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Note

In vitro neuraminidase inhibitory concentration (IC_{50}) of four neuraminidase inhibitors in the Japanese 2017–18 season: Comparison with the 2010–11 to 2016–17 seasons[☆]

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ABSTRACT

To assess the extent of susceptibility to the four most commonly used neuraminidase inhibitors (NAIs) of the viruses epidemic in the 2017-18 Japanese influenza season, we measured the 50% inhibitory concentration (IC_{50}) for influenza virus isolates from patients and compared them with the results from the 2010–11 to 2016-17 seasons.

Viral isolation was done with specimens obtained prior to treatment, and the type and subtype was determined by RT-PCR using type- and subtype-specific primers. The IC_{50} was determined by a neuraminidase inhibition assay using a fluorescent substrate.

A total of 237 virus isolates, 50 A(H1N1)pdm09, 92 A(H3N2), and 95 B were measured. No A(H1N1)pdm09 with highly reduced sensitivity for oseltamivir was found in the 2017-18 season. No isolates with highly reduced sensitivity to the four NAIs have been found for A(H3N2) or B from the 2010–11 to 2017-18 seasons. The geometric mean IC_{50}s of the four NAIs were quite consistent during the eight studied seasons. These results indicate that the sensitivity to the four commonly used NAIs has been maintained.

Treatment of influenza with the neuraminidase inhibitors (NAIs) oseltamivir phosphate (Tamiflu®, oseltamivir), zanamivir hydrate (Relenza®, zanamivir), peramivir hydrate (Rapiacta®, peramivir), and laninamivir octanoate hydrate (Inavir®, laninamivir) has become common among Japanese primary care providers; thus, the possible emergence of resistant virus to any of these NAIs is of great concern for physicians. A national antiviral resistance surveillance system has been developed by National Institute of Infectious Diseases [1]. Global surveillance of the susceptibility of human influenza virus to NAIs is being continued [2,3]; however, the background of medical conditions, such as antiviral usage may differ in the clinical setting of the countries involved. In this study, we investigate the IC_{50}s of the viruses circulating in the Japanese 2017–2018 season, using virus isolated from influenza patients who visited the clinics participating this study and compare the results with those we have reported in the previous seven influenza seasons, from 2010 to 11 to 2016–17 [4].

This study was approved by the institutional review board of Hara-doi hospital. This study was registered to UMIN (UMIN000030136). A total of 19 clinics participated. Patients were enrolled from December 2017 to April 2018. Specimen for viral isolation were collected with informed consent from patients who showed a positive result with a rapid influenza antigen detection kit. Informed consent was obtained from a parent if the patient was under 19 years.

Nasal aspirates, nasopharyngeal swabs, or self-blown nasal discharge were obtained prior to treatment. Influenza virus isolation was done with Madin-Darby canine kidney (MDCK)- SIAT1 cells that was developed as a modified derivative with an increased expression of a2,6-linked sialic acid receptors [5]. The type and subtype of influenza was determined by RT-PCR using type- and subtype-specific primers [6]. The Yamagata and Victoria lineages of

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Table 1
Geometric mean (GM) IC_{50} for the four neuraminidase inhibitors by season.

Drug	Season	A(H1N1)pdm09			A(H3N2)			B		
		No.	IC_{50} GM, nM	(95% CI)	No.	IC_{50} GM, nM	(95% CI)	No.	IC_{50} GM, nM	(95% CI)
Oseltamivir	2010/11	185	0.86	(0.76, 0.98)	54	0.73	(0.65, 0.82)	30	33.11	(28.78, 38.09)
	2011/12	0	–		283	0.77	(0.75, 0.80)	42	15.28	(13.60, 17.16)
	2012/13	5	0.85	(0.76, 0.95)	316	1.07	(1.04, 1.10)	8	19.07	(15.36, 23.67)
	2013/14	172	0.76	(0.69, 0.83)	49	0.76	(0.72, 0.81)	106	19.50	(18.00, 21.12)
	2014/15	0	–		200	0.77	(0.75, 0.79)	19	19.50	(18.19, 20.90)
	2015/16	210	0.77	(0.71, 0.83)	20	0.86	(0.76, 0.98)	82	15.95	(14.82, 17.16)
	2016/17	6	0.70	(0.59, 0.83)	249	0.70	(0.67, 0.72)	21	16.12	(14.52, 17.89)
	2017/18	50	0.71	(0.69, 0.73)	92	0.74	(0.72, 0.77)	95	11.12	(10.28, 11.96)
Zanamivir	2010/11	185	0.73	(0.69, 0.78)	54	1.64	(1.51, 1.79)	30	11.21	(9.98, 12.61)
	2011/12	0	–		283	1.97	(1.91, 2.04)	42	7.24	(6.72, 7.80)
	2012/13	5	1.37	(0.90, 2.08)	316	2.45	(2.39, 2.52)	8	14.65	(11.14, 19.26)
	2013/14	172	1.17	(1.13, 1.21)	49	2.03	(1.88, 2.20)	106	7.82	(7.42, 8.24)
	2014/15	0	–		200	2.00	(1.94, 2.06)	19	9.71	(9.02, 10.45)
	2015/16	210	1.30	(1.26, 1.35)	20	2.22	(2.07, 2.37)	82	7.15	(6.94, 7.37)
	2016/17	6	1.02	(0.91, 1.15)	249	1.73	(1.69, 1.77)	21	5.46	(5.01, 5.96)
	2017/18	50	1.51	(1.43, 1.59)	92	2.12	(2.06, 2.19)	95	7.51	(7.29, 7.72)
Peramivir	2010/11	185	0.38	(0.34, 0.42)	54	0.66	(0.61, 0.71)	30	3.96	(3.44, 4.55)
	2011/12	0	–		283	0.83	(0.81, 0.85)	42	2.89	(2.69, 3.10)
	2012/13	5	0.76	(0.48, 1.20)	316	0.97	(0.94, 0.99)	8	3.90	(3.26, 4.66)
	2013/14	172	0.44	(0.41, 0.47)	49	0.70	(0.66, 0.74)	106	2.87	(2.78, 2.97)
	2014/15	0	–		200	0.68	(0.66, 0.70)	19	4.21	(3.85, 4.61)
	2015/16	210	0.47	(0.45, 0.50)	20	0.71	(0.61, 0.82)	82	2.38	(2.32, 2.45)
	2016/17	6	0.36	(0.28, 0.47)	249	0.57	(0.55, 0.58)	21	2.04	(1.92, 2.17)
	2017/18	50	0.54	(0.52, 0.57)	92	0.73	(0.71, 0.76)	95	2.67	(2.51, 2.84)
Laninamivir	2010/11	185	1.37	(1.27, 1.47)	54	3.22	(2.91, 3.56)	30	21.25	(19.11, 23.64)
	2011/12	0	–		283	3.48	(3.37, 3.60)	42	15.97	(14.27, 17.87)
	2012/13	5	2.15	(1.92, 2.41)	316	4.69	(4.59, 4.79)	8	21.41	(18.78, 24.40)
	2013/14	172	1.69	(1.64, 1.74)	49	3.22	(2.97, 3.49)	106	14.90	(14.18, 15.65)
	2014/15	0	–		200	3.23	(3.11, 3.35)	19	17.97	(16.47, 19.61)
	2015/16	210	1.85	(1.79, 1.91)	20	3.69	(3.30, 4.13)	82	16.64	(15.86, 17.45)
	2016/17	6	1.40	(1.10, 1.79)	249	2.72	(2.64, 2.81)	21	11.90	(10.62, 13.34)
	2017/18	50	1.90	(1.74, 2.06)	92	3.40	(3.23, 3.57)	95	13.58	(13.07, 14.09)

influenza B virus were discriminated by real-time RT-PCR using specific primers and probes for the hemagglutinin gene [7]. The 50% inhibitory concentration (IC_{50}) of oseltamivir, zanamivir, peramivir, and laninamivir were determined for each isolate by a fluorescence-based neuraminidase inhibition assay [8].

In the 2017–2018 season, 237 specimens were available for analysis; 50 influenza A(H1N1)pdm09, 92 A(H3N2), and 95 B. Except one of Victoria linage, B viruses were all Yamagata lineage. The overall mean age was 26.3 ± 21.4 years. The mean ages of the patients were 22.2 ± 20.2 years, 29.9 ± 22.6 years, and 25.1 ± 20.3 years, for A(H1N1)pdm09, A(H3N2), and B, respectively.

The GMs of the IC_{50} for A(H1N1)pdm09, A(H3N2), and B in the 2017-18 season are listed Table 1. The GM IC_{50}s of all four NAIs were higher in B compare to A(H1N1)pdm09 and A(H3N2), as previously observed, and the difference was statistically significant ($p < 0.001$ for all NAIs, ANOVA). The variability of the extent of the IC_{50} difference by virus type/subtype of these each NAIs may be relevant to that the duration of fever after the first administration of oseltamivir was longer for B patients than A patients [9].

Distribution of the IC_{50} values of the four NAIs in the 2017-18 season are depicted by virus type/subtype using Box and Whisker plot analysis along with the results of the seasons from 2010 to 11 to 2016–17: for A(H1N1)pdm09 in Fig. 1A, for A(H3N2) in Fig. 1B, and for B in Fig. 1C.

No A(H1N1)pdm09 virus with highly reduced sensitivity was observed in the 2017-18 season in this study. Although 1.7% of A(H1N1)pdm09 had highly reduced sensitivity to oseltamivir in the Japanese national surveillance report for the 2017–18 season [1]; it is unlikely that the use of oseltamivir was a driving force in its appearance or the increase of oseltamivir resistant A(H1N1)pdm09 viruses.

No virus with highly reduced sensitivity to any of the four NAIs was detected among A(H3N2) and B isolates through the 2010–11 to 2017–18 seasons of our studies. Several amino acid substitutions related to resistance to NAIs are reported [2,3,10–12]. We reported almost no amino acid change in the neuraminidase catalytic site of the A(H3N2) and B isolates in the seasons from the 2011–12 to 2016–17 [13]. It is unlikely that the use of the four NAIs was a driving force in appearance of NAI resistant viruses.

The distribution of IC_{50} of the four NAIs was similar throughout the 2010–11 to 2017–18 study periods without any apparent trend (Fig. 1). The IC_{50} GMs of the four NAIs in the eight seasons ranged within a two fold difference (Table 1). The IC_{50} of laninamivir was slightly higher than that of the other NAIs especially peramivir, but no significant difference was found. Laninamivir is inhaled one time, which results in a very high concentration in patient's nasopharyngeal area, reaching a level of almost or over 100 times of the IC_{50} of laninamivir for the reference viruses. Peramivir is by drip-infusion, and plasma concentration decreases gradually over time, but the plasma concentration is maintained at a much higher level than the IC_{50} of peramivir. The observed IC_{50} levels of the four NAIs in this study suggest that the widespread NAI usage for the treatment of influenza patients has not affected the sensitivity of the circulating influenza viruses to these commonly used NAIs.

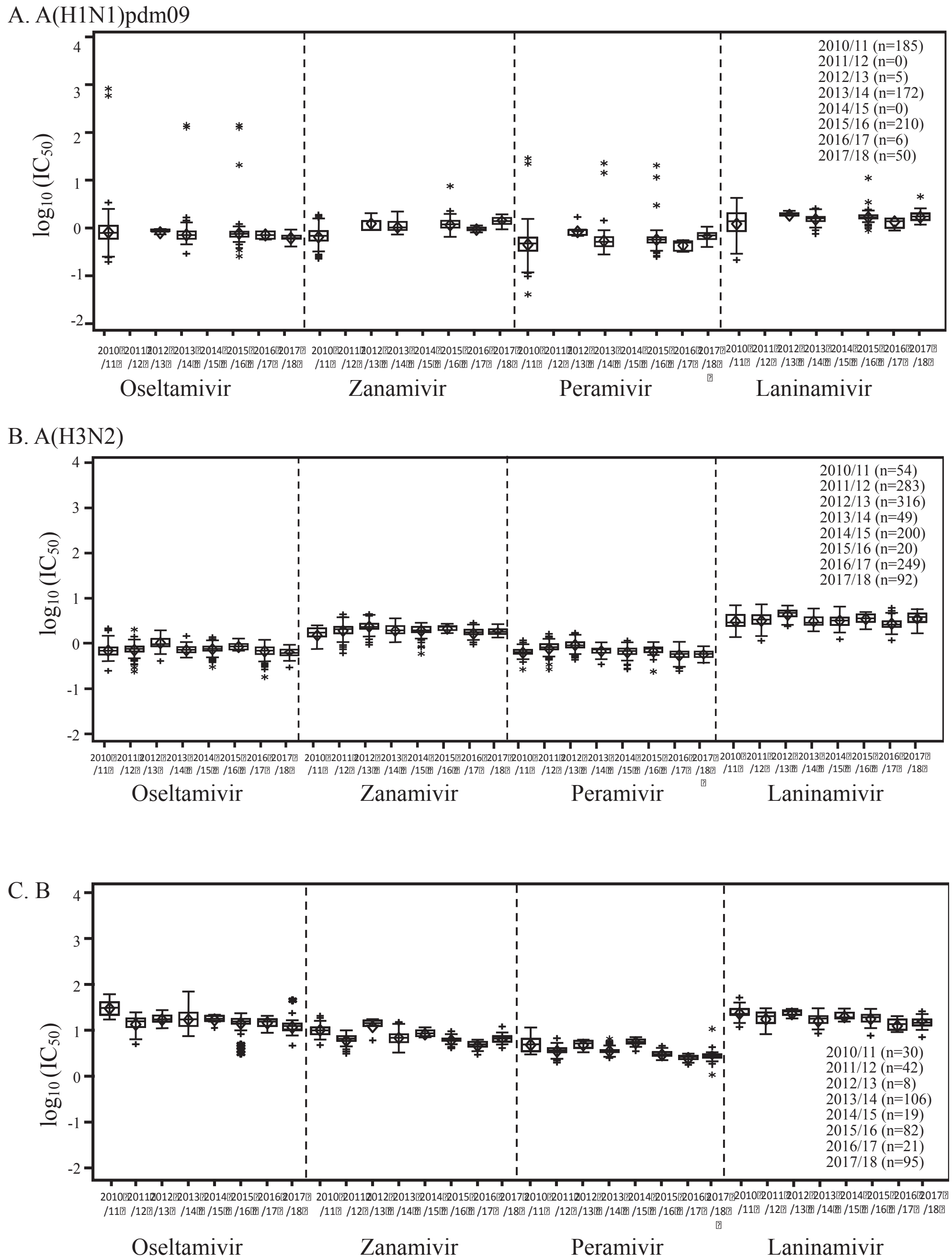

Fig. 1. Box and whisker plot analysis of the IC_{50}s of the four tested NAIs by virus type/subtype. A(H1N1)pdm09 in Fig. 1A, A(H3N2) in Fig. 1B, and B in Fig. 1C. The bottom and top of the box are Q1 and Q3, and the band near the middle of the box is the median. The ends of the whiskers are the lowest datum still within 1.5 interquartile ranges (IQR) of the lower quartile and the highest datum still within 1.5 IQR of the upper quartile. ◊: arithmetic mean. +: 1.5 to 3 interquartile ranges from the box. *: more than 3 interquartile ranges from the box.

Conflicts of interest

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