in the serotypes contained in PCV13 [8]. In 2010, PRSP accounted for 32.0% of the serotype strains that were contained in PCV13 and 9.8% of those that were not contained in PCV13, showing a great difference. It was considered that the frequency of PRSP decreased because the serotypes with a high resistance rate reduced as a result of the dissemination of PCV. Regarding the susceptibility of individual antibiotics, the results showed no significant change from those in the past. However, there was one strain showing a TFLX MIC as high as 16 μg/mL. The result of this surveillance was a record high since in 2012 the TFLX MIC ranged from ≤0.06 μg/mL to 0.5 μg/mL. Recently, it is reported that strains isolated from adult patients showed low sensitivity to quinolones, [10] so it should be observed carefully whether such strain will increase in the future. Regarding *H. influenzae*, combined BLNAI and BLNAR accounted for 28.8% in 2001, which shifted between 50% and 60% since 2004 and remained 58.1% in 2015, showing no significant change. BLPACR accounted for 1.6% in 2007, 4.8% in 2010, 4.1% in 2012, and 9.1% in this surveillance, showing an increase. Regarding the susceptibility of individual antibiotics, the results showed no significant change from those in the past. However, there was one strain showing a TFLX MIC as high as 4 μg/mL. As the TFLX MIC in 2012 ranged from ≤0.06 μg/mL to 1 μg/mL, it revealed the emergence of a strain with low susceptibility, although it was only one strain. Recently, it is reported that some strains of *H. influenzae* from adult patients showed low sensitivity to quinolones [11], so it should also be observed carefully whether such strain will increase in the future.

In November 2008, the Hib vaccine became available commercially. Similar to PCV, the national government started the Urgent Vaccination Promotion Project in November 2010. With the reduction in self-pay for vaccination, the vaccination rate increased, and in April 2013, Hib vaccination was included in the routine immunization schedule. The frequency of Hib decreased from 3.6% in 2007, 4.8% in 2010, and 1.2% in 2012, and it was not detected in this surveillance. The frequency of Hib decreased with the dissemination of Hib vaccination.

In the past surveillance, We might obtain a significant difference in the background factors such as the age category and pre-administration antimicrobial agents, in *S. pneumonia* and *H. influenzae*. However, we didn't found in neither backgronud factor in this surveillance. We thought with a reason that the isolates in this surveillance (*S. pneumoniae* : 104, *H. influenzae* : 129) had less number of the isolates than other years

Regarding *M. catarrhalis*, one strain out of 111 (0.9%) showed low susceptibility to three quinolones in 2012: LVFX 4 μg/mL, TFLX 2 μg/mL and GRNX 1 μg/mL [6]. This surveillance showed increase in strains with low susceptibility; 3 (5.6%) in LVFX, 2 (3.7%) in TFLX, and 2 (3.7%) in GRNX. It is important to maintain a strong focus on this trend toward the increasing drug resistance of *M. catarrhalis* in order to prevent further increase of drug-resistant *M. catarrhalis*.

6. Conclusion

1. Compared to the results of previous nationwide surveillance, the status of resistant strains of *S. pneumoniae* showed a significant reduction in PRSP.
2. Compared to the results of previous nationwide surveillance, the status of resistant strains of *H. influenzae* showed no significant change. However, there was a tendency that BLPACR was increasing.
3. Compared to the results of nationwide surveillance in 2012, the results of susceptibility test of *M. catarrhalis* showed no significant change.
4. Regarding the quinolones, although it was one isolate, there was a *S. pneumoniae* isolate with MIC as high as 16 μg/mL to TFLX and a *H. influenzae* isolate with MIC as high as 4 μg/mL to TFLX, and the number of *M. catarrhalis* isolates with MIC of 2 μg/mL to TFLX have increased from one in 2012 to two. Quinolone-resistant strains need to be monitored continuously in the future.

Conflicts of interest

Hiroshi Sakata has received speaker honoraria from Meiji Seika Pharma Co., Ltd.

Akira Watanabe has received speaker honoraria from Sumitomo Dainippon Pharma Co., Ltd., UCB Japan Co. Ltd., MSD K.K., Kobayashi Pharmaceutical Co., Ltd., Shionogi & Co., Ltd., Daiichi Sankyo Co., Ltd., Taisho Toyama Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Co., Chugai Pharmaceutical Co., Ltd., Toyama Chemical Co., Ltd., Pfizer Japan Inc., Janssen Pharmaceutical K.K.; payments for manuscript drafting and editing from Iyaku (Medicine and Drug) Journal Co., Ltd.; donations from Astellas Pharma Inc., Daiichi Sankyo Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd.; and grant support from Kyorin Pharmaceutical Co., Ltd., Shionogi & Co., Ltd., Daiichi Sankyo Co., Ltd., Taisho Toyama Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Meiji Seika Pharma Co., Ltd.

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Seiji Hori has received speaker's honorarium from Kyorin Pharmaceutical Co., Ltd.; and grant support from Daiichi Sankyo Co., Ltd.

Masaaki Odajima is an employee of Kyorin Pharmaceutical Co., Ltd.

Yoshitaka Katakuse is an employee of Toyama Chemical Co., Ltd.

Toshikazu Hasegawa is an employee of Towa Pharmaceutical Co. Ltd.

Nobuko Maki is an employee of Taisho Toyama Pharmaceutical Co., Ltd.

Koichi Wada is an employee of Sawai Pharmaceutical Co., Ltd.

Yoshitake Sato and Yoshio Yamaguchi have no potential conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2018.11.004.

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