Research paper

Incidence of antiviral drug resistance markers among human influenza A viruses in the Eastern Mediterranean Region, 2005–2016

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ABSTRACT

Background: Two classes of antiviral drugs are available for influenza antiviral therapy: the adamantanes and the neuraminidase inhibitors (NAIs). Due to the emergence of adamantane-resistant variants, the use of these drugs has been largely limited in the world. The NAIs became the drugs of choice for treatment of influenza A infections. However, amino acid substitutions in the NA protein might lead to reduced sensitivity to NAIs.
Methods: The frequency and distribution of matrix protein 2 (M2) and neuraminidase (NA) variants which confer resistance to antiviral drugs was investigated in the Eastern Mediterranean Region (EMR) between 2005 and 2016. A total of 314 M2 and 1209 NA protein sequences from influenza A/H1N1, A/H1N1pdm09, A/H3N2, and A/H5N1 available in the public database were analyzed.
Results: Eighty-six percent of the influenza A viruses detected in the EMR were resistant to adamantanes, among which, H3 strains exhibited the highest (95.32%) level of adamantane resistance. Approximately 98.51% (265/269) of influenza A/H1N1 and H3N2 resistant viruses had the S31N substitution in their M2 sequences. The V27A mutation was the only resistance marker found in A/H5N1 viruses and was detected at a frequency of 7.40% among the investigated viruses. Other resistant mutations L26F, A30T, G34E, and L38F were not detected in any of the variants. We found that 2.81% (n = 34) of the detected NA sequences from influenza A viruses possessed at least one NAI-resistant mutation and the vast majority of resistant viruses 79.41% (27/34) bear the H274Y mutation. The frequency of NAI-resistant viruses was 3.29% (24/729) for the H1N1pdm09, 10.64% (5/47) for the seasonal H1N1, and 4.06% (5/123) for H5N1 viruses. None of the H3N2 viruses analyzed during the study period were resistant to NAIs.
Conclusion: Our study reveals the emergence and spread of antiviral drug resistant influenza A viruses in the EMR and emphasizes the importance of continuous surveillance to maintain the effective use of the current antivirals.

1. Introduction

Influenza is a highly contagious respiratory infection with typical symptoms including sudden onset fever, fatigue, sore throat, cough, malaise, and myalgia (Truter, 2012). The majority of influenza infections are self-limiting in low-risk individuals. However, the infection can cause complications like bronchitis, encephalitis, primary viral or secondary bacterial pneumonia leading to hospitalization and occasionally death (Fiore et al., 2010; Short et al., 2013). Replication of the virus in the lower respiratory tract may lead to severe pneumonia, hypoxemia, shock and acute respiratory disease syndrome (ARDS)

(Bautista et al., 2010; Fraaij and Heikkinen, 2011; Kaiser et al., 2003). Approximately, 5–20% of the world populations are infected with influenza virus annually.

Influenza complications are more likely to occur in high risk groups such as children, elderly, pregnant women, smokers, and those with chronic diseases such as diabetes (Van Kerkhove et al., 2011).

Influenza viruses are enveloped, negative-sense RNA viruses with segmented genomes belonging to the *Orthomyxoviridae* family (Shaw and Palese, 2013). Influenza A viruses are subtyped based on their two surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) (Palese and Shaw, 2007; Wright and Neumann, 2007). Six subtypes,

including H1, H2, H3, H5, H7, and H9 have been identified in humans (Choi et al., 2004; Fouchier et al., 2004; Munoz-Medina et al., 2015); however, only three of those (H1, H2 and H3) acquired the capacity of sustained transmission among the human population (Munoz-Medina et al., 2015). The *incidence* of *influenza virus* subtypes *varies* among different countries and regions (Clark and Lynch, 2011).

The influenza A virus matrix protein 2 (M2) is an essential component of the influenza viral envelope. It functions as a proton pump and facilitates the release of viral RNA into the host cell (Moorthy et al., 2014). The adamantanes, amantadine and its derivative rimantadine, represent the first class of antivirals clinically approved for the treatment of influenza A infection, which act by blocking the M2 proton channel of influenza A viruses (Hay et al., 1985; Hayden and Hay, 1992). Amantadine was the first of the two adamantanes to be approved for clinical use in 1966 followed by rimantadine in 1993 (Arruda and Hayden, 1996; JAMA, 1967) Adamantanes were used as the first generation of effective antiviral drugs against community outbreaks of influenza A viruses for more than 30 years. Although the therapeutic efficacy of amantadine and rimantadine is well proven, the universal emergence of influenza viruses that are resistant to this class restricted their value in treatment (Bean et al., 1989; Gu et al., 2015; Hay et al., 1985; Van Voris et al., 1981). Adamantane-resistant influenza A viruses were first reported during the 1980 epidemic (Heider et al., 1981). The resistance to both amantadine and rimantadine drugs was then detected at varying frequencies among the seasonal H1N1 and H3N2 subtypes (Hussain et al., 2017). Almost 15.6% of the H1N1 and 90.6% of the H3N2 global isolates were adamantane-resistant during the 2005–2006 season (Deyde et al., 2007). The rapid global surge of the adamantane-resistant influenza A viruses prompted the Centers for Disease Control and Prevention (CDC), USA, to issue an advisory against the use of these drugs in treating influenza A virus infections (Centers for Disease Control and Prevention (CDC), 2006).

Structural studies on M2 in complex with adamantanes revealed two potential sites of interaction; a single drug molecule binds to the core of the pore and four drug molecules bind to the lipid-exposed surface of the channel close to the cytoplasmic ends of the helices (Pielak et al., 2009). Naturally occurring mutations in the M2 protein that confer resistance to adamantane drugs are classified into three categories according to their location in the protein. They include pore-facing mutations (V27A, A30T, S31N, G34E), the N-terminal interhelical-facing mutation (L26F) and C-terminal interhelical-facing mutations (L38F, D44A) (Gu et al., 2011; Hay et al., 1985). S31 N, is the most prevalent mutation among all current drug resistant influenza A viruses (Wang et al., 2013).

Neuraminidase inhibitors (NAIs) are the second class of approved anti-influenza drugs that bind to the NA enzyme active site and inhibit its enzymatic function, which is critical for the release of viral progeny from infected cells (Kapoor and Dhama, 2014; Oh and Hurt, 2014). NAIs are currently the class of choice in treatment and prophylaxis of influenza infections. Orally administered oseltamivir (Tamiflu) and zanamivir (Relenza) were the first approved NAIs and are used worldwide (Kim et al., 1997; von Itzstein et al., 1993). In addition, intravenous peramivir and inhalation laninamivir (only available in Japan) became recently available for treatment of influenza (Oh and Hurt, 2014).

The NA protein is a homotetrameric glycoprotein with an enzymatically active head that cleaves sialic acid at the glycosidic bond on the host cell (Colman et al., 1983). The active site of NA protein contains eleven framework residues in addition to eight functional residues in influenza A and B viruses (Burmeister et al., 1992; Yen et al., 2006). Amino acid mutations in the NA enzymatic framework or functional residues may lead to resistance to NAIs. Substitutions that confer NAI-resistance are located in the NA active site or its proximity; E119A, E119G, E119D, E119V, R292K, and N294S resistant-substitutions are found in the N2 and N9 subtypes and H274Y and N294S are found in the N1 subtype (Aoki and Boivin, 2009; McKimm-Breschkin, 2002;

Rameix-Welti et al., 2008). A natural occurring substitution (Q136K) was found in A/H1N1 and A/H3N2 viruses and was associated with reduced susceptibility to zanamivir and peramivir (Hurt et al., 2009a). The most common resistant mutation is the H274Y in the N1 subtype (Samson et al., 2013). Influenza A/H1N1 viruses containing this substitution emerged during the 2007–2008 influenza season (Meijer et al., 2009). These oseltamivir-resistant viruses became predominant during the 2008 influenza season in the Southern Hemisphere and circulated at an incidence ranging between 10 and 70% in the Northern Hemisphere (Hurt et al., 2009b). The I222R and S246N resistant substitutions emerged shortly after the A/H1N1pdm09 outbreak. These mutations, when combined with the H274Y mutation, further elevated the resistance to the NAIs (Hurt et al., 2011; Nguyen et al., 2010).

The emergence of oseltamivir resistance was found to be rare in recent influenza A viruses. However, some clinical *studies reported* a high frequency of drug resistance among post-treatment isolates recovered from children who received oseltamivir (Kiso et al., 2004; LeGoff et al., 2012; van der Vries et al., 2013; Ward et al., 2005). A novel influenza A/H1N1pdm09 variant, possessing a S246N mutation, with mildly reduced oseltamivir and zanamivir sensitivity, was detected in more than 10% of community specimens in Singapore and more than 30% of samples from northern Australia during the early months of 2011 (Hurt et al., 2011). Whitley et al. recently reported that the highest rate of oseltamivir-resistant influenza viruses was observed in children between 1 and 5 years of age (10.1%) during antiviral therapy (Whitley et al., 2013). Studies from Japan reported that drug-resistance emerge in 16% and 18% in post-treatment A/H1N1pdm09 and A/H3N2 isolates, respectively (Kiso et al., 2004; Ward et al., 2005).

In addition to adamantanes and NAIs, a new class (polymerase inhibitors) of antiviral drugs was recently approved (Omoto et al., 2018). Baloxavir marboxil is the first in class PA endonuclease inhibitor that is highly effective against influenza A viruses. Resistance to this drug ranged from 2.2% to 9.7% in phase II and phase III clinical trials, respectively (Hayden et al., 2018).

The rapid emergence and spread of resistance to currently available antivirals emphasizes on the importance of understanding the scope of this problem globally. The Eastern Mediterranean Region (EMR) of the World Health Organization (WHO) includes 22 countries: Afghanistan, Bahrain, Djibouti, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Oman, Pakistan, Palestine, Qatar, Saudi Arabia, Somalia, Sudan, Syria, Tunisia, United Arabs Emirates (UAE) and Yemen. Due to its central location, the EMR is considered among the critical geographic regions for circulation and transmission of influenza A viruses (Kayali et al., 2013). Therefore, characterization of antiviral drug resistant markers is critical for the monitoring and control of influenza in this region. To assess antiviral drug resistance among influenza viruses in the EMR, we analyzed the incidence and geographic distribution of mutations conferring resistance to adamantanes and NAIs among H1, H3 and H5 human influenza A viruses detected in EMR region from 2005 through 2016. We also discuss the possible reasons underlying the incidence of NAIs- resistance and high adamantanes-resistance incidence in this region.

2. Methods

2.1. Sequence data collection and analysis

All available and complete amino acid sequences of the M2 and NA proteins of seasonal influenza A/H1N1, A/H1N1pdm09, A/H3N2, and A/H5N1 viruses were obtained from the Influenza Resource Database (IRD; http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) and the Epiflu platform of the Global Initiative on Sharing All Influenza Data (GISAID; http://platform.gisaid.org/) databases. A total of 314 M2 and 1209 NA protein sequences from human influenza viruses that were detected in the EMR countries between 2005 through 2016 were downloaded. Multiple sequence alignments of the M2 and NA genes

Table 1
Incidence of adamantane-resistant variants among influenza A viruses in the EMR countries.

Type/Subtype	Date of collection	Protein sequences (n)	Resistant variant (n)[a]	Frequency of (%)
A/H1N1 seas.	2005–2008	9	0	0
A/H1N1pdm09	2009–2016	171	165	96.49
A/H3N2	2005–2016	107	102	95.32
A/H5N1	2006–2015	27	2	7.40
Overall	2005–2016	314	269	85.66

[a] Possess at least one of the following mutations in the M2 protein that confer resistance to adamantane drugs: V27A, S31N, and V27A/S31N.

were performed with the Clustal Omega alignment tool at EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/clustalo) (McWilliam et al., 2013). The transmembrane regions of the M2 ion channel proteins and the NA proteins were then screened for resistance-conferring mutations.

The annual incidence of resistance was calculated as the proportion of the resistant variants divided by the total number of reported sequences during a specific period of time or in a given country.

3. Results

3.1. Frequency of adamantane-resistance

A total of 314 M2 protein sequences of influenza A viruses that were detected in the EMR region between 2005 and 2016 were screened for the mutations known to confer adamantane-resistance. Overall, 269/314 (85.66%) of the assessed influenza A viruses possessed at least one adamantane-resistant mutation (Table 1). The frequency of adamantane-resistant mutations was 96.49% (165/171) for the H1pdm09 viruses, 95.32% (102/107) for the H3, and 7.40% (2/27) for the H5 viruses. No amantadine-resistant mutations were detected among seasonal A/H1N1 viruses in the EMR (0/9).

The frequency of the adamantane-resistant A/H1N1seas variants increased from 0% during the 2005–2006 influenza season to 95.83% during the 2008–2009 season. As of 2011, 100% of all detected A/H3N2 and A/H1N1pdm09 viruses were resistant to adamantanes (Fig. 1).

The majority (265/269, 98.51%) of the resistant variants possessed the S31N mutation and 0.74% (2/269) carried the V27A mutation. The former mutation was detected in the A/H1N1pdm09 and A/H3N2 viruses, while the latter was detected among the A/H5N1 viruses. Two sequences (0.74%) possessed the V27A/S31N resistant mutations; one each in an A/H1N1pdm09 and A/H3N2 viruses that originated from

Table 2
Incidence of adamantine-resistant mutations among influenza A subtypes.

Resistant mutation	Frequency by subtype		
	H1pdm09	H3	H5
L26F	–	–	–
V27A	–	–	2
A30T	–	–	–
S31 N	164	101	–
G34E	–	–	–
L38F	–	–	–
L26F/S31N	–	–	–
V27A/S31N	1	1	–
G34E/S31N	–	–	–
Overall (n)	165	102	2

Iran during the 2014/2015 and 2011/2012 seasons, respectively. The substitutions L26F, A30T, G34E, and L38F were not detected in any of the H1, H3 and H5 viruses (Table 2).
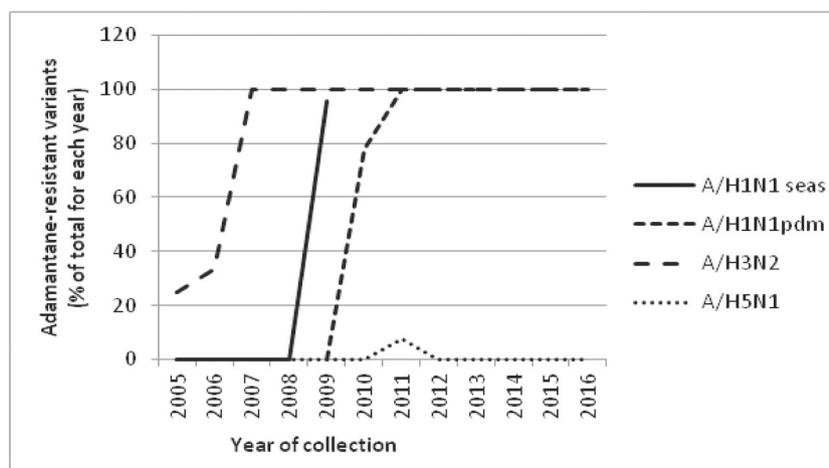
3.2. Geographical distribution of adamantane-resistant influenza A variants

Geographical analysis indicated that adamantane-resistant influenza A/H1N1pdm09 and A/H3N2 viruses were detected across the whole EMR (Table 2). The largest proportion (48/165; 29.09%) of the adamantane-resistant influenza A/H1N1pdm09 viruses was detected from Oman, followed by 13.94% (23/165) from Bahrain, 13.94% (23/165) from Pakistan, and 12.12% (20/165) from Morocco. The majority of the detected adamantane-resistant influenza A/H3N2 variants originated from Iran (31/102, 30.40%), Egypt (13/102, 12.74%), and Jordan (12/102, 11.76%). Adamantane-resistant influenza A/H5 variants were only detected in Egypt, where this virus is mainly circulating in the region (Table 3).

3.3. Incidence of NAI-resistance by influenza A subtypes

A total of 1209 NA protein sequences from the EMR were screened for the mutations known to confer NAI-resistance or reduced susceptibility during 2005–2016. The incidence of NAI-resistance over the study period is presented in Table 4. Overall, 34/1209 (2.81%) of the detected NA sequences from influenza A viruses possessed at least one NAI-resistant mutation. The frequency of NAI-resistant mutations was 10.64% (5/47) for the seasonal A/H1N1, 3.29% (24/729) for the A/H1N1pdm09, 0% (0/310) for the A/H3N2, and 4.06% (5/123) for the A/H5N1 viruses (Fig. 2).

Several *subtype-specific* NA mutations conferring *resistance* to *NAIs*

Fig. 1. Increase in adamantane-resistance in the EMR during the past decade.

Table 3
Distribution of adamantane-resistant influenza A variants among the EMR countries.

Country[a] (total)	Frequency of adamantane-resistant variants from different HA subtypes (%)			
	H1N1 seas.	H1N1pdm09	H3N2	H5N1
n = 314	n = 9	n = 171	n = 107	n = 27
Afghanistan (5)	0	1 (100%)	4 (100%)	0
Bahrain (25)	0	23 (100%)	2 (100%)	0
Djibouti (1)	0	0	1 (100%)	0
Egypt (48)	0	6 (100%)	13/14 (92.58%)	2/27 (7.40%)
Iran (52)	0	13/17 (76.47%)	31/35 (88.57%)	0
Iraq (17)	0	10/11 (90.91%)	4 (100%)	0
Jordon (22)	0	10 (100%)	12 (100%)	0
Kuwait (11)	0	1/2 (50%)	6 (100%)	0
Lebanon (7)	0	3 (100%)	4 (100%)	0
Morocco (29)	0	20 (100%)	9 (100%)	0
Oman (54)	0	48 (100%)	6 (100%)	0
Pakistan (25)	0	23 (100%)	2 (100%)	0
Qatar (8)	0	4 (100%)	1 (100%)	0
Saudi Arabia (4)	0	2 (100%)	2 (100%)	0
Tunisia (3)	0	1 (100%)	2 (100%)	0
United Arab Emirates (3)	0	0	3 (100%)	0

[a] No data was available from Libya, Palestine, Somalia, Sudan, Syrian Arab Republic, and Yemen.

have been previously described (Abed et al., 2006; Aoki et al., 2007; Kamali and Holodniy, 2013; McKimm-Breschkin, 2013). A summary of the effects of these mutations on the susceptibility of influenza viruses to each of the approved NAIs is presented in Table 4. Of the 729 detected A/H1N1pdm09 viruses, 3.29% (n = 24) possessed the H274Y mutation that confers resistance to oseltamivir. In addition, 0.96% (7/729) of the A/H1N1pdm09 variants had the S246N mutation that was associated with reduced susceptibility to both oseltamivir and zanamivir. Among the seasonal A/H1N1 viruses, 6.38% (3/47) possessed the H274Y mutation and 4.25% (2/47) carried the Q136K amino acid substitution. The latter mutation was previously reported to induce reduced susceptibility to zanamivir and peramivir (Hurt et al., 2009a). For influenza A/H3N2 viruses, two out of 310 NA sequences had the D151E mutation, which confers reduced susceptibility to oseltamivir (Yen et al., 2006). All of the detected influenza A/H5N1 viruses had the H252Y mutation, previously shown to decrease the binding affinity of oseltamivir to NA (McKimm-Breschkin, 2013). In addition, the I222V/T

and N294S substitutions that confer resistance to oseltamivir (Ilyushina et al., 2010; McKimm-Breschkin, 2013) were detected in two and three A/H5N1 viruses, respectively.

3.4. Geographical distribution of NAI-resistant influenza A variants

The frequencies of NAI resistant variants in different EMR countries are presented in Table 5. The majority of the NAI resistant influenza A/H1N1pdm09 H274Y variants were detected from three EMR countries (Egypt, Iran, and Oman). Of these variants, 29.16% (7/24) were from Oman, 20.83% (5/24) were detected in Iran, and 20.83% (5/24) were detected in Egypt. For seasonal A/H1N1 viruses, the NAI-resistant viruses reported during our study period originated from Egypt and Iran. None of detected A/H3N2 viruses were resistant to NAIs. For influenza A/H5N1 viruses, all the resistant variants originated from Egypt (100%, 5/5).

4. Discussion

Antivirals against influenza play a critical role in the management and prevention of influenza. Although effective influenza vaccines are available for prevention of seasonal influenza, treatment and prophylaxis (in case of pandemics) relies on antiviral therapy. Currently, most circulating influenza A viruses are resistant to adamantanes, which limited the therapeutic options for influenza to NAIs (Nelson et al., 2009; World Health Organization, 2013). The aim of this study was to investigate the incidence and genetic markers of antiviral drug resistance among influenza viruses in the EMR. In this study, 314 influenza A viruses (H1 seas., H1pdm09, H3 and H5) detected in the EMR region from 2005 to 2016 were assessed for resistance to adamantanes. Genotypic analysis of these viruses revealed a steady increase of adamantane-resistance in the EMR. The frequency of adamantane-resistant variants varied by country and subtype. This variability could be attributed at least partially to the large data gaps in the Region and suboptimal reporting. This urges the need to enhance influenza surveillance systems in the EMR and to improve reporting. Resistance to adamantanes reached 100% by 2011 among H1 and H3 viruses in the EMR. This is consistent with the globally reported trend of universal adamantane-resistance among circulating influenza A viruses (H1 and H3) during the same period (Barr et al., 2008; Deyde et al., 2007; Dong et al., 2015; Sheu et al., 2011). Egypt is the only country in the EMR region where A/H5N1 viruses are endemic and human cases are reported (Centre for Health Protection, 2017; Kim, 2018). A low level of adamantane-resistant variants was reported among human A/H5N1 in Egypt.

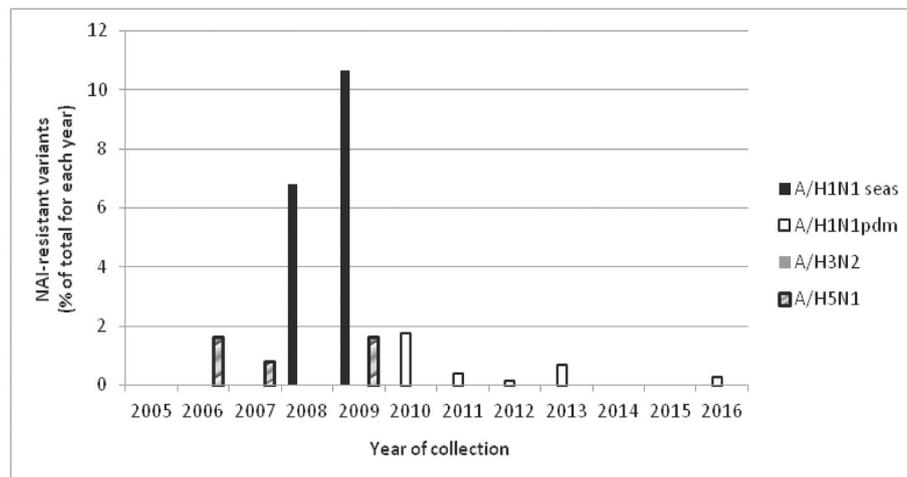Consistent with data reported from other regions, the S31N

Table 4
Incidence of NA mutations reported to affect susceptibility to NAIs.

Subtypes	Date of collection	Incidence of resistance (%)	Amino acid substitution	Positive viruses (n)	Effect on susceptibility to NAIs			References
					Oseltamivir	Zanamivir	Peramivir	
H1N1 seas.	2005–2009	5/47 (10.64)	Q136K	2	S[a]	R	R	((McKimm-Breschkin, 2013); (Boivin, 2013))
			H274Y	3	R	S	R	(McKimm-Breschkin, 2013)
H1N1pdm09	2009–2016	24/729 (3.29)	S246 N	7	RS	RS	S	(Hurt et al., 2011)
			H274Y	24	R	S	R	((McKimm-Breschkin, 2013); (The WHO GISRS antiviral susceptibility expert working group (AVWG), 2016))
H3N2	2005–2016	0/310 (0)	D151E	2	RS	S	–	(Yen et al., 2006)
H5N1	2006–2015	5/123 (4.06)	I222V	1	RS/R	S	S	((McKimm-Breschkin, 2013); (Hurt et al., 2009c))
			I222T	1	RS/R	S	S	(McKimm-Breschkin, 2013)
			H252Y	123	RS	S	S	(McKimm-Breschkin, 2013)
			N294S	3	R	RS	RS	((McKimm-Breschkin, 2013); (Ilyushina et al., 2010); (Earhart et al., 2009))

[a] R = Resistant, RS = Reduced susceptibility, S = Susceptible, "–" = No effect.

Fig. 2. Alteration of NAI-resistance rate in the EMR during the past decade.

Table 5
Distribution of NAI-resistant mutations among the EMR countries.

Countries[a] (total)	Frequency of NAI-resistant mutations (%)					
	H1N1 seas[b]		H1N1pdm09	H5N1		
n = 1209	n = 5		n = 24	n = 5		
	Q136K	H274Y	H274Y	I222V	I222T	N294S
Afghanistan (7)	0	0	0	0	0	0
Bahrain (25)	0	0	0	0	0	0
Djibouti (10)	0	0	0	0	0	0
Egypt (281)	0	3/8 (37.5%)	5/102 (4.90%)	1/121 (0.83%)	1/121 (0.83%)	3/121 (2.47%)
Iran (201)	2/29 (6.89%)	0	5/108 (4.62%)	0	0	0
Iraq (31)	0	0	1/22 (4.54%)	0	0	0
Jordon (184)	0	0	0	0	0	0
Kuwait (12)	0	0	1/2 (50%)	0	0	0
Lebanon (54)	0	0	1/45 (2.22%)	0	0	0
Morocco (80)	0	0	3/51 (5.88%)	0	0	0
Oman (161)	0	0	7/113 (6.19%)	0	0	0
Pakistan (25)	0	0	0	0	0	0
Palestine (30)	0	0	0	0	0	0
Qatar (12)	0	0	0	0	0	0
Saudi Arabia (43)	0	0	0	0	0	0
Tunisia (31)	0	0	0	0	0	0
United Arab Emirates (22)	0	0	1/19 (5.26%)	0	0	0

[a] No data was available from Libya, Somalia, Sudan, Syrian Arab Republic, and Yemen.
[b] In case of H3N2, none of the analyzed strains carried a substitution that confers resistance to NAIs.

substitution was the most common resistant marker in the EMR among H1 and H3 viruses, while the V27A was detected among the H5 viruses (Dong et al., 2015; El-Shesheny et al., 2014; Garcia and Aris-Brosou, 2014; Hussain et al., 2017; Marinova-Petkova et al., 2014). Recently, Hurt et al. described the detection of adamantane-susceptible influenza A/H3N2, which constituted 3.3% of circulating viruses in Australia (Hurt et al., 2017). The potential for re-emergence of susceptible influenza A strains warrants continued examination of influenza A viruses for resistant markers. The successful return of adamantane-sensitive strains could revive the utility of this class of antivirals in clinical practice.

Given the universal resistance to adamantanes among contemporary human viruses, NAIs are currently the drugs of choice for treatment and prophylaxis against influenza infections (Hurt, 2014; Nguyen-Van-Tam et al., 2015). In 2007 and onward, the utility of oseltamivir was threatened by the emergence and quick spread of the H274Y substitution among seasonal A/H1N1 viruses (Ives et al., 2002; Moscona, 2009). However, the oseltamivir-resistant seasonal A/H1N1 virus was soon replaced by the susceptible, novel A/H1N1pdm09 virus in 2009

during which oseltamivir use skyrocketed in the absence of an effective vaccine (European Centre for Disease Prevention and Control, 2009; Garten et al., 2009). The frequency of oseltamivir-resistant seasonal A/H1N1 viruses was 10.64% between 2005 and 2009 in the EMR. In case of the H1N1pdm09 viruses, the frequency of the resistant viruses was comparably less at 3.29% between 2009 and 2016. No NAI-resistant markers were detected among the H3N2 viruses during the study period. This data is in agreement with the recent reports by the WHO showing that NAI-resistant variants constitute a minority among the recent human influenza viruses, but still significant enough to cause concern and warrant ongoing monitoring (Gubareva et al., 2017; Hurt et al., 2016).

Our study provides an important outlook of the frequency and distribution of adamantane- and NAI-resistant influenza virus variants throughout the EMR during 2005 through 2016. However, our study is limited by its reliance on the data reported to the database rather than systematic surveillance of circulating influenza viruses. Although the data might not represent the exact incidence of resistant strains in each country and the Region as a whole, but it is likely to reflect the general

trends of resistance in the Region.

Our data clearly highlights the large data gaps in the Region and in general the suboptimal reporting compared to other WHO regions. The availability of surveillance data including monitoring of drug resistance is critical for developing regional guidelines for the management of influenza and also for early detection of resistant variants at the global level. However, not all countries of the EMR region report influenza sequences. Therefore, there is a critical need to upgrade and advance the influenza surveillance systems in the Region and improve reporting. Several countries in the Region have been enhancing their capacities for influenza surveillance in collaboration with the WHO EMR office (EMRO) by establishing or expanding the capacities of existing national influenza centers. These efforts will bridge the gaps in data and complete the global outlook of influenza.

Funding

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