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Surveillance

Nationwide surveillance of 6 otorhinolaryngological infectious diseases and antimicrobial susceptibility pattern in the isolated pathogens in Japan

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ABSTRACT

The Japanese Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Committee has conducted a nationwide surveillance on antimicrobial susceptibility patterns and rates of isolation in 6 otolaryngological diseases. The surveillance program was conducted in the otorhinolaryngological departments of 29 universities, and their 26 affiliated hospitals. Patients suffering from acute otitis media, chronic otitis media, acute nasal sinusitis, chronic nasal sinusitis, acute tonsillitis, and peritonsillar abscess between January 2011 and June 2012 were investigated. The collected swab or incision samples were cultivated for microbial identification, and the drug susceptibility of detected bacteria was measured at the Kitasato University Research Center for Infections and Antimicrobials. The surveillance focused on three gram-positive bacteria (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*), three gram-negative bacteria (*Haemophilus influenzae*, *Moraxella Catarrhalis*, and *Pseudomonas aeruginosa*), and three anaerobic bacteria (*Peptostreptococcus* spp., *Prevotella* spp., and *Fusobacterium* spp.). Bacterial susceptibility to 39 antimicrobial drugs was investigated. We compared bacterial isolation ratio of each disease in this surveillance from those of past 4 times surveillance which we performed formerly, and we also compared percentage of main drug resistant strains from those of past 4 times surveillance. The age composition between this time and former surveillances was not statistically significant by student-t test.

We were unable to completely resolve the rise in resistant bacteria, such as methicillin-resistant *S. aureus*, penicillin-resistant *S. pneumoniae*, penicillin-intermediate resistant *S. pneumoniae*, beta-lactamase non-producing ampicillin-resistant *H. influenzae*, beta-lactamase producing ampicillin-resistant *H. influenzae*, and beta-lactamase producing amoxicillin clavulanic acid-resistant *H. influenzae*. We suggest promoting the proper usage of antimicrobial drugs in order to avoid the spread of these bacteria.

1. Introduction

The Japan Society for Infectious Diseases in Otorhinolaryngology has, thus far, conducted four nationwide surveillances for otorhinolaryngological diseases [1–5]. The resultant data, reported in the society journal, have found wide application in the handling of otorhinolaryngological field diseases for approximately 20 years. These results provide useful information regarding the proper use of antimicrobial drugs. Recent years have seen an increase in antibiotic-resistant *Streptococcus pneumoniae* and

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Haemophilus influenzae strains. Therefore, it is important to obtain information on the annual trends in detected bacteria and their susceptibility toward antimicrobial drugs. However, due to the high costs involved, our society has been unable to implement a nationwide surveillance on resistant bacteria on a standalone basis.

In 2009, three academic societies, the Japanese Society of Chemotherapy (which expanded its surveillance projects in 2006), the Japanese Association for Infectious Diseases, and the Japanese Society for Clinical Microbiology, jointly launched the Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Program. As part of this program, our society was able to conduct a nationwide surveillance in the field of otolaryngology on a standalone basis in 2011. The total expenditure for this surveillance was borne by the Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Committee. The results have been presented in national and international academic conferences. In this paper, we report the results of our surveillance in the field of otolaryngology.

2. Subjects and methods

The surveillance was implemented in collaboration with the otorhinolaryngological departments at 29 universities, and their 26 affiliated hospitals and practitioners in Japan (Table 1). Patients suffering from acute otitis media, chronic otitis media (including cases of acute exacerbation), acute nasal sinusitis, chronic nasal sinusitis (including cases of acute exacerbation), acute tonsillitis, and peritonsillar abscess, who received medical diagnosis at the participating facilities between January 2011 and June 2012 were included in the study. Postoperative cases were excluded and patients were sampled only once. The accumulated fluid from the middle ear or fresh pus from the external ear canal was collected aseptically in otitis media patients. In either case, samples were collected using Seed Swab γ No. 2 (Eiken Chemical Co., Ltd., Tokyo, Japan). For sinusitis, fresh pus from nasal sinuses was collected using Seed Swab γ No. 2, or the pus or accumulated fluid was collected by direct puncture of the maxillary sinus using an Anaerobic Porter II (Terumo Clinical Supply Co., Ltd., Tokyo, Japan). For acute tonsillitis, Seed Swab γ No. 2 was used to swab deep in the crypt. Pus samples from peritonsillar abscess patients were collected by puncturing or incising the sterilized abscess using the Anaerobic Porter II. The collected samples and the patient backgrounds were sent to the Kitasato University Research Center for further testing. Several bacterial strains were subjected to identification [6,7] and antibiotic-susceptibility testing. These included gram-positive bacteria (*S. pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*), gram-negative bacteria (*H. influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*), and anaerobic bacteria (*Peptostreptococcus* spp., *Prevotella* spp., and *Fusobacterium* spp.). Bacteria were cultivated in both aerobic and anaerobic culture media. These were separated and identified, and their minimum inhibition concentrations (MIC) were measured [8,9] by the trace liquid dilution method (aerobic) [10,11] or agar plate dilution method (anaerobic) [12], according to the procedures described by the Clinical and Laboratory Standards Institute (CLSI).

The strains of *S. aureus* were classified into MSSA (methicillin sensitive *S. aureus*; MIC of MPIPC $\leq$ 2 μg/mL) and MRSA (MIC of MPIPC $\geq$ 4 μg/mL) in accordance with the guidelines set by CLSI Document M100-S23 [9].

Resistant strains of *S. pneumoniae* were determined in accordance with both the current standard [9] and the former standard (CLSI M100-S17) [8]. In this report, the former method was applied to compare the past results of resistant bacteria. In other words, bacteria were classified as: PSSP (penicillin susceptible *S. pneumoniae*; MIC of PCG $\leq$0.06 μg/mL), PISP (penicillin intermediate *S. pneumoniae*: 0.125 $\leq$ MIC of PCG $\leq$1 μg/mL) and PRSP (penicillin resistant *S. pneumoniae*: MIC of PCG $\geq$2 μg/mL) [8].

H. influenzae was classified as follows: BLNAS (β-lactamase negative ampicillin susceptible strains of *H. influenzae*: ABPC; MIC $\leq$1 μg/mL), BLNAI (β-lactamase negative, ampicillin resistant strains of *H. influenzae;* MIC = 2 μg/mL), BLNAR (MIC $\geq$ 4 μg/mL), and BLPAR (β-lactamase productive strain) [9].

M. catarrhalis was classified into two groups according to the susceptibility toward ABPC: resistant strains (MIC $\geq$ 1 μg/mL) and susceptible strains (MIC $\leq$ 0.5 μg/mL) [11].

Indigenous bacteria (α-*Streptococcus* spp., *Neisseria* spp., etc.) and bacteria other than the ones listed above were excluded from the MIC measurement. Thirty-nine antimicrobial drugs were used for the measurement of drug susceptibility: 8 penicillins – Benzylpenicillin (PCG), Ampicillin (ABPC), Amoxicillin (AMPC), Amoxicillin-clavulanic acid (CVA/AMPC), Piperacillin (PIPC), Ampicillin-sulbactam (SBT/ABPC), Piperacillin-tazobactam (TAZ/PIPC), and Oxacillin (MPIPC); 1 penem Faropenem (FRPM); 7 cephems – Cefditoren (CDTR), Cefcapene (CFPN), Cefteram (CFTM), Ceftriaxone (CTRX), Cefmenoxime (CMX), Cefpirome (CPR), and Flomoxef (FMOX); 6 carbapenems – Tebipenem (TBPM), Doripenem (DRPM), Panipenem (PAPM), Meropenem (MEPM), Biapenem (BIPM), and Imipenem/Cilastatin (IPM/CS); 2 macrolides – Clarithromycin (CAM) and Azithromycin (AZM); 1 tetracycline – Minocycline (MINO); 7 new quinolones – Levofloxacin (LVFX), Tosufloxacin (TFLX), Prulifloxacin (PUFX), Garenoxacin (GRNX), Moxifloxacin (MFLX), Sitafloxacin (STFX), and Ciprofloxacin (CPFX); 1 oxazolidinone – Linezolid (LZD); 2 glycopeptides – Vancomycin (VCM) and Teicoplanin (TEIC); 2 aminoglycosides – Amikacin (AMK) and Arbekacin (ABK); 1 lincomycin – Clindamycin (CLDM); and 1 other – Fosfomycin (FOM). The appropriate antimicrobial drug was selected for each subject strain. For the β-lactamase productivity in *M. catarrhalis*, and *H. influenzae* was confirmed using Cefinase (Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. The metallo-β-lactamase productivity was confirmed by conducting the Cica-Beta-Test (Kanto Chemical Co., Inc., Tokyo, Japan) and SMA Disk (Eiken Chemical Co., Ltd.) test, as per the manufacturer's instructions.

We compared bacterial isolation ratio of each disease in this surveillance from those of past 4 times surveillance which we performed formerly, and we also compared percentage of main drug resistant strains (methicillin resistant *S. aureus*: MRSA; penicillin resistant *S. pneumoniae*: PRSP; penicillin intermediate *S. pneumoniae*: PISP; oniaeillin intermedia ampicillin resistant *H. influenzae*:BLNAR; ABPC resistant *M. catarrhalis*) from those of past 4 times surveillance.

The age composition between this time and former surveillances was not statistically significant by student-t test.

3. Results

3.1. Frequency of detected bacteria by disease

We observed 184 cases of acute otitis media (bacteria detected in 140 cases, 76.1%; 195 strains detected), 120 cases of chronic otitis media (bacteria in 107 cases, 89.2%; 130 strains), 129 cases of acute sinusitis (98 cases, 76.0%; 167 strains), 91 cases of chronic sinusitis (65 cases, 71.4%; 101 strains), 116 cases of acute tonsillitis (116 cases, 100%; 135 strains), 89 cases of peritonsillar abscess (87 cases, 97.8%; 233 strains). Among the total 729 cases, 961 bacterial strains were detected from 613 cases (84.1%) (Table 2). As shown in Table 3, the frequencies of detected bacteria were as follows: 114 stains (12.1%)

Table 1
Participating institutions.

1.	Dept, of Otolaryngology Head & Neck Surg., Asahikawa Med. Univ.
2.	Nonaka Tracheoesophago- ENT Clinic
3.	Dept. of Otolaryngology, Sapporo Med. Univ.
4.	KKR Sapporo Medical Center.
5.	Dept. of Otolaryngology Head & Neck Surg., Akita Univ. Sch. of Med.
6.	Sannohe ENT Clinic.
7.	Dept. of Otolaryngology Head & Neck Surg., Yamagata Univ. Faculty of Med.
8.	Dept. of Otolaryngology Head & Neck Surg., Tohoku Univ. Sch. of Med.
9.	Tohoku Rosai Hospital.
10.	Dept. of Otolaryngology, Gunma Univ. Sch. of Med.
11.	Tone Central Hospital.
12.	Dept. of Otorhinolaryngology, Juntendo Univ. Faculty of Med.
13.	Sugita ENT Clinic.
14.	Dept. of Otorhinolaryngology, Tokyo Women's Med. Univ.
15.	Dept. of Otolaryngology, Toho Univ. Faculty of Med.
16.	Dept. of Otolaryngology, Kyorin Univ. Sch. of Med.
17.	Fussa Municipal Hospital
18.	Dept. of Otorhinolaryngology, Kitasato Univ. Sch. of Med.
19.	Momiyama ENT Clinic.
20.	Dept. of Otolaryngology Head & Neck Surg., Niigata Univ. Faculty of Med.
21.	Urano ENT Clinic
22.	Dept, of Otorhinolaryngology, Shinshu Univ. Sch. of Med.
23.	Sato ENT Clinic.
24.	Dept. of Otolaryngology Head & Neck Surg., Kanazawa Univ. Grad. Sch. Of Med. Sciences
25.	Kushi ENT Clinic.
26.	Dept. of Neuro-otolaryngology, Nagoya City Univ. Grad. Sch. of Med. Sciences
27.	Japanese Red Cross Nagoya Second Hospital
28.	Dept. of Otolaryngology Head & Neck Surg., Sec. Hosp., Fujita Health Univ. Sch. of Med.
29.	Sakai ENT Clinic
30.	Dept. of Otorhinolaryngology Head & Neck Surg., Mie Univ. Grad, Sch.of Med.
31.	Amesara ENT Clinic.
32.	Dept. of Otorhinolaryngology, Wakayama Med. Univ.
33.	Dept. of Otorhinolaryngology, Kansai Med. Univ.
34.	Kumazawa ENT Clinic
35.	Dept. of Otorhinolaryngology, Shimane Univ.Faculty of Med.
36.	National Hospital Organization Hamada Medical Center
37.	Dept. of Otorhinolaryngology Head & Neck Surg., Ehime Univ. Sch. of Med.
38.	Dept. of Otolaryngology Head & Neck Surg., Kochi Med. Univ.
39.	Sawada EYE and EAR Clinic.
40.	Department of Otolaryngology Head & Neck Surg., Okayama Univ. Med. Sch.
41.	Fujimoto ENT Clinic
42.	Department of Otolaryngology Head & Neck Surg., Hiroshima Univ. Faculty of Med.
43.	ENT Kunimoto Clinic.
44.	Department of Otolaryngology, Yamaguchi Univ.Grad. Sch. of Med.
45.	Hiyoshi ENT Clinic.
46.	Department of Otolaryngology Head & Neck Surg., Saga Univ. Med. Sch., Faculty of Med.
47.	Iwanaga ENT clinic.
48.	Department of Otolaryngology Head & Neck Surg., Oita Med. Univ. Faculty of Med.
49.	Ichimiya ENT Clinic
50.	Department of Otolaryngology Head & Neck Surg., Kagoshima Univ. Grad. Sch. of Med. and Dent. Sciences
51.	Goto Chuou Hospital
52.	Department of Otorhinolaryngology Head & Neck Surg., Grad. Sch. of Med., Univ. of the Ryukyu
53.	Naha City Hospital.
54.	Fujimaki ENT Clinic
55.	Komatsu ENT Clinic

Participating 55 institutions listed geographical order from north to south.

of *S. pneumoniae*, 107 strains (11.5%) of *H. influenzae*, 63 strains (6.8%) of *S. pyogenes*, 55 strains (5.9%) of *M. catarrhalis*, and 114 strains (12.4%) of *S. aureus*. The results obtained by disease are elucidated as follows.

3.1.1. Acute otitis media

The most frequently detected bacterial strains were *S. pneumoniae* and *H. influenzae* (frequency of detection 29.7% and 27.2%, respectively). When cases of children of 15 years of age and

Table 2
Bacterial isolates from each disease.

Disease	Acute otitis media	Chronic otitis media	Acute sinusitis	Chronic sinusitis	Acute tonsillitis	Peritosillar abscess	Total
No. of samples	184	120	129	91	116	89	729
No. of bac. isolated samples	140	107	98	65	116	87	613
Ratio of bacteria isolation	76.1%	89.2%	76.0%	71.4%	100%	97.8%	84.1%
No. of detected bacteria	195	130	167	101	135	233	961
No. of normal flora	0	0	0	1	59	1	61

No. of samples: total number of samples corrected from each disease.
No. of bac. Isolated samples: total number of samples which bacteria was isolated from each disease.

Table 3
Number and ratio of major isolated strains for each disease.

Bacteria	Diseases						
	Acute otitis	Chronic otitis	Acute sinusitis	Chronic sinusitis	Acute tonsillitis	Peritonsillar abscess	Total
S. pneumoniae	58(29.7%)	2(1.5%)	38(22.8%)	11(10.9%)	5(3.7%)	0	114
S. pyogenes	4(2.1%)	3(2.3%)	1(0.6%)	1(1.0%)	36(26.7%)	18(7.7%)	63
S. aureus	26(13.3%)	50(38.5%)	12(7.2%)	20(19.8%)	3(2.2%)	2(0.9%)	113
H. influenzae	53(27.2%)	4(3.1%)	35(21.0%)	11(10.9%)	2(1.5%)	2(0.9%)	107
M. catarrhalis	22(11.3%)	0	27(16.2%)	6(5.9%)	0	0	55
P. aeruginosa	1(0.5%)	8(6.2%)	1(0.6%)	4(4.0%)	1(0.7%)	0	15
Anaerobic bacteria	0	0	21(12.6%)	11(10.9%)	0	136(58.4%)	168
Normal flora	0	0	0	1(1.0%)	59(43.7%)	1(0.4%)	61
Non-objective bacteria	31(15.9%)	63(48.5%)	32(19.2%)	36(35.6%)	29(21.5%)	74(31.8%)	265
Total	195	130	167	101	135	233	961

Non-objective bacteria: total number and percentage of bacteria which is not listed above bacteria groups.

under were considered, these percentages increased up to 30.8% and 32.7%, respectively. The incidence of *S. pneumoniae* and *H. influenzae* cases has seen a slight decrease and increase, respectively, in recent years. Throughout the age-bar, the detection rates of *S. aureus* and *M. catarrhalis* were 13.3% and 11.3%, respectively. However, among children of 15 years of age and under, the rates decreased in *S. aureus* and increased in *M. catarrhalis* (Tables 2 and 3).

Table 4 lists bacterial detection with respect to age. Zero and 1 year old infants demonstrated high detection percentages of 48.3% and 43.9%, respectively, for *H. influenzae*. This rate decreased sharply with the increase in age. *S. pneumoniae* was detected at a uniform high rate throughout all ages. These two strains accounted for approximately 70% of bacterial detection in children (2 years of age and under). *M. catarrhalis* was detected at an average approximate rate of 10% throughout all ages, with only minute age-related changes. *S. aureus* was frequently detected in children and adults, particularly in those of advanced age.

3.1.2. Chronic otitis media

As seen in Table 3, *S. pneumoniae* was rarely detected, and the detection rate of *H. influenzae* increased steadily (3.1%). *S. aureus* and *P. aeruginosa* accounted for 38.5% and 6.2%, respectively. The bacterial detection in this infection was different from that seen for acute otitis media, due to the range of bacteria detected.

3.1.3. Acute sinusitis

As shown in Table 3, the types of bacteria detected and the change in detection rate for acute sinusitis are similar to those seen in acute otitis media. *S. pneumoniae* (22.8%), *H. influenzae* (21.0%), and *M. catarrhalis* (16.2%) showed a decreasing trend during the previous surveillance. However, we observed an increase in bacterial count, accounting for 76.0% of the detection rate (Table 2). Anaerobic bacteria accounted for 12.6% of detected bacteria, and *S. aureus* count showed a decrease compared to the previous study (Table 3). Bacteria detected with respect to age-groups are listed in Table 5. *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* accounted for 27.9%, 29.6%, and 26.2% of the total bacterial count, respectively, in the 5 years and under age-group. *S. aureus* expression increased with a corresponding increase in age, and anaerobic bacteria were mostly observed in adults.

Table 5
Isolates from patients with acute nasal sinusitis listed by age.

Bacteria	Age				
	0~5	6~19	20~59	60~	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
S. aureus	3 (4.9)	2 (8.7)	4 (6.1)	3 (17.6)	12 (7.2)
S. pyogenes	1 (1.6)				1 (0.6)
S. pneumoniae	17 (27.9)	4 (17.4)	15 (22.7)	2 (11.8)	38 (22.8)
H. influenzae	18 (29.6)	6 (26.1)	10 (15.2)	1 (5.9)	35 (21.0)
M. catarrhalis	16 (26.2)	3 (13.0)	6 (9.1)	2 (11.8)	27 (16.2)
P. aeruginosa				1 (5.9)	1 (0.6)
Fusobacterium spp.		1 (4.3)	6 (9.1)	1 (5.9)	8 (4.8)
Prevotella spp.			5 (7.6)	1 (5.9)	6 (3.6)
Other anaerobes		1 (4.3)	4 (6.1)	2 (11.8)	7 (4.2)
Others	6 (9.8)	6 (26.1)	16 (24.2)	4 (23.5)	32 (19.2)
Total	61 (100)	23 (100)	66 (100)	17 (100)	167 (100)

S. pneumoniae, H. influenza and *M. catarrhalis* are major isolates from acute nasal sinusitis. Anaerobes were detected 12.6% totally.

3.1.4. Chronic sinusitis

Based on the results of the surveillance listed in Table 3, the rates of detection of *S. pneumoniae* and *H. influenzae* (10.9% for both)

Table 4
Isolates from patients with acute otitis media listed by age.

Bacteria	Age					
	0	1	2~5	6~15	16~	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
S. aureus	5 (17.2)		6 (10.3)	2 (18.2)	13 (32.5)	26 (13.3)
S. pyogenes			2 (3.4)	1 (9.1)	1 (2.5)	4 (2.1)
S. pneumoniae	6 (20.7)	20 (35.1)	18 (31.0)	5 (45.5)	9 (22.5)	58 (29.7)
H. influenzae	14 (48.3)	25 (43.9)	12 (20.7)	1 (9.1)	1 (2.5)	53 (27.2)
M. catarrhalis	2 (6.9)	6 (10.5)	10 (17.2)	1 (9.1)	3 (7.5)	22 (11.3)
P. aeruginosa			1 (1.7)			1 (0.5)
Others	2 (6.9)	6 (10.5)	9 (15.5)	1 (9.1)	13 (32.5)	31 (15.9)
Total	29 (100)	57 (100)	58 (100)	11 (100)	40 (100)	195 (100)

S. pneumoniae and *H. influenza* are major isolates from acute otitis media.
Percentage of *S. aureus* is increasing according to age.

Table 6
Isolates from patients with acute tonsillitis listed by age.

Bacteria	Age				
	0~5	6~19	20~59	60~	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
S. aureus			2 (1.9)	1 (9.1)	3 (2.2)
S. pyogenes	1 (20)	3 (25)	32 (29.9)		36 (26.7)
S. pneumoniae	1 (20)	1 (8.3)	2 (1.9)	1 (9.1)	5 (3.7)
Other streptococcus spp.	3 (60)	5 (41.7)	47 (43.9)	4 (36.4)	59 (43.7)
H. influenzae			2 (1.9)		2 (1.5)
M. catarrhalis					
P. aeruginosa			1 (0.9)		1 (0.7)
Others		3 (25)	21 (19.6)	5 (45.4)	29 (21.5)
Total	5 (100)	12 (100)	107 (100)	11 (100)	135 (100)

S. pyogenes is isolated 26.7% in this surveillance.

were similar to those seen in previous surveillances. *S. aureus*, *M. catarrhalis*, *P. aeruginosa,* and anaerobic bacteria were detected at rates of 19.8%, 5.9%, 4.0%, and 10.9%, respectively. No significant change was observed, when compared to past results.

3.1.5. Acute tonsillitis

Data from the surveillance (Table 3) shows that normal bacterial flora (intraoral indigenous bacteria centered on α-*Streptococcus*) was widely detected, accounting for 43.7% of bacterial count. The major bacterial strain causing this disease, *S. pyogenes*, accounted for 26.7% of the total bacterial count. *S. pneumoniae* and *H. influenzae* were observed at comparatively low rates of 3.7% and 1.5%, respectively. A low percentage (2.2%) of *S. aureus* was also detected. With respect to age (Table 6), only 17 strains were isolated from the age group 19 years and under, which was fewer than those seen in past surveillances. However, *S. pneumoniae*, *S. pyogenes,* and indigenous *Streptococcus* spp. were commonly detected among young people. *S. pyogenes* and other *Streptococcus* spp. were chiefly detected in adults and seniors.

3.1.6. Peritonsillar abscess

We observed that anaerobic bacteria accounts for 136 strains (58.4%) of the detected bacteria isolated from patients suffering from this disease; 68 strains (29.2%) of *Prevotella* spp., 31 strains (13.3%) of *Fusobacterium* spp., 4 strains (1.7%) of *Peptostreptococcus* spp., and 33 strains (14.2%) of other anaerobic bacteria were observed (Table 3). We also observed 18 strains (7.7%) of *S. pyogenes,* 2 strains (0.9%) each of *H. influenzae* and *S. aureus*, and 74 strains (31.8%) of other aerobic bacteria. All patients were over 18 years, and we observed no differences in detection rate based on age.

3.2. Drug susceptibility pattern of major bacterial strains

We tested for susceptibility patterns against various drugs for only six of the major isolated bacterial strains – *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis,* and *P. aeruginosa*.

3.2.1. S. aureus

We measured drug susceptibility pattern in 112 out of the total 113 isolated *S. aureus* strains. As shown in Fig. 1, the isolation frequency of MRSA in this surveillance was 25.9%. Table 7 shows MIC50 and MIC90 values of 17 antimicrobial drugs against *S. aureus* (83 MSSA and 29 MRSA strains). The MIC50 values showcased by all drugs were relatively good. On the other hand, FRPM, GRNX, STFX, and MFLX resulted in relatively good MIC90 values of 2 μg/mL, anti-MRSA drugs ABK, VCM, and TEIC displayed a MIC90 of 1 μg/mL, and the MIC90 of LZD was 2 μg/mL.

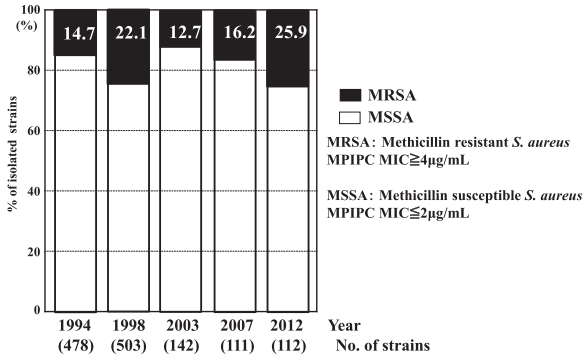

Fig. 1. Expression of isolated *S. aureus* over the past 18 years. Proportions of MRSA isolated from otorhinolaryngological field were about 20%, and it is increasing up to 25.9%, recently.

Table 7
MIC 50 and MIC 90 values of antibiotics against *S. aureus* (112 strains: MSSA: 83 strains, MRSA: 29 strains).

Antibiotics	Total (112 strains)		
	Range	MIC50	MIC90
MPIPC	0.125 to >256	0.5	128
AMPC	≦0.06 to 128	1	16
PIPC	0.25 to >256	2	64
CDTR	0.125 to 64	0.5	32
CFPN	0.125 to >256	1	>256
CMX	0.25 to >256	1	64
FRPM	0.125 to >256	0.25	2
MINO	0.125 to 16	0.25	8
TFLX	≦0.06 to >32	≦0.06	>32
GRNX	≦0.06 to 64	≦0.06	2
STFX	≦0.06 to 16	≦0.06	2
MFLX	≦0.06 to 64	≦0.06	2
MEPM	≦0.06 to 16	0.125	4
ABK	0.25 to 2	0.5	1
VCM	0.5 to 1	0.5	1
TEIC	0.25 to 2	0.5	1
LZD	1 to 4	2	2

Anti-MRSA drugs revealed good MICs on the whole.

3.2.2. S. pyogenes

MIC50 and MIC90 values of 16 antimicrobial drugs acting on 63 strains of *S. pyogenes* are displayed in Table 8. All drugs displayed a favorable MIC50. CAM, AZM, and MIN0 were shown to be

Table 8
MIC 50 and MIC 90 values of antibiotics against *S. pyogenes* (63 strains).

Antibiotics	Total (63 strains)		
	Range	MIC50	MIC90
ABPC	≦0.06	≦0.06	≦0.06
AMPC	≦0.06	≦0.06	≦0.06
CVA/AMPC	≦0.06	≦0.06	≦0.06
CDTR	≦0.06	≦0.06	≦0.06
CFPN	≦0.06	≦0.06	≦0.06
CFTM	≦0.06	≦0.06	≦0.06
CMX	≦0.06	≦0.06	≦0.06
CPR	≦0.06	≦0.06	≦0.06
FRPM	≦0.06	≦0.06	≦0.06
CAM	≦0.06 to 128	≦0.06	>128
AZM	≦0.06 to 128	0.125	>128
LVFX	0.5 to 16	1	2
GRNX	≦0.06 to 2	0.125	0.25
STFX	≦0.06 to 0.25	≦0.06	0.125
TFLX	≦0.06 to 32	0.25	1
MINO	80.125 to 16	0.25	16

Antibiotics except for MINO and LVFX revealed good MICs.

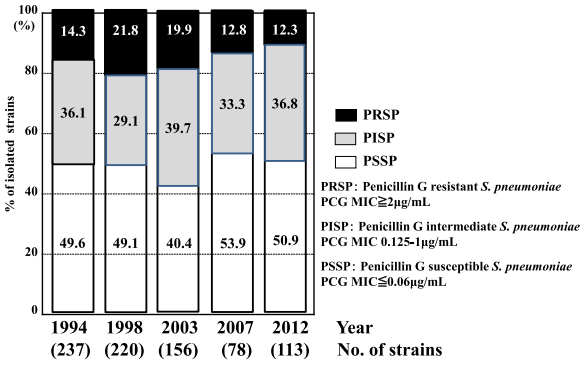

Fig. 2. Expression of isolated penicillin intermediate/resistant *S. pneumoniae* over the past 18 years. PRSP expression decreased gradually, while PISP and PSSP expressions have remained constant.

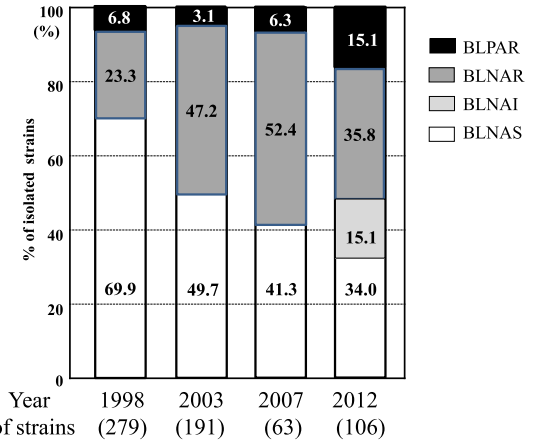

Fig. 3. Expression of isolated penicillin resistant *H. influenzae* over the past 18 years. Before 2007: BLPAR: β-lactamase producing ABPC resistant strain; BLNAR: ABPC MIC >2 μg/mL; BLNAS: ABPC MIC <1 μg/mL. On 2012: BLPAR: β-lactamase producing ABPC resistant strain; BLNAR: ABPC MIC >4 μg/mL; BLNAI: ABPC MIC = 2 μg/mL; BLNAS: ABPC MIC <1 μg/mL. BLPAR: β-lactamase producing ABPC resistant; BLNAR: β-lactamase negative ABPC resistant; BLNAI: β-lactamase negative ABPC-intermediate; BLNAS: β-lactamase negative ABPC susceptible.

ineffective by analysis of MIC90. Almost all antimicrobial drugs other than the ones mentioned above showed high susceptibility, and MIC90 values for all β-lactams were 0.06 μg/mL or below. The MIC90 of LVFX was worse; however, GRNX and STFX were better.

3.2.3. *S. pneumoniae*

Drug susceptibility pattern was measured in 113 out of the 114 isolated *S. pneumoniae* strains. In this surveillance, PRSP and PISP accounted for 12.3% and 36.8% (total 49.1%) of the bacterial strains, as shown in Fig. 2. We observed that the rate of resistant bacteria increased with a decrease in age. For infants under 1 year of age, 77.8% of the observed bacteria were resistant. Among children 5 years of age and under, we observed an average 58.5% of resistant bacteria, with a drop in the rate to 37.5% in patients 6 years and older. Table 9 shows a comparison of the MIC90 values of 18 major drugs against 113 strains of *S. pneumoniae* observed to those seen in 78 strains of a former surveillance in 2007. Among the oral drugs, TBPM showed a MIC ≤0.06 μg/mL, followed by TFLX, CDTR, and FRPM, which expressed MIC90 of 0.25, 0.5, and 0.5 μg/mL, respectively. MIC90 of penicillins and cephems were ≥1 μg/mL, with the susceptibility decreasing with the increase in resistant bacteria. Susceptibility of macrolides showed a noticeable decline. Many highly-resistant bacteria were observed, and neither CAM nor AZM is recommended for use. Among the injection drugs, PAPM showed the best result, with a MIC90 value of 0.25 μg/mL. VCM also showed a good result.

3.2.4. *H. influenzae*

As demonstrated in Fig. 3 and Table 10, we measured the drug susceptibility pattern of 106 out of 107 isolated *H. influenzae* strains. The rate of occurrence of each group with respect to the whole was 15.1%, 35.8%, 15.1%, and 34% for BLPAR, BLNAR, BLNAI, and BLNAS, respectively. The incidence of BLPAR infections has increased in recent years. BLPACR (β-lactamase positive clavulanic acid/amoxicillin resistant strains of *H. influenzae*) was observed to account for 5 strains (4.7%) out of the total. Resistant strains of *H. influenzae* were frequently detected, especially among children (≤2 years of age), accounting for 71.4%. Table 10 compares the MIC90 values of 18 major drugs against 106 and 63 strains of *H. influenzae* in this surveillance and a 2007 surveillance, respectively. MIC90 values for ABPC and AMPC were 32 μg/mL, and that for a compound drug with β-lactamase antagonist was 8 μg/mL. Those showed worse susceptibility. MIC90 for FRPM was 4 μg/mL, which showed also worse susceptibility. MIC90 values were 0.5 μg/mL for CDTR, CMX, and CTRX, 1 μg/mL for TBPM, and 0.5 μg/mL for MEPM, all antibiotics showing comparatively good susceptibility. The MIC90 values for quinolones were ≤0.06 μg/mL.

3.2.5. *M. catarrhalis*

As seen in Fig. 4, resistant strains account for nearly all of the strains (53; 96.4%), while only 2 susceptible strains (3.6%) were

Table 9
MIC90 of antibiotics against S. pneumoniae as seen in 2007 and 2012 (78 and 113 strains).

Antibiotics	MIC90 2007 78	MIC90 2012 113	Antibiotics	MIC90 2007 78	MIC90 2012 113
PCG	2	2	TBPM	–	≦0.06
AMPC	2	2	PAPM	0.125	0.25
CVA/AMPC	2	2	MEPM	0.5	0.5
SBT/ABPC	4	4	GRNX	≦0.06	≦0.06
FRPM	0.5	0.5	LVFX	2	2
CDTR	1	0.5	TFLX	0.25	0.25
CFPN	1	1	CAM	128	>128
CMX	0.25	0.5	AZM	32	>128
CTRX	0.5	1	VCM	–	0.25

Antibiotics except for penicillins and macrolides revealed good MICs on the whole.

Table 10
MIC90 of antibiotics against H. influenzae as seen in 2007 & 2012 (63 and 106 strains).

Anitibiotics	MIC90 2007 63	MIC90 2012[a] 106	Anitibiotics	MIC90 2007 63	MIC90 2012[a] 106
ABPC	128	32	CTRX	0.25	0.5
AMPC	128	32	TBPM	–	1
CVA/AMPC	16	8	MEPM	0.5	0.5
SBT/ABPC	16	8	GRNX	< 0.06	< 0.06
FRPM	8	4	LVFX	< 0.06	< 0.06
PIPC	32	8	TFLX	< 0.06	< 0.06
[a]TAZ/PIPC	–	0.125	CAM	16	8
CDTR	0.5	0.5	AZM	4	2
CFPN	4	2	MINO	0.5	1
CMX	0.5	0.5			

TAZ/PIPC, CDTR, CMX, CTRX, MEPM and quinolones revealed better MICs on the whole.
Concentration of TAZ was fixed to 4μ/mL throughout in this surveillance.
[a] Concentration of TAZ was fixed to 4μ/mL throughout.

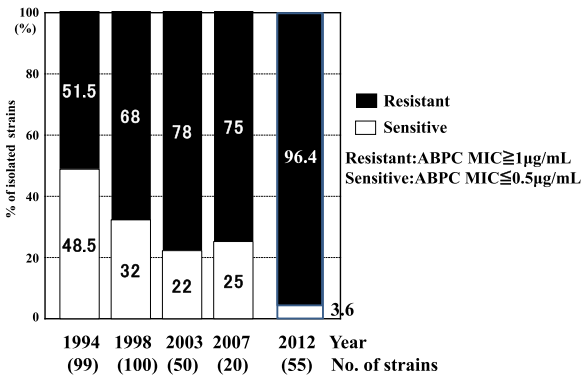

Fig. 4. Expression of isolated ABPC resistant *M. catarrhalis* over the past 18 years. Resistant strains have been increasing up to 96.4%, 2012. All 55 strains isolated from this surveillance produced β-lactamase.

observed. In this surveillance, all 55 strains produced β-lactamase. Table 11 compares the MIC90 values of 18 drugs against 55 and 20 strains of *M. catarrhalis* seen in this surveillance and a former surveillance (2007), respectively. β-lactamase inhibitor compounds (SBT/ABPC and CVA/AMPC) were observed to hold good susceptibility. FRPM and cephems also showed relatively good MIC90 values, ranging from 1 to 2 μg/mL. Other drugs also showed favorable MIC90 values.

3.2.6. P. aeruginosa

MIC50 and MIC90 values of 11 antimicrobial drugs toward 15 strains of *P. aeruginosa* are listed in Table 12. The MIC90 values for TFLX, PUFX, and STFX, ranged from 0.5 to 1 μg/mL. Among the injection drugs, MIC90 value for DRPM was relatively favorable, at 1 μg/mL.

4. Discussion

4.1. Frequency and transition of detected bacteria from major diseases

This surveillance has investigated both acute and chronic diseases (otitis media and chronic sinusitis). The frequency of detected bacteria and susceptibility toward antimicrobial drugs was compared with those of past studies. Therefore, we have identified various changes in bacterial manifestation.

We observed a decrease in the detection rate of *S. aureus* in all diseases. *S. Aureus* and *Staphylococcus* spp. (Coagulase-negative

Table 11
MIC90 of antibiotics against *M. catarrhalis* as seen in 2007 & 2012 (20 and 55 strains).

Antibiotics	MIC90 2007	MIC90 2012	Anitibiotics	MIC90 2007	MIC90 2012
	20	55		20	55
ABPC	8	16	CMX	1	1
AMPC	8	8	CTRX	–	< 0.06
CVA/AMPC	0.25	0.5	MEPM	–	< 0.06
SBT/ABPC	0.25	0.25	LVFX	< 0.06	0.125
FRPM	0.5	1	TFLX	< 0.06	< 0.06
PIPC	8	–	GRNX	< 0.03	< 0.06
[a]TAZ/PIPC	–	< 0.06	CAM	0.25	0.25
CDTR	–	1	AZM	< 0.06	0.125
CFPN	0.5	1	MINO	–	0.25
CFTM	2	2			

Antibiotics except for ABPC and AMPC revealed good MICs on the whole.
Concentration of TAZ was fixed to 4μ/mL throughout in this surveillance.
[a] Concentration of TAZ was fixed to 4μ/mL throughout.

Table 12
MIC 50 and MIC 90 values of antibiotics against *P. aeruginosa* (15 strains).

Antibiotics	Total		
	Range	MIC50	MIC90
PIPC	1 to 16	4	8
[a]TAZ/PIPC	0.5 to 16	4	8
MEPM	≦0.06 to 2	0.25	2
DRPM	≦0.06 to 1	0.25	1
TFLX	0.125 to 1	0.5	1
LVFX	0.25 to 4	1	2
PUFX	≦0.06 to 2	0.125	1
STFX	≦0.06 to 0.5	0.25	0.5
MFLX	1 to 4	2	4
AMK	1 to 8	2	4
FOM	4 to > 256	64	>256

The MIC90 values for DRPM, TFLX, PUFX, and STFX, ranged from 0.5 to 1 μg/mL.
Concentration of TAZ was fixed to 4μ/mL throughout in this surveillance.
[a] Concentration of TAZ was fixed to 4μ/mL throughout.

Staphylococcus (CNS)) were frequently isolated in otolaryngological diseases; however, these are indigenous bacteria often contaminating the sample. The sampling precision has improved in this surveillance due to the improvement in awareness regarding sampling at all facilities. This may have helped reduce the contamination of samples with *S. aureus* and CNS.

The rates of detection of *S. pneumoniae*, *H. influenzae,* and *M. catarrhalis* in acute otitis media have increased, seen in over 75% of children (≤15 years), and in approximately 80% of the samples from young patients. The treatment of acute otitis media of infants should be initiated with a focus on the three abovementioned strains. Acute otitis media develops when bacteria from nasopharynx are infected through eustachian tube. In this surveillance program, no bacteria were detected in 24% of acute otitis media cases. However, if *S. pneumoniae*, *H. influenzae* or *S. pyogenes* was detected in the sample collected from the nasopharynx, they can be asserted, based on former research [4], to be the pathogen of otitis media with a probability > 80%.

No substantial changes have been observed in the detected bacteria in chronic otitis media over the last 14 years. *S. aureus* and *P. aeruginosa* were detected at rates of 38.5% and 6.2%, respectively. In this surveillance, fungi and gram-negative bacteria accounted for 48.5% of the total isolated bacteria. *S. aureus* plays an important role in chronic otolaryngological diseases.

S. pneumoniae, *H. influenzae,* and *M. catarrhalis* were the three most important nasal sinusitis pathogens. Though detected in very few children, *S. pyogenes* is assumed to be one of the most important pathogens. Anaerobic bacteria were detected at a ratio of 12.6%. The involvement of anaerobic bacteria in sinusitis was taken into account in an anatomical disease environment. *S. aureus* was frequently detected in seniors, *S. pneumoniae* and *M. catarrhalis* in a relatively wider range of ages, and *H. influenzae* in children and young people. Anaerobic bacteria were detected in very few patients (≤19 years).

S. pneumoniae and *H. influenzae*, two of the major pathogens of acute upper respiratory infection, were each detected at a rate of 11% in chronic sinusitis, showing only a small annual change over the last 20 years. *M. catarrhalis* and *S. pyogenes* were detected in a few cases with acute exacerbation of disease. *S. aureus* expression showed an increase (19.8%) compared to that seen in a previous study, suggesting its importance in chronic infectious diseases. Anaerobic bacteria expression was relatively high (10.9%), warranting further studies.

Due to the effects of the improvement in detection precision, isolation frequency of *Streptococcus* spp. increased significantly in acute tonsillitis cases. Other *Streprtococcus* spp. accounted for 43.7% of the total bacteria isolated. Many of these were α-*Streptococcus* spp.,

normal oropharyngeal bacterial flora, and may have been detected as the true pathogen. However, in general, virus and atypical pathogens not detected by regular bacteriological examinations, could be the true causative microorganisms. Furthermore, the detection rate of *S. pyogenes* was high (26.7%). Therefore, we conclude that *S. pyogenes* is the most important pathogen causing acute tonsillitis.

Anaerobic bacteria showed a high isolation frequency of 58.4% in peritonsillar abscesses. This could be due to the improvements in conditions such as sample collection, transport medium, and isolation culture, used for the detection of anaerobic bacteria. In another report [5], isolation frequency of anaerobic bacteria in peritonsillar abscess patients accounted for approximately 60%, suggesting that the results from recent years reflect a relatively unchanged scenario.

4.2. Resistance of major detected bacterial strains

The susceptibility of some detected bacteria against antimicrobial drugs were analyzed. The detection frequency of MRSA has shown a constant increase and decrease since 1994, followed by an increasing trend following the 2003 surveillance, as seen in Fig. 1. Therefore, it is important to pay close attention to future trends. The MIC of anti-MRSA drugs against *S. aureus* (Table 7) showed a relatively good antimicrobial activity of quinolones against the same.

We propose that any drugs, excluding CAM, AZM, MINO, and LVFX can be used against *S. pyogenes* (Table 8).

The biggest issue that we currently face is the increase in resistant *S. pneumoniae* and *H. influenzae,* based on the results of the surveillances conducted thus far. *S. pneumoniae* and *H. influenzae* are the most important pathogens for acute otitis media and acute sinusitis. An increase in the drug resistance could largely affect treatment strategies. These strains have a high isolation frequency of resistant bacteria at young ages. This strongly implies that the exchange of resistant bacteria among infants, especially grouped infants attending nursery schools and kindergartens, is a source of infection [13].

As shown in Fig. 2, the ratio of resistant *S. pneumoniae* has fluctuated between 46.1% and 59.6% in the previous nationwide surveillances, and the expression of PISP has seen repeated increase and decrease. However, the ratio of PRSP has been continuously decreasing since the second surveillance in 1998. One of the reasons could be the increased awareness towards selection of penicillins, centering on AMPC as the first-choice drug. This may also may be due to the inhibitory effect of the 7-valent vaccine for *S. pneumoniae* (Prevenar R) introduced in February 2010. Furthermore, the vaccine was altered to Prevenar 13 R in November 2013, and is currently designated as a routine vaccination. Since further inhibitory effect is expected in the future, future trends in bacterial detection should be carefully monitored. Strains resistant to penicillin, macrolides, and quinones have increased in recent years [14–16]. Therefore, there are only a few oral antimicrobial drugs, which can be used in the treatment of *S. pneumoniae*. The first-choice drug for cases displaying moderate level infection (or higher) should be AMPC, AMPC/CVA, or CDTR-PI, as mentioned earlier. These drugs do not result in sufficient concentration in the blood on a normal dosage. Therefore, for AMPC, a high dose of 60–90 mg/kg should be used. Increased dose administration is also recommended for CDTR-PI. The MIC90 (0.5 μg/mL) concentration of FRPM is expected to be effective against *S. pneumoniae* and its resistant bacteria, and could be considered as one of the treatment options. TBPM-PI (the world's first oral carbapenem for children available since August 2009), TFLX (oral respiratory quinolone for children with high antimicrobial activity toward *S. pneumoniae*), and GRNX, and MFLX (for adults) are recommended as second-choice drugs, in case of resistant bacteria.

Resistant strains for *H. influenzae*, has been increasing over the past 14 years, and further attention must be paid to future trends. 3.1% of BLPAR (survey before last), 6.3% (in the last survey), and 15.1% in this surveillance, showing a gradual increase. Furthermore, the recent increase in detection ratio of BLPACR among BLPAR, Kiyama et al. [17] reported that the ratio increased from 0.7% in 2000 to 2.6% in 2006. Likewise, Sugawara et al. [18] have reported that the ratio increased from 1.5% in 2006 to 6.7% in 2009. Our data shows that BLPACR accounted for 3 strains (4.8%) out of 63 strains in surveillance in 2007, and 5 strains (4.7%) out of 106 strains in our surveillance. Although almost no change has been observed, problems may arise in the future, and due caution must be exercised. In recent years, various guidelines concerning this field have suggested a limited use of recommended antimicrobial drugs, frequently recommending CVA/AMPC as the first- or second-choice drug. This may be the cause of the increase in BLPACR; and therefore warrants further inspection. MIC90 values of the drugs are shown in Table 10. Penicillins, which we recommend as the first-choice drug is not effective at normal dosages. Increased dosages of CDTR-PI and TBPM-PI could make them more effective. Considering the recent increase in BLPACR, FRPM should be considered as one of the treatment options. AZM could be used when other drugs are ineffective, with reference to the susceptibility. New quinolones are strong and effective even against resistant bacteria.

All of the strains of *M. catarrhalis* from this surveillance produce β-lactamase, and are ABPC resistant. Although the bacteria itself is not very phlogogenic, with other pathogens existing together, β-lactams are inactivated by the β-lactamase produced by this bacteria, and the pathogens will be rampant. This is why *M. catarrhalis* is called an indirect pathogen. However, as shown in Table 11, this bacteria can be cured without difficulty if drugs stable in the presence of β-lactamase are chosen.

For *P. aeruginosa*, new quinolones are effective as oral antimicrobial drugs. Its susceptibility has improved than that seen in past surveillances, especially in PUFX and STFX, which showed relatively a good MIC90 value of 0.5 μg/mL. Nevertheless, the numbers of strains gathered in the last and this surveillances are only 14 and 15, respectively. Though the susceptibility is clearly improving in numbers, the precision of this value should be further investigated and evaluated. Among the 15 strains gathered in this surveillance, MBL-producing strains have not been detected.

5. Conclusions

The results of the nationwide Three Academic Societies Joint Antimicrobial Susceptibility Surveillance on otolaryngological infections are reported as above. In this surveillance, we have observed an increase in the manifestation of resistant bacteria, such as MRSA, PRSP, PISP, BLNAR, BLPAR, and BLPACR. As mentioned before, we have been unable to comprehensively treat these infections, prompting the need for the proper usage of antimicrobial drugs.

Conflict of interest

Kenji Suzuki has received speaker's honorarium from Taisho Toyama Pharm. Co., Ltd., Meiji Seika Pharma Co., Ltd., Maruho Co., Ltd., Dainippon Sumitomo Pharm. Co., Ltd, and Pfizer Japan Inc. Akira Watanabe has received speaker's honorarium from MSD Japan, Glaxo SmithKline K.K., Shionogi & Co. Ltd., Daiichi-Sankyo, Taisho Toyama Pharmaceutical and Pfizer Japan Inc.; grant support from Kyorin Pharmaceutical, Shionogi & Co. Ltd., Taisho Pharmaceutical, Toyama Chemical Co. Ltd., Daiichi-Sankyo, Dainippon Sumitomo Pharma, Taiho Pharma and Meiji Seika Pharma. Junichi Kadota has received speaker's honorarium from Taisho Toyama

Pharmaceutical Co.,Ltd., Pfizer Japan Inc., MSD K.K., Kyorin Pharmaceutical Co.,Ltd., Daiichi Sankyo Co., Ltd., Glaxo SmithKline K.K., payments for a manuscript drafting and edting from Nankodo Co., Ltd. and donation from Astellas Pharma Inc. Mitsuo Kaku has received speaker's honorarium from Taisho Toyama Pharmaceutical Co.,Ltd., Shionogi & Co., Ltd., Pfizer Japan Inc. and Sumitomo Dainippon Pharma Co., Ltd. and donation from Astellas Pharma Inc. Satoshi Iwata has received speaker's honorarium from Astellas Pharma Inc., Pfizer Japan Inc., Taisho Toyama Pharmaceutical Co.,Ltd., MSD K.K., Meiji Seika Pharma Co., Ltd., Daiichi Sankyo Co., Ltd. and Japan Vaccine Co., Ltd., donation from Taisho Toyama Pharmaceutical Co.,Ltd. and supported, in part, by a fund from Nikon Corporation. Kyoichi Totsuka is a consultant to Toyama Chemical Co. Ltd. Hideaki Hanaki is a member of a laboratory endowed chair from Kohjin Bio Co., Ltd.

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