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Note

Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* clinical isolates from children with acute otitis media in Japan from 2014 to 2017

Kosuke Nagai [a, 1], Osamu Kimura [b, 1], Hisanori Domon [a, c], Tomoki Maekawa [a, c, d], Daisuke Yonezawa [c, e], Yutaka Terao [a, c, *]

[a] Division of Microbiology and Infectious Diseases, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[b] Shiokaze Clinic, Niigata, Japan
[c] Research Centre for Advanced Oral Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[d] Division of Periodontology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[e] Division of Oral Science for Health Promotion, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

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ABSTRACT

Increase in antimicrobial resistance (AMR) among pathogenic bacteria is a serious threat to public health. Surveillance studies to monitor shifting trends in resistance are important and guide the selection of appropriate antimicrobial agents for a particular organism. Furthermore, these studies help in dissemination of accurate information regarding AMR to the public. In this study, we investigated the antimicrobial susceptibility patterns of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* clinical isolates from outpatient children with acute otitis media in Japan from 2014 to 2017. A total of 8693 strains (2415 of *S. pneumoniae*, 3657 of *H. influenzae*, and 2621 of *M. catarrhalis)* were clinically isolated, and their antimicrobial susceptibilities to benzylpenicillin (PCG), ampicillin (ABPC), amoxicillin-clavulanic (AMPC/CVA), azithromycin (AZM), ceftriaxone (CTRX), and levofloxacin (LVFX) were investigated. Based on the minimum inhibitory concentration (MIC) breakpoints, the average proportion of *S. pneumoniae* isolates non-susceptible to PCG and AZM was 38.2% and 82.0% respectively. The average proportion of *H. influenzae* isolates non-susceptible to ABPC, CVA/AMPC, and CTRX was 61.9%, 43.5%, and 49.4%, respectively. The high prevalence of these resistant organisms is attributed to frequent use of antibiotic agents in Japan. Moreover, the proportion of LVFX-non-susceptible *H. influenzae* isolates increased in this four-year study. Here, we report updates regarding the AMR trends amongst the major pathogens that cause acute otitis media in Japan. Continuing surveillance of antimicrobial susceptibility and application of control measures against further transmission are required to decrease the emergence of resistant strains.

Antimicrobial resistance (AMR) is a global public health concern. The development of AMR is accelerated by the widespread use of antibiotics in primary health care. Therefore, national strategies have been adopted by many countries to foster the judicious prescription and consumption of antibiotics [1]. In Japan, the government has adopted an “Action Plan” to reduce the use of antibiotics, especially oral cephalosporins, quinolones, and macrolides, to two-thirds of the current level by 2020, in an effort to combat AMR [2].

AMR has become a problem in patients with acute otitis media, because their treatment relies largely on the administration of antibiotics. The most common bacteria associated with acute otitis

Abbreviations: ABPC, ampicillin; AMR, antimicrobial resistance; AMPC/CVA, amoxicillin-clavulanic; AZM, azithromycin; CI, confidence intervals; CLSI, Clinical Laboratory Standards Institute; CTRX, ceftriaxone; EUCAST, European Committee on Antimicrobial Susceptibility Testing; LVFX, levofloxacin; MIC, minimum inhibitory concentration; TFLX, tosufloxacin; PCG, benzylpenicillin.

* Corresponding author. Division of Microbiology and Infectious Diseases, Niigata University Graduate School of Medical and Dental Sciences, 2-5274, Gakkocho-dori, Chuo-ku, Niigata-shi, Niigata, 951-8514, Japan.
E-mail address: terao@dent.niigata-u.ac.jp (Y. Terao).

[1] Contributed equally as first authors to this work.

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media are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* [3]. The increase in AMR of these three isolates has complicated the selection of antibiotics for their treatment. Moreover, antimicrobial resistance among these three organisms varies from region to region. Therefore, it is important to understand the AMR tendency at a regional level.

A surveillance study of the susceptibilities to antibiotics of common pathogens that cause acute otitis media is valuable to guide empirical therapeutic choices and to influence prescription habits. In this study, we investigated the antimicrobial susceptibility patterns of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* clinical isolates from children with acute otitis media in Japan from 2014 to 2017.

All samples were collected with a sterile swab from the nasopharynx of outpatient children (0–15 years old, no duplications), diagnosed with acute otitis media at the Shiokaze Clinic (Niigata, Japan) from 2014 to 2017. Acute otitis media was diagnosed by one otolaryngologist when all of the following were present: acute onset of signs and symptoms including fever, otalgia, and/or irritability; the presence of middle ear fluid; and symptoms of middle ear inflammation (tympanic membrane erythema or otalgia). The specimens were immediately sent to the Kotobiken Medical Laboratories Niigata Branch (Niigata, Japan) and then cultured on Columbia agar with 5% sheep blood, Brucella agar with 5% horse blood, and Chocolate agar plates with bacitracin (Becton Dickinson, Franklin Lakes, NJ, USA). The plates were incubated at 36 °C in 95% air and 5% CO_2 for up to 48 h. Identification was performed by conventional microbiological methods as follows: colony morphology, growth on Columbia agar, susceptibility to optochin, and bile solubility for *S. pneumoniae*; growth on Chocolate agar with bacitracin, colony morphology and non-hemolytic reaction on horse blood agar, and the requirement for X (hemin) and V (nicotinamide adenine dinucleotide) factors for *H. influenzae*; and colony morphology, positive oxidase, and DNase tests for *M. catarrhalis*. A total of 8693 strains (2415 of *S. pneumoniae*, 3657 of *H. influenzae*, and 2621 of *M. catarrhalis*) were clinically isolated.

Antimicrobial susceptibility testing was performed by a broth microdilution method using dry plate Eiken (Eiken Chemical, Tokyo, Japan), according to the Clinical Laboratory Standards Institute (CLSI) guidelines [4]. In this study, the following antibiotics were tested over a final concentration range from 0.06 to 8 μg/ml: benzylpenicillin (PCG), ampicillin (ABPC), amoxicillin-clavulanic (AMPC/CVA), azithromycin (AZM), ceftriaxone (CTRX), and levofloxacin (LVFX). The minimum inhibitory concentration (MIC) value was defined as the lowest concentration at which there was prominent or complete inhibition of growth, compared to the drug-free controls. All the isolates were classified as susceptible or non-susceptible to antibiotics, according to their MIC breakpoints. The MIC breakpoints for resistance were used based on the CLSI recommendations [4]. When CLSI breakpoints were not available, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used [5].

The antimicrobial susceptibilities of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* are shown in Tables 1–3, respectively. In this study, the average proportion of *S. pneumoniae* isolates non-susceptible to PCG was 38.2%. The proportion of PCG-non-susceptible *S. pneumoniae* isolates decreased compared with that reported in a previous surveillance study [6]. It has been reported that the proportion of PCG-non-susceptible *S. pneumoniae* isolates is decreasing at an annual rate of approximately 2% in Japan, because the Japanese government has promoted the use of oral cephalosporin and other antimicrobials for *S. pneumoniae* [2]. Consistent with a previous study demonstrating that over 70% of *S. pneumoniae* clinical isolates were resistant to macrolides in Asian countries [7], the proportion of AZM-non-susceptible *S. pneumoniae* isolates was 82.0%. The widespread use of macrolides in clinical practice and the clonal spread of macrolide-resistant strains are the major reasons for the high prevalence of

Table 1
The proportion of antimicrobial susceptibility of *S. pneumoniae*.

S. pneumoniae	Year	Susceptible		Non-susceptible	
		n	% (95% CI)	n	% (95% CI)
PCG	2014	393	67.1 (63.2–70.7)	193	32.9 (29.3–36.8)
	2015	431	59.5 (55.9–63.0)	293	40.5 (37.0–44.1)
	2016	399	59.6 (55.9–63.3)	270	40.4 (36.7–44.1)
	2017	269	61.7 (57.1–66.1)	167	38.3 (33.9–42.9)
	Total	1492	61.8 (59.8–63.7)	923	38.2 (36.3–40.2)
CVA/AMPC	2014	576	98.3 (96.9–99.1)	10	1.7 (0.9–3.1)
	2015	708	97.8 (96.4–98.6)	16	2.2 (1.4–3.6)
	2016	648	96.9 (95.2–97.9)	21	3.1 (2.1–4.8)
	2017	427	97.9 (96.1–98.9)	9	2.1 (1.1–3.9)
	Total	2359	97.7 (97.0–98.2)	56	2.3 (1.8–3.0)
AZM	2014	69	11.8 (9.4–14.6)	517	88.2 (85.4–90.6)
	2015	140	19.3 (16.6–22.4)	584	80.7 (77.6–83.4)
	2016	149	22.3 (19.3–25.6)	520	77.7 (74.4–80.7)
	2017	76	17.4 (14.2–21.3)	360	82.6 (78.7–85.8)
	Total	434	18.0 (16.5–19.6)	1981	82.0 (80.4–83.5)
CTRX	2014	580	99.0 (97.8–99.5)	6	1.0 (0.5–2.2)
	2015	721	99.4 (98.6–99.8)	4	0.6 (0.2–1.4)
	2016	663	99.1 (98.1–99.6)	6	0.9 (0.4–1.9)
	2017	431	99.1 (97.7–99.6)	4	0.9 (0.4–2.3)
	Total	2395	99.2 (98.7–99.5)	20	0.8 (0.5–1.3)
LVFX	2014	586	100 (99.3–100)	0	0 (0–0.7)
	2015	725	100 (99.5–100)	0	0 (0–0.5)
	2016	668	99.9 (99.2–100)	1	0.1 (0–0.8)
	2017	434	99.8 (98.7–100)	1	0.2 (0–1.3)
	Total	2413	99.9 (99.7–100)	2	0.1 (0–0.3)

S. pneumoniae isolates were classified as susceptible or non-susceptible to antibiotics, according to the MIC breakpoints. The upper and lower 95% confidence intervals (CI) presented in this study relate to the percentage of susceptibility. AMPC/CVA, amoxicillin-clavulanic; AZM, azithromycin; CTRX, ceftriaxone; LVFX, levofloxacin; MIC, minimum inhibitory concentration; PCG, benzylpenicillin.

Table 2
The proportion of antimicrobial susceptibility of *H. influenzae*.

H. influenzae	Year	Susceptible		Non-susceptible	
		n	% (95% CI)	n	% (95% CI)
ABPC	2014	458	49.4 (46.2–52.6)	469	50.6 (47.4–53.8)
	2015	428	39.1 (36.2–42.0)	668	60.9 (58.0–63.8)
	2016	311	29.6 (26.9–32.4)	741	70.4 (67.6–73.1)
	2017	198	34.0 (30.3–38.0)	384	66.0 (62.0–69.7)
	Total	1395	38.1 (36.6–39.7)	2262	61.9 (60.3–63.4)
CVA/AMPC	2014	703	75.8 (73.0–78.5)	224	24.2 (21.5–2.07)
	2015	625	57.0 (54.1–59.9)	471	43.0 (40.1–45.9)
	2016	436	41.4 (38.5–44.4)	616	58.6 (55.6–61.5)
	2017	304	52.2 (48.2–56.3)	278	47.8 (43.7–51.8)
	Total	2068	56.5 (54.9–58.1)	1589	43.5 (41.9–45.1)
AZM	2014	903	97.4 (96.2–98.3)	24	2.6 (1.7–3.8)
	2015	1066	97.3 (96.1–98.1)	30	2.7 (1.9–3.9)
	2016	1046	99.4 (98.8–99.7)	6	0.6 (0.3–1.2)
	2017	580	99.7 (98.8–99.9)	2	0.3 (0.1–1.2)
	Total	3595	98.3 (97.8–98.7)	62	1.7 (1.3–2.2)
CTRX	2014	571	61.6 (58.4–64.7)	356	38.4 (35.3–41.6)
	2015	554	50.5 (47.6–53.5)	542	49.5 (46.5–52.4)
	2016	434	41.3 (38.3–44.3)	618	58.7 (55.7–61.7)
	2017	292	50.2 (46.1–54.2)	290	49.8 (45.8–53.9)
	Total	1851	50.6 (49.0–52.2)	1806	49.4 (47.8–51.0)
LVFX	2014	925	99.8 (99.2–99.9)	2	0.2 (0.1–0.8)
	2015	1092	99.6 (99.1–99.9)	4	0.4 (0.1–0.9)
	2016	1022	97.1 (96.0–98.0)	30	2.9 (2.0–4.0)
	2017	563	96.7 (95.0–97.9)	19	3.3 (2.1–5.0)
	Total	3602	98.5 (98.0–98.8)	55	1.5 (1.2–2.0)

H. influenzae isolates were classified as susceptible or non-susceptible to antibiotics, according to the MIC breakpoints. The upper and lower 95% confidence intervals (CI) presented in this study relate to the percentage of susceptibility. ABPC, ampicillin; AMPC/CVA, amoxicillin-clavulanic; AZM, azithromycin; CTRX, ceftriaxone; LVFX, levofloxacin; MIC, minimum inhibitory concentration.

Table 3
The proportion of antimicrobial susceptibility of *M. catarrhalis*.

M. catarrhalis	Year	Susceptible		Non-susceptible	
		n	% (95% CI)	n	% (95% CI)
CVA/AMPC	2014	645	100 (99.4–100)	0	0 (0–0.6)
	2015	761	100 (99.5–100)	0	0 (0–0.5)
	2016	709	99.9 (99.2–100)	1	0.1 (0–0.8)
	2017	505	100.0 (99.2–100)	0	0.0 (0–0.8)
	Total	2620	100.0 (99.8–100)	1	0.0 (0–0.2)
AZM	2014	617	95.7 (93.8–97.0)	28	4.3 (3.0–6.2)
	2015	732	96.2 (94.6–97.3)	29	3.8 (2.7–5.4)
	2016	704	99.2 (98.2–99.6)	6	0.8 (0.4–1.8)
	2017	495	98.0 (96.4–98.9)	10	2.0 (1.1–3.6)
	Total	2548	97.2 (96.5–97.8)	73	2.8 (2.2–3.5)
CTRX	2014	629	97.5 (96.0–98.5)	16	2.5 (1.5–4.0)
	2015	735	96.6 (95.0–97.7)	26	3.4 (2.3–5.0)
	2016	684	96.3 (94.7–97.5)	26	3.7 (2.5–5.3)
	2017	495	98.0 (96.4–98.9)	10	2.0 (1.1–3.6)
	Total	2543	97.0 (96.3–97.6)	78	3.0 (2.4–3.7)
LVFX	2014	645	100 (99.4–100)	0	0 (0–0.6)
	2015	761	100 (99.5–100)	0	0 (0–0.5)
	2016	710	100 (99.5–100)	0	0 (0–0.5)
	2017	505	100 (99.2–100)	0	0 (0–0.8)
	Total	2621	100 (99.9–100)	0	0 (0–0.1)

M. catarrhalis isolates were classified as susceptible or non-susceptible to antibiotics, according to the MIC breakpoints. The upper and lower 95% confidence intervals (CI) presented in this study relate to the percentage of susceptibility. AMPC/CVA, amoxicillin-clavulanic; AZM, azithromycin; CTRX, ceftriaxone; LVFX, levofloxacin; MIC, minimum inhibitory concentration.

macrolide resistance in Japan. Therefore, the empirical use of a macrolide alone may not be an appropriate choice for the treatment of acute otitis media, caused by *S. pneumoniae*. The average proportion of *S. pneumoniae* isolates non-susceptible to CVA/AMPC, CTRX, and LVFX was 2.3%, 0.8%, and 0.1% respectively. CVA/AMPC, CTRX, and LVFX demonstrated the same levels of antimicrobial activity against *S. pneumoniae* as that in other developed countries (>90%) [8]. Accordingly, these antibiotics except for LVFX could be as a better choice for the treatment of *S. pneumoniae* infections (LVFX is not approved for children in Japan). From the viewpoint of disease prevention, the 7-valent pneumococcal vaccine (PCV7) was introduced in 2010 and was replaced with the PCV13 in 2013 in Japan. PCVs are effective against antibiotic-non-susceptible *S. pneumoniae* included in these vaccines. However, PCVs reportedly induce serotype replacement and non-vaccine serotypes may increase and become AMR [9]. Therefore, continued surveillance is needed to monitor the trends of *S. pneumoniae* infection.

The average proportion of *H. influenzae* isolates non-susceptible to ABPC, CVA/AMPC, and CTRX was 61.9%, 43.5%, and 49.4%, respectively. The high prevalence of these resistant organisms is attributed to frequent use of these antibiotic agents in Japan. Moreover, the proportion of LVFX-non-susceptible *H. influenzae* isolates increased from 0.2% in 2014 and 0.4% in 2015 to 2.9% in 2016 and 3.3% in 2017. In accordance with the acceptance of oral fluoroquinolone tosufloxacin (TFLX) not only for adults, but also for children, since January 2010 in Japan, many pediatricians and otolaryngologists prescribe TFLX, owing to its clinical effectiveness [10]. Frequent use of TFLX could lead to an increase in fluoroquinolone-resistant *H. influenzae*. Therefore, TFLX should only be used in appropriate cases, after the antimicrobial susceptibility has been examined.

M. catarrhalis, previously considered a harmless commensal organism, has gained recognition as a pathogen over the past three decades and has shown an increased prevalence of β-lactamase production. β-lactamase-positive *M. catarrhalis* isolates have increased rapidly in recent years, and account for more than 80–90% of the isolates in European countries [11], and nearly 98% in Taiwan [12]. In this study, about 95% of the *M. catarrhalis* isolates showed >1 μg/ml MIC for PCG (data not shown). Although Table 3 shows that most of *M. catarrhalis* isolates (95–100%) were susceptible for CVA/AMPC, AZM, CTRX, and LVFX, careful monitoring of the trends in the antibiotic susceptibility of *M. catarrhalis* should be continued.

Our study has the following limitations. First, although recent study reported that the frequency of isolation of β-lactamase-negative ampicillin resistant (BLNAR) strains of *H. influenzae* in Japanese children has been increasing rapidly [13], we did not analyze whether *H. influenzae* isolates produce β-lactamase or not. Therefore, we understand that the further research is needed to determine the current prevalence of BLNAR *H. influenzae*. In addition, we investigated the antimicrobial susceptibility about the clinical isolates from patients with acute otitis media, which isolates could contain normal inhabitant. Therefore, we were unable to reliably determine whether all these samples are causative bacteria of otitis media.

In this four-year study, we report updates on the AMR trends amongst the major pathogens that cause acute otitis media in Japan. Continuing surveillance of the antimicrobial susceptibility and application of control measures against further transmission are required to decrease the emergence of resistant strains.

Conflicts of interest

None of the authors have conflicts of interest associated with this study to report.

Authorship statement

All authors meet the ICMJE authorship criteria.

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