Antiviral Research 84 (2009) 199–202

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short communication

Amantadine-resistant influenza A viruses isolated in South Korea from 2003 to 2009

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ARTICLE INFO

Article history:
Received 9 April 2009
Received in revised form 17 August 2009
Accepted 21 August 2009

Keywords:
Amantadine resistance
Influenza A virus
Phylogenetic analysis
South Korea

ABSTRACT

To investigate the frequency of amantadine resistance among influenza A viruses isolated in Korea during the 2003–2009 seasons, 369 (16.8%) 2199 A/H1N1 viruses and 780 (14.8%) of 5263 A/H3N2 viruses were randomly selected. The M2 and HA1 genes of each isolate were amplified by reverse transcription-polymerase chain reaction and followed by nucleotide sequencing. The results showed that the resistance rate to amantadine among A/H1N1 viruses increased significantly from 2004–2005 (33.3%) to 2007–2008 (97.8%) and then decreased dramatically in 2008–2009 (1.9%). The A/H1N1 isolates recently detected in 2008–2009 turned amantadine-sensitive containing two new substitutions at specific sites (S141N, G185A) in HA1. Compared with A/H1N1 viruses, the amantadine resistance among the A/H3N2 viruses increased from 2003–2004 (9.7%) to 2005–2006 (96.7%) and decreased in 2006–2007 (57.4%). During 2006–2007, both of amantadine-resistant and -sensitive A/H3N2 viruses co-circulated but clustered in different branches phylogenetically. All of A/H3N2 isolates tested during 2007–2009 appeared to cluster in the same group being resistant to amantadine.

Influenza infects approximately 20% of the world's population and more than half a million individuals die every year of influenza-associated complications (Englund, 2002). Vaccination and antiviral treatments are essential for the prevention and control of influenza infection. With the use of antiviral drugs for the clinical management of influenza, a high prevalence of adamantane resistance in influenza A viruses was observed in both countries where these drugs have been used and where they have not been used (Bright et al., 2005; CDC, 2006; Hayden, 2006; Deyde et al., 2007; Barr et al., 2008; Saito et al., 2008; Nelson et al., 2009).

To investigate the prevalence of amantadine resistance in Korea, we tested 1149 influenza A viruses (369 A/H1N1 and 780 A/H3N2) isolated from throughout the country by the Korean Influenza Surveillance Scheme (KISS). Influenza isolates in this study were collected between October 1, 2003 (2003–2004 season) and December 13, 2008 (2008–2009 season). The matrix gene (339 bp) and HA gene (1179 bp of A/H1 and 1141 bp of A/H3) were amplified by PCR. The sequences were aligned with the MegAlign program (DNASTAR Inc. Software, Madison, WI, USA). We analyzed the amino acid substitutions at positions 26, 27, 30, 31, or 34 within the transmembrane domain of the M2 protein, known to confer resistance to amantadine (Abed et al., 2005). We also phylogenetically analyzed the HA1-coding region (978 bp of A/H1 and 987 bp of A/H3) in both of the influenza A/H1N1 and A/H3N2 viruses. All the sequence of the Korean isolates used in this study was submitted to GenBank (accession nos. FJ794081–FJ794172) and was compared with the reference sequences from GenBank (accession nos. CY031342, CY030230, EU100724, AY289929, EU103822, EU501153, CY034108, CY031795, CY031799, and CY035022).

The resistance to amantadine among the influenza A/H1N1 viruses was 0% (0/1) in 2003–2004, 33.3% (6/18) in 2004–2005, which might be attributable to the relatively small number of viruses isolated. The resistance rates for A/H1N1 viruses increased significantly from 8.5% (11/129) in the 2005–2006 season, 81.1% (60/74) in the 2006–2007 season, to 97.8% (91/93) in the 2007–2008 season. However, the resistance rate for A/H1N1 viruses decreased dramatically to 1.9% (1/54) during the 2008–2009 season. In contrast, the frequency of A/H3N2-resistant viruses was 9.7% (9/93) during the 2003–2004 influenza season, but increased significantly to 51.9% (42/81) during the 2004–2005 influenza season and to 96.7% (89/92) in the 2005–2006 influenza season. The resistance rates increased consistently from 2003 to 2006. However,

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0166-3542/$ – see front matter.
doi:10.1016/j.antiviral.2009.08.006

the resistance rate of A/H3N2 viruses decreased by half to 57.4% (226/394) during the 2006–2007 season and then increased to 100% (109/109) in the 2007–2008 season until December 13, 2008. The frequency of amantadine-resistant H3N2 isolates (100%, 11/11) was much higher than that of amantadine-resistant H1N1 isolates (1.9%, 1/54) in the 2008–2009 season. Of 1149 viruses tested (369 A/H1N1 and 780 H3N2), all of them had the S31N substitution and only two (one in 2005–2006 and one in 2006–2007) had a double V27A/S31N substitution. Our data show that the S31N substitution is the most common mutation for the acquisition of amantadine resistance in influenza viruses. The amantadine resistance identified by sequencing was confirmed by virus yield reduction assay (Bright et al., 2005).

Sequence and phylogenetic analyses of the HA1-coding region showed that amantadine-resistant A/H1N1 viruses during the 2006–2007 and 2007–2008 seasons clustered in clade 2C, represented by A/Hong Kong/2652/2006, and contained substitutions at S26N and R188 M (Fig. 1). However, the recent A/H1N1 isolates collected during 2008–2009 belong to clade 2B and these amantadine-sensitive strains are circulating extensively

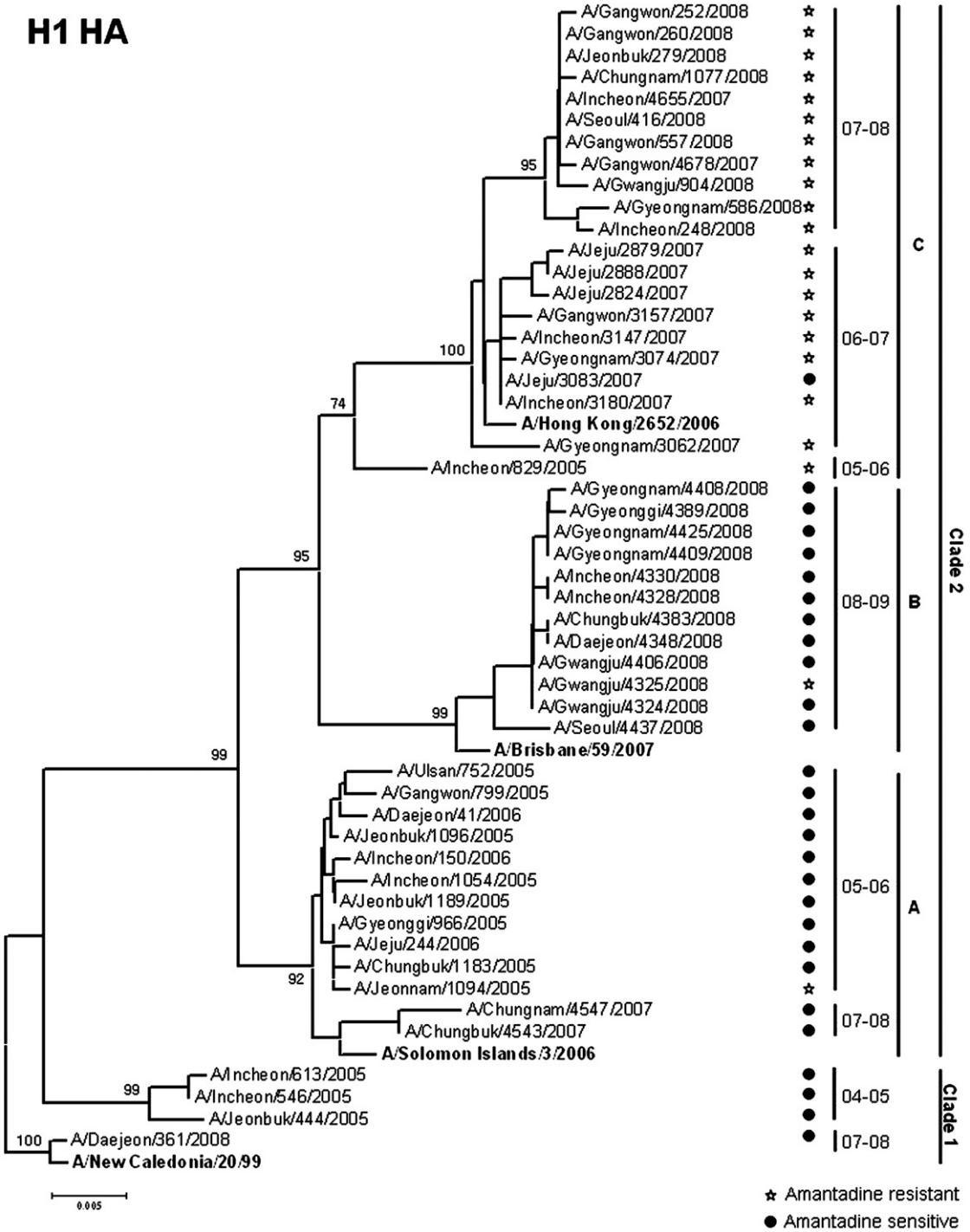

Fig. 1. Phylogenetic tree analysis of the HA1-coding region of the hemagglutinin (HA) gene of human influenza A/H1N1 viruses isolated in Korea during 2003–2009. Bootstrap values are shown for nodes having values >70%. Reference strains are denoted in bold. A star sign and dotted circle symbol indicate amantadine-resistant strains and amantadine-sensitive strains, respectively. Scale bar indicates 5 substitutions per 1000 nucleotides.

at present. The 2008–2009 A/H1N1 isolates were in the same cluster as A/Brisbane/59/2007, which is the 2008–2009 influenza vaccine strain, but differ from A/Brisbane/59/2007 by changes at two new positively selected sites (S141N, G185A) in HA1. These two amino acid changes in sensitive strains occurred at the antigenic sites Ca (residue 141) and near Sb (residue 185). This may have contributed to the extensive spread of A/H1N1 isolates up to the 50th week in the 2008–2009 season. These 2008–2009 A/H1N1 isolates collected during December 2008 showed high homology (98.8–99.4%) at the amino acid level of HA1 to viruses circulating in the first quarter of 2008 in the USA and several European countries (A/Washington/01/2008, A/Bucuresti/229/2008, A/Lisbon/3/2008, A/North Carolina/02/2008, A/New Jersey/10/200/, A/Norway/773/2008), whereas the 2007–2008 A/H1N1 isolates collected in December 2007 showed homology (96.0–96.6%) to viruses circulating in the first quarter of 2008 in the USA and several European countries. It is unclear whether the strains circulating in Korea in the 2008–2009 season were derived from the USA or other European countries. More recent data on the HA sequences of A/H1N1 in other countries, especially in Asia, are required to clarify the epicenters of these viral epidemics.

Amantadine-resistant A/H3N2 viruses with double mutations at S193F and D225N, designated clade N (Saito et al., 2007), appeared at the end of the 2004–2005 season (Fig. 2). During the 2003–2004 and 2004–2005 seasons, amantadine-resistant A/H3N2 viruses contained aspartic acid at residue 225. The amantadine-resistant isolates and amantadine-sensitive isolates during the 2004–2005 season were genetically close to A/Wisconsin/67/2005 and A/California/7/2004, respectively. Two subgroups of A/H3N2

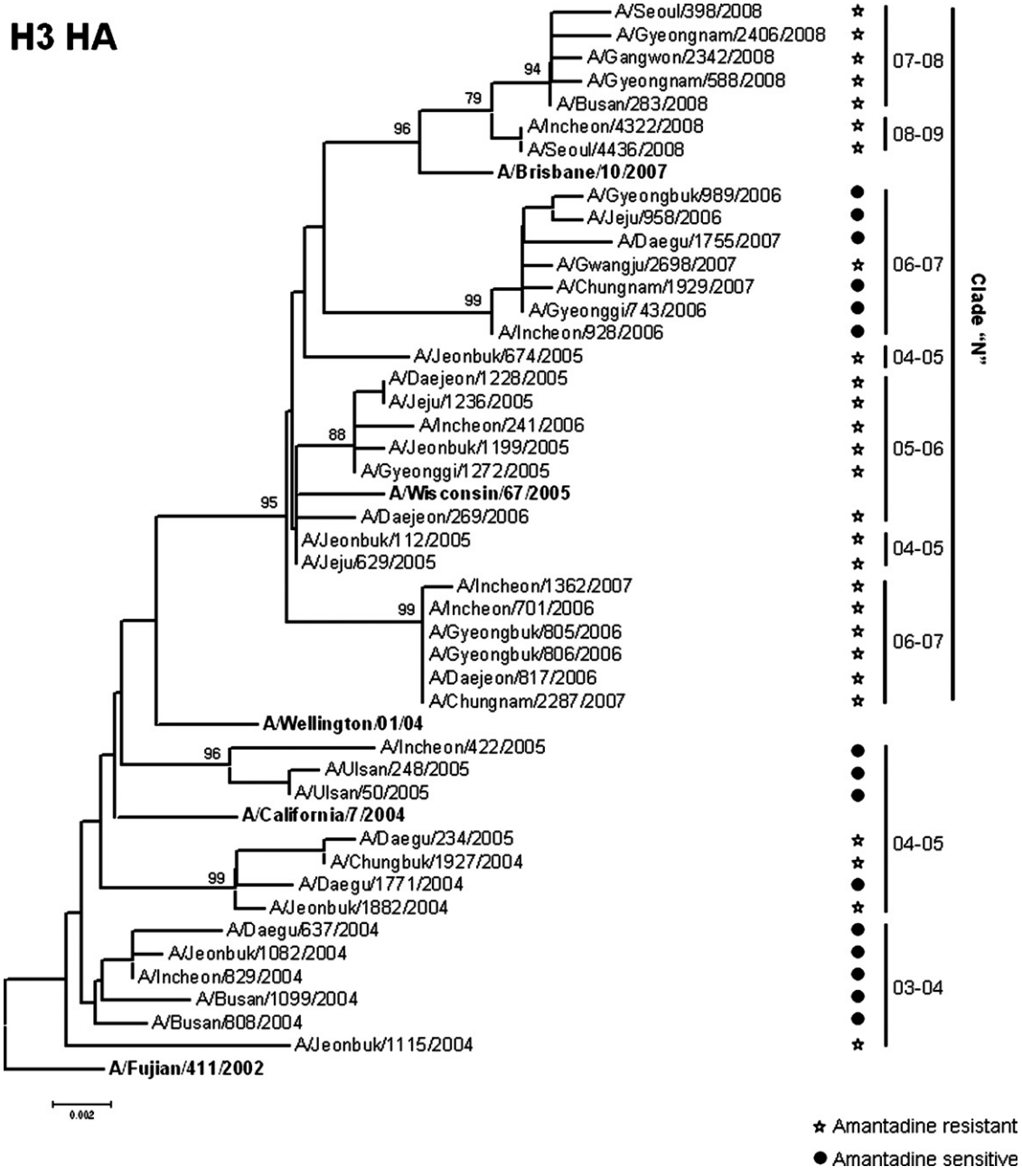

Fig. 2. Phylogenetic tree analysis of the HA1-coding region of the hemagglutinin (HA) gene of human influenza A/H3N2 viruses isolated in Korea during 2003–2009. Bootstrap values are shown for nodes having values >70%. Reference strains are denoted in bold. A star sign and dotted circle symbol indicate amantadine-resistant strains and amantadine-sensitive strains, respectively. Scale bar indicates 2 substitutions per 1000 nucleotides.

were circulating in the 2004–2005 season with resistance rates of 51.9%. Nearly all of the amantadine-resistant and -sensitive viruses in the beginning and middle of the 2004–2005 season had aspartic acid at residue 225 of HA1, whereas all the amantadine-resistant viruses at the end of the 2004–2005 season had a mutation at residue 225, of HA1 from aspartic acid to asparagine (D225N), and were grouped together with A/Wisconsin/67/2005. During the 2006–2007 season, both amantadine-resistant viruses and amantadine-sensitive viruses co-circulated with a resistance rate of 57.4%, but were clustered phylogenitically on different branches. The amantadine-sensitive viruses as well as the amantadine-resistant viruses in 2006–2007 had the amino acid changes S193F and D225N and these amantadine-sensitive N-lineage strains might be due to reassortment event, as reported previously (Furuse et al., 2009). The recent A/H3N2 isolates from 2008–2009 cluster in the same group as the isolates from 2007–2008 and are resistant to amantadine.

In Korea, amantadine has been licensed as an antiviral drug for clinical use since 1974 and has therefore been used for the treatment of influenza A virus infection for more than 30 years. A doctor's prescription is required for its use. However, the Korea Food and Drug Administration recommended restrictions on the use of amantadine as an anti-influenza drug at the beginning of 2006 based on the CDC report (CDC, 2006). Based on the resistance rates of influenza A/H1N1 and A/H3N2 viruses during six seasons, we assume that resistant viruses are circulating without selection pressure from the drug and the resistance rate of the isolates collected each season might depend on the degree of the resistant strains among the circulating viruses. Here, our data provide the information on the adequate use of antiviral drugs in a rapid manner for influenza treatment in Korea through the antiviral resistance monitoring. This study raises concerns about the increasing incidence of amantadine-resistant influenza A viruses circulating in Korea and draws attention to the importance of tracking the emergence and worldwide spread of drug-resistant viruses.

Acknowledgements

This study was supported by the intramural research fund (code#2006-N00252-00) from the Korea Centers for Disease Control and Prevention. We thank Ok Park, Hyekyung Park, Joo-Sun Lee, and Soo-Gin Beak from the Division of Infectious Diseases Surveillance at Korea CDC for the data on the incidence of ILI. We also thank 16 Public Institutes of Health & Environmental Research for diagnosis of influenza virus and sentinel clinic doctors for collecting specimens.

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