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## A clinical approach to the threat of emerging influenza viruses in the Asia-Pacific region

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### ABSTRACT

Seasonal influenza epidemics and periodic pandemics are important causes of morbidity and mortality. Patients with chronic co-morbid illness, those at the extremes of age and pregnant women are at higher risks of complications requiring hospitalization, whereas young adults and obese individuals were also at increased risk during the A(H1N1) pandemic in 2009. Avian influenza A(H5N1) and A(H7N9) viruses have continued to circulate widely in some poultry populations and infect humans sporadically since 1997 and 2013, respectively. The recent upsurge in human cases of A(H7N9) infections in Mainland China is of great concern. Sporadic human cases of avian A(H5N6), A(H10N8) and A(H6N1) have also emerged in recent years while there are also widespread poultry outbreaks due to A(H5N8) in many countries. Observational studies have shown that treatment with a neuraminidase inhibitor (NAI) for adults hospitalized with severe influenza is associated with lower mortality and better clinical outcomes, especially when administered early in the course of illness. Whether higher than standard doses of NAI would provide greater antiviral effects in such patients will require further investigation. High-dose systemic corticosteroids were associated with worse outcomes in patients with severe influenza. There is an urgent need for developing more effective antiviral therapies for treatment of influenza infections.

**Key words:** avian influenza, respiratory tract infections, seasonal, treatment, viral.

**Abbreviations:** ACH, air changes per hour; AGP, aerosol-generating procedure; ARDS, acute respiratory distress syndrome; CAP, community-acquired pneumonia; CAPD, continuous ambulatory peritoneal dialysis; CrCl, creatinine clearance; HA, haemagglutinin; HD, haemodialysis; HFR,

hospitalization fatality risk; HR, hazard ratio; IMV, invasive mechanical ventilation; LPM, live poultry market; LRT, lower respiratory tract; NA, neuraminidase; NAI, NA inhibitor; NIV, non-invasive ventilation; NP, nasopharyngeal; qd, once a day; RCT, randomized controlled trial; RIDT, rapid influenza diagnostic test; rRT-PCR, real time reverse transcription polymerase chain reaction; RSV, respiratory syncytial virus; URT, upper respiratory tract; WHO, World Health Organization.

### INTRODUCTION

Severe acute respiratory infections such as avian influenza A(H5N1)<sup>1</sup> and A(H7N9) viruses<sup>2</sup> with pandemic potential have continued to circulate widely in some poultry populations and infect humans sporadically. Infection caused by these viruses may result in severe disease and high case fatality rates because most humans have no background immunity to these viruses. Sporadic human cases due to avian A(H5N6),<sup>3</sup> A(H10N8)<sup>4</sup> and A(H6N1)<sup>5</sup> have also emerged in recent years. The global circulation of oseltamivir-resistant seasonal influenza, the emergence of A(H1N1)pdm09 virus in 2009 followed by its continual circulation,<sup>6</sup> the rising number of A(H7N9) infections in humans<sup>2</sup> and the ongoing spread of A(H5N8) in recent months in the poultry populations in many countries in Asia, Africa, Europe and Middle East with pandemic potential<sup>7</sup> all point to an urgent need for developing more effective antiviral therapies to reduce morbidity and mortality. This article reviews the epidemiology, clinical, diagnostic and treatment aspects of these important and emerging influenza viruses that are posing threats to human health in the Asia-Pacific region.

### EPIDEMIOLOGY

#### Seasonal influenza and pandemic influenza

Seasonal influenza is a major health problem. For instance, in Hong Kong, influenza A and B accounted for 39.4% and 10.2%, respectively, of hospital admissions confirmed to have viral infections.<sup>8</sup> The average annual incidence of hospitalization with laboratory-confirmed influenza A infection was 107.7/10 000 and

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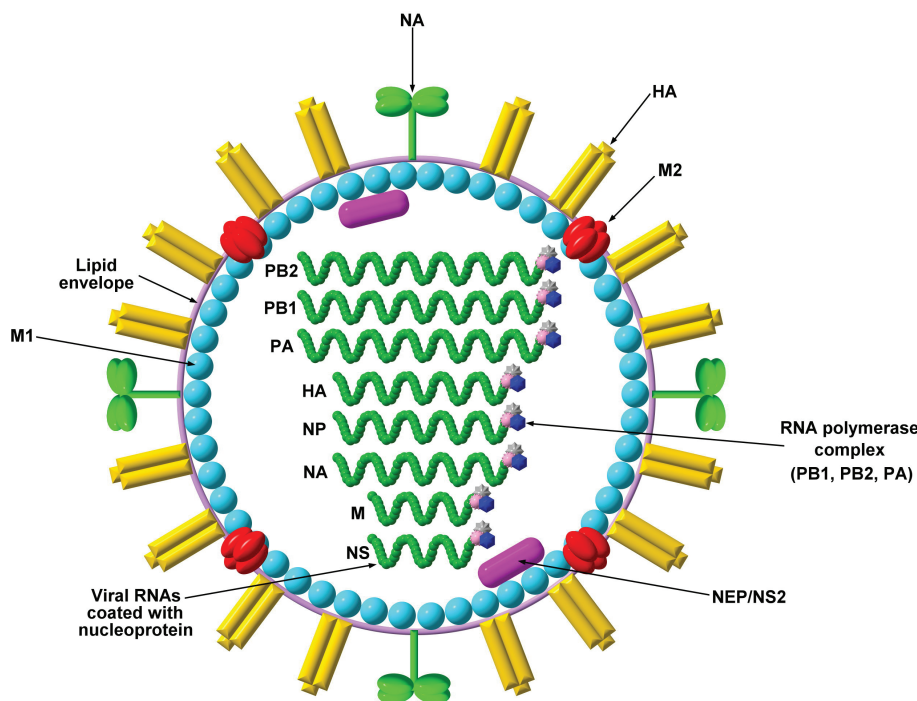
18.3/10 000 among children aged <5 years and elderly  $\geq 65$  years, respectively. Furthermore, influenza A was the most common (45.5%) virus detected from patients who died of respiratory viral infections. Although influenza B is usually associated with a less degree of disease burden, its impact on human health is substantial. Our previous study conducted in Hong Kong revealed that 24% of laboratory-confirmed influenza-associated hospital admissions were due to influenza B, and was much higher (41.9%) among older children and adolescents (aged 5–14 years).<sup>9</sup> This observation is similar to data in Europe and North America.

In the temperate region, influenza exhibits an annual seasonal peak in winter. However, the seasonality in tropical and subtropical regions is more variable. For instance, in Hong Kong, a subtropical city, most of the time, influenza exhibits two peaks during January–March and June–August, and occasionally, the two peaks merge without an obvious gap.<sup>8,10</sup> Such bimodal and variable seasonality of influenza is also observed in countries within the subtropical and tropical regions, and poses a challenge to implement annual influenza vaccination campaign as well as a question on whether the Northern or Southern Hemisphere version of influenza vaccine would be more appropriate.

A schematic diagram of the structure of influenza virion is shown in Figure 1. Influenza represents one of the few examples of viruses with segmented genome. This, together with the widespread infection with

different subtypes in various animal species, allows robust evolution through the process of ‘reassortment’. The prerequisite for reassortment is a simultaneous infection with more than one strain of viruses in a host referred to as the ‘mixing vessel’ that allows replication and swabbing of gene segments between different strains of viruses. Water birds are well-known mixing vessels, but the reassortants are more likely to have a tropism restricted to avian species. These new reassortants may pose problems in the land birds and poultry, and subsequently be of human concern. Swine is another well-known mixing vessel. The unique property of swine is their susceptibility to both avian and human influenza viruses. Thus, reassortants from swine are more likely to gain tropism for infection in humans. Field observations made as early as 1990s have pointed out that influenza outbreaks that began with either pigs or people were then rapidly transferred to each other.<sup>11</sup> Swine are likely to play the role of a mixing vessel in creating pandemic strains. Although the 1918 pandemic strain was most probably an entirely avian-like virus,<sup>12</sup> the 1957 and 1968 pandemic strains were reassortants of human- and avian-origin viruses.<sup>13</sup>

While the most recent pandemic, A(H1N1)pdm09, was not as severe as those that occurred before, there is no guarantee that the next pandemic will be as ‘mild’. At present, a few subtypes (H5, H7 and H9) are of particular concern. Cross-species infections,



**Figure 1** Structure of influenza A virion. The virion of influenza A contains a host-derived lipid envelope, embedding the haemagglutinin (HA, found as trimer), neuraminidase (NA, found as tetramer) and matrix protein 2 (M2, found as tetramer). HA is required for attachment (binding between the virion and the sialic acid residues on the host cell surface). NA is used for cleaving sialic acid receptors from the host cell membrane for new virion release. M2 is an ion channel for virion internal acidification, contributing to viral uncoating. The HA : NA ratio ranges from 4:1 to 5:1. Underlying the viral envelope, there is a layer of the matrix protein 1 (M1). Inside the virion, a nuclear export protein (NEP/NS2) is also found. Eight single-stranded, negative-sense viral RNA molecules are coated with nucleoproteins and bound by the RNA polymerase complex: polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase acidic protein (PA).

especially the recent strong wave of human A(H7N9) infection in Mainland China underscores that the next pandemic could be imminent.<sup>14,15</sup> Countries should have pandemic preparedness in place, including antiviral stockpiling and pandemic vaccine preparations. These would be better achieved under joint multigovernment industrial partnership.

## Avian influenza viruses

### H5N1 viruses

Human cases of the highly pathogenic avian influenza A(H5N1) were first detected in Hong Kong in 1997.<sup>1</sup> The number of affected countries increased between 2003 and 2008, with expansion from east and southeast Asia, then to west Asia and Africa, with a sharp rise in Egypt since November 2014.<sup>16</sup> As of 16 March 2017, there have been 453 deaths out of 858 human cases in 16 countries since 2003.<sup>17</sup> A Canadian tourist died of A(H5N1)-related meningoencephalitis in Alberta in January 2014 after visiting Beijing in December 2013 without any obvious exposure to infected avian sources or environmental contamination.<sup>18</sup> A history of exposure to dead or sick poultry/wild birds occurs in over 60% of cases of human A(H5N1) infection. The incubation period for A(H5N1) infection ranges from 2 to 8 days but may be as long as 17 days. The clinical spectrum may range from asymptomatic infection, mild influenza-like illness to severe pneumonia, with multiorgan failure.<sup>19</sup> Some of the A(H5N1) human cases have been linked to consumption of dishes made of raw, contaminated poultry blood. However, slaughter, defeathering, handling carcasses of infected poultry and preparing poultry for consumption, especially in household settings, appear to be important risk factors.<sup>19,20</sup>

Among the fatal cases, the median duration from symptom onset to death was 9–10 days (range: 6–30 days).<sup>20</sup> Viral RNA was present in the blood of fatal A(H5N1) cases and this was associated with higher pharyngeal viral loads. Nasopharyngeal swab samples, bronchoalveolar lavage and cerebrospinal fluid samples were positive in the Canadian case for influenza A(H5N1) virus by various molecular testing methods, including sequencing.<sup>18</sup> In addition, lymphopenia and high chemokine/cytokine levels have been observed in severe A(H5N1) infection and these correlated with pharyngeal loads.<sup>21</sup>

A new reassortant genotype of A(H5N1) containing the haemagglutinin (HA) and neuraminidase (NA) genes from clade 1.1.2 and the internal genes from clade 2.3.2.1 was detected in 2013 and associated with the highest number of cases ( $n = 26$ ) and deaths ( $n = 14$ ) in Cambodia.<sup>22</sup> Complete viral genome analysis of the fatal case in Canada has shown an HA gene of clade 2.3.2.1c and was a reassortant with an A(H9N2) subtype lineage polymerase basic 2 gene without mutations conferring resistance to adamantanes or NA inhibitors (NAI).<sup>18</sup> Human-human transmission remains rare as a meta-analysis has shown that only 1–2% of more than 12 500 study participants from 20 studies exposed to patients with A(H5N1) infection had serological evidence for prior A(H5N1) infection.<sup>23</sup> Delay in the delivery of appropriate

treatment to patients with influenza A(H5N1) infection in Indonesia was mainly related to delay in diagnosis rather than late presentation to healthcare settings.<sup>24</sup> Age, country, per capita government health expenditure and delay from symptom onset to hospitalization were the risk factors for mortality related to A(H5N1) infection, emphasizing the importance of early diagnosis, treatment and supportive care.<sup>25</sup> A systematic review has shown that females were at higher risk of death (OR: 1.75, 95% CI: 1.27–2.44) following A(H5N1) infection, whereas young age in particular <5 years was protective (OR: 0.44, 95% CI: 0.25–0.79).<sup>26</sup>

### H7N9 viruses

Human infections with a novel avian influenza A(H7N9) virus were first reported in China in March 2013 in patients hospitalized with severe pneumonia.<sup>27</sup> There have been five seasonal epidemics in China since the virus was first discovered in 2013. The estimated risk of death (hospitalization fatality risk, HFR) among hospitalized cases of A(H7N9) infection in the second epidemic was 48% (95% credibility interval: 42–54%), which was slightly higher than the corresponding risk of 36% in the first wave. In the second epidemic, the HFR was estimated at 36% (95% CI: 28–45%) in patients aged <60 years but at 59% (95% CI: 51–67%) among those aged ≥60 years.<sup>28</sup>

An upsurge in human A(H7N9) infections has been observed since November 2016 in Mainland China. During this fifth epidemic wave, the number of human cases is higher than during the previous two waves during 2014–2015 and 2015–2016. The majority of recently reported human cases are associated with exposure to infected live poultry or contaminated environments, including wet markets where live poultry are sold. In addition, the human cases are more geographically widespread and cases are also reported from rural areas, unlike in previous epidemics (Fig. 2).<sup>29,30</sup> The phylogenetic analysis on HA genes of isolates collected from humans suggested that the virus is evolving and with the more recent isolates clustered to a separate branch (Supplementary Figure S1).<sup>31,32</sup> As of 5 April 2017, 1364 laboratory-confirmed cases of human infection with avian influenza A(H7N9) virus have been reported to World Health Organization (WHO).<sup>33</sup> Human cases of A(H7N9) infection exported from the Mainland of China have been confirmed in Hong Kong ( $n = 21$ ), Taiwan ( $n = 4$ ), Macau ( $n = 2$ ), Canada ( $n = 2$ ) and Malaysia ( $n = 1$ ) in visitors who developed illness after returning from the Mainland of China to their home cities.<sup>29,30,33</sup>

The incubation period of human infection with the A(H7N9) virus ranges from 1 to 10 days, with an average of 5 days. The median time from poultry exposure to disease onset was 6 days, whereas the median time from illness onset to hospitalization, acute respiratory distress syndrome (ARDS) development, antiviral therapy and death were 4, 7, 6 and 21 days, respectively.<sup>34</sup> Preexisting co-morbid conditions occurred in >60% of these cases. The prominent clinical features on admission were those of a severe influenza syndrome with fever, cough, fatigue and dyspnoea, whereas the most striking laboratory findings were marked lymphopenia



**Figure 2** Geographic distribution of the fifth wave of human infection with avian influenza A(H7N9) in Mainland China.<sup>29</sup> Cases include those reported from October 2016 to 25 March 2017. Two cases in Beijing were imported from Hebei and Liaoning. Two cases in Yunnan Province were imported from Jiangxi.

and thrombocytopenia. Elevated cytokine levels have been observed in patients and the excessive cytokine responses may contribute to the clinical severity of A(H7N9) infection.<sup>35,36</sup>

Limited human to human transmission with at least two epidemiologically linked cases due to close household contact with the index patients has been reported in a number of family clusters in both A(H5N1)<sup>20,37,38</sup> and A(H7N9) infections.<sup>34,39,40</sup> Nosocomial transmission of A(H7N9) infection between two patients sharing a hospital room,<sup>41</sup> and co-transmission of avian influenza A(H7N9) and A(H1N1)pdm09 viruses between two patients with haematological disorders sharing the same room with bed distance <1 m have been reported.<sup>42</sup> Comparisons of the clinical features between human cases of A(H5N1) and A(H7N9) are shown in Table 1.<sup>34,43</sup>

Closure of live poultry markets (LPM) in the Mainland of China has tremendously reduced the risk of A(H7N9) infection temporarily but closure is difficult to sustain due to cultural preference for live poultry. An ecologic modelling study estimated that closure of LPM reduced the mean daily number of A(H7N9) virus infections in the four most affected cities by 97–99%.<sup>44</sup> A retrospective serological study of blood specimens taken during January–May and October–November in 2012 from 1544 subjects who worked in LPM, farms, slaughter houses or kept backyard poultry in Eastern China before the epidemic occurred in 2013 revealed no evidence of A(H7N9) infection.<sup>45</sup>

**Other novel avian influenza subtypes**

The first human case of avian A(H6N1) infection was reported in a 20-year-old female with pneumonia in

Taiwan in May 2013, with subsequent full recovery following treatment with oseltamivir.<sup>5</sup>

The first human case of avian A(H5N6) was confirmed in a 49-year-old male in Sichuan Province,

**Table 1** Comparisons of clinical and epidemiological features between A(H7N9) and A(H5N1) infections in humans<sup>16,34,43</sup>

	A(H7N9) (n = 139) <sup>34</sup>	A(H5N1) (n = 907) <sup>16</sup>
Median age (years)	61 (2–91)	19 (5–32)
Male (%)	71	431 (47)
Co-morbid illness	79/108 (73%)	5/41 (12%) <sup>43</sup>
Urban residence	101 (73%)	19 (44%) <sup>43</sup>
Rural residence	38 (27%)	24 (56%) <sup>43</sup>
Exposure to poultry	82%	82.5%
Occupational exposure to poultry	9 (6%)	15 (1.7%)
Visited wet poultry markets	70/107 (65%)	82/907 (9%)
Exposure to sick or dead poultry	63/107 (59%)	439/907 (48.4%)
Exposure to backyard poultry	NA	188/907 (20.7%)
Onset of illness to hospitalization (median, days)	4	4
Onset of illness to ARDS (median, days)	7	7.5 <sup>43</sup>
Onset of illness to death (median, days)	21	11 <sup>43</sup>
Case fatality rate in hospital	34%	53%

ARDS, acute respiratory distress syndrome; NA, not applicable.



**Figure 3** Geographic distribution of human infection with avian influenza A(H5N6) in Mainland China.<sup>29</sup> Cases include those reported from 2014 till 25 March 2017. One case in Jiangxi was imported from Guangdong.

China, in May 2014 with a fatal outcome<sup>3</sup> and a second case was confirmed in a 58-year-old male with history of exposure to live poultry in Guangdong Province in December 2014.<sup>46</sup> The virus was a reassortant that contained seven genes from A(H5N1) and the NA gene from an A(H6N6) virus circulating in ducks.<sup>47</sup> Influenza A(H5N6) outbreaks in birds and poultry have been reported in Laos, Vietnam and Mainland China since 2014. In 2016, A(H5N6) outbreaks in poultry have continued to occur in Vietnam and Mainland China (Guizhou, Jiangxi and Hunan). Since 2014, 16 human cases of avian influenza A(H5N6) have occurred in Mainland China with six deaths (Fig. 3).<sup>29</sup> The latest case was reported on 1 December 2016.<sup>48</sup>

In addition, China has confirmed three human infections, two fatal, with avian A(H10N8) viruses that contain the internal genes from A(H9N2), as does A(H7N9).<sup>4,49</sup> Similar to the A(H7N9) virus, the A(H6N1) and A(H10N8) viruses have low pathogenicity in poultry and are therefore more difficult to detect in birds in contrast to the highly pathogenic A(H5N1) virus.

Highly pathogenic avian influenza A(H5N8) viruses belonging to clade 2.3.4.4 of the A/goose/Guangdong/1/1996 lineage were detected in 2014 in wild birds and poultry in China, Japan, South Korea, Netherlands, Germany, Italy, Russia, the UK and Northern Ireland. The virus was detected sporadically in Canada and the USA in wild birds and poultry until mid-2015. The A(H5N8) viruses were also detected in Taiwan, China and in Hungary and Sweden in 2015. Since June 2016, many more countries in both Europe and Asia have detected infections in wild birds and/or domestic poultry with A(H5N8). Many of these recent detections were associated with mortality in wild birds.<sup>7,50</sup> Although

human infection with A(H5N8) has not been detected, the ongoing spread of this virus to different countries in recent months is worrisome and may increase the risk of human infection.

## CLINICAL MANAGEMENT

### Initial assessment

Visitors who have returned from areas affected by avian influenza and developed respiratory symptoms and fever within 10 days after their return should be enquired about their relevant history (travel history, occupation, contact history and clustering) to facilitate early diagnosis and treatment. It is important to obtain any history of contact with poultry (live or sick/dead poultry as A(H7N9) virus may cause no or mild symptoms in poultry in contrast to A(H5N1)), exposure to wet markets or known avian influenza cases, and laboratory exposure including staff handling with spillage. Patients with influenza-like illness who present with dyspnoea, tachypnoea, evidence of hypoxaemia and pulmonary infiltrates on chest radiograph should be hospitalized.<sup>51</sup> Early case identification and isolation precautions would facilitate clinical management which may reduce the risk of severe infection and related complications for seasonal epidemic, zoonotic and pandemic influenza, in addition to reducing the risk of influenza transmission and mitigating the impact of outbreaks on the healthcare system.

Common findings in severe influenza infection include low or normal white cell counts, lymphopenia and thrombocytopenia, and increased serum transaminases, lactate dehydrogenase (LDH), creatine kinase and urea/creatinine.<sup>6,20,27,51</sup> In the Influenza Clinical

Information Network (FLUCIN) Study, early elevation of C-reactive protein  $\geq 100$  mg/L was an independent predictor of severe outcome. In a study of 1770 patients hospitalized with community-acquired pneumonia (CAP), procalcitonin concentration had a strong association with need for invasive mechanical ventilation (IMV) or inotrope support (in 6.5% of those studied).<sup>52</sup> Higher levels of certain biomarkers on presentation linked to inflammation, coagulation or immune function (interleukin 6 (IL-6), Cluster of Differentiation 163 (CD163), interferon gamma-induced protein 10 (IL-10), lipopolysaccharide binding protein (LBP), IL-2, monocyte chemoattractant protein-1 (MCP-1) and interferon gamma-induced protein 10 (IP-10)) have been associated with disease progression in both outpatients and those hospitalized with influenza.<sup>53</sup>

### Laboratory investigations

Laboratory diagnosis plays an important role in the management of influenza. The narrow window of an effective antiviral intervention for both seasonal and avian influenza demands a rapid turnaround time.<sup>6,54</sup> Nowadays, most laboratories employ either real time reverse transcription polymerase chain reaction (rRT-PCR)-based methods or antigen detection-based assays. While the rRT-PCR approach is most sensitive and can be applied to a wide variety of specimens including the less invasive throat swab samples, it requires a certain degree of laboratory setup and technical skills, which are not readily available in many healthcare settings. The antigen-based rapid influenza diagnostic test (RIDT), although less sensitive, is still the test of choice particularly as a point-of-care test. Our experience on a few RIDT suggested that the sensitivity could be lower for patients who presented more than 2 days after the onset of illness, for young children compared to elderly, and for cases with pneumonia compared to those without.<sup>55</sup> It is therefore crucial to evaluate the performance with respect to the setting that RIDT will be applied.

The specimen of choice is critical. While in most situations, nasopharyngeal aspirate/swab is the preferred choice of specimen for seasonal influenza, false-negative results may occur in patients with severe pneumonia where the viral load remains high in the lower respiratory tract (LRT) but has become undetectable in the upper respiratory tract (URT).<sup>56</sup> Similarly, LRT specimens such as tracheal aspirates or sputum are the specimen of choice for diagnosis of avian influenza infection in humans as the viral load in the URT is typically low. The development and availability of more affordable and reliable molecular diagnostic tests including multiplex PCR would facilitate future clinical management of patients with severe CAP due to viral or bacterial aetiology.<sup>57</sup> The advantages and limitations of different diagnostic tests for influenza are summarized in Table 2.<sup>55,57</sup>

### Antiviral therapy

The M2 inhibitors (amantadine and rimantadine) and the NAI (oseltamivir, peramivir, zanamivir and laninamivir) are the main antiviral agents approved for the

prevention of and treatment for influenza. In general, antiviral treatment should be commenced as soon as possible for any patient hospitalized with confirmed/suspected influenza with severe, complicated or progressive illness, and also for outpatients at higher risk for influenza complications.<sup>58</sup>

The M2 inhibitors (adamantanes) are not effective against influenza B viruses and recently circulating influenza A(H3N2) and influenza A(H1N1)pdm09 viruses, which are resistant due to an S31N mutation in the M2 ion channel. However, as some avian influenza A(H5N1) strains are still susceptible,<sup>59</sup> combination of an adamantane with an NAI may enhance antiviral activity for susceptible isolates.<sup>60</sup> Oseltamivir is effective in reducing mortality (OR: 0.17;  $P = 0.04$ ) in influenza A(H5N1) infection when given before the onset of respiratory failure,<sup>61</sup> and may provide some survival benefit (49% reduction in mortality) when treatment is started within 6–8 days after symptom onset.<sup>62</sup> Several observational studies have shown that treatment with oseltamivir for adults hospitalized with severe influenza is associated with lower mortality and better clinical outcomes, especially when antiviral treatment has been initiated within 2 days of illness onset but even as late as 4–5 days after symptom onset.<sup>63,64</sup> A systematic review has shown that in prophylactic studies, oseltamivir could reduce the proportion of symptomatic influenza whereas in treatment studies it modestly reduced the time to first alleviation of symptoms by 16.8 h, but it caused nausea, vomiting, headaches and renal/psychiatric side effects.<sup>65</sup> A meta-analysis of randomized controlled trials (RCT) has shown that oseltamivir in adults with influenza shortens time to clinical symptom alleviation by 21%, reduces risks of LRT complications and hospitalization, but increases the occurrence of nausea and vomiting.<sup>66</sup> Oseltamivir resistance is infrequent in A(H5N1), and <3% in circulating A(H1N1)pdm09, A(H3N2) and B viruses but it is important to monitor for antiviral resistance when managing patients with severe influenza.<sup>67</sup>

All H7N9 viruses are amantadine resistant due to the S31N substitution in the M2 ion channel protein. In patients hospitalized with severe A(H7N9) infection, reduction of viral load following oseltamivir treatment was associated with improved outcome, whereas the emergence of virus resistant to NAI harbouring an R292K mutation was associated with poor outcomes and lack of response to oseltamivir and peramivir, and reduced susceptibility to zanamivir and laninamivir (50- and 25-fold rises in inhibitory concentration by 50% (IC<sub>50</sub>), respectively). Two patients with severe A(H7N9) infection and R292K mutation requiring extracorporeal membrane oxygenation had received systemic corticosteroid treatment which might have contributed to treatment failure and a fatal outcome.<sup>68</sup>

The recommended treatment duration of oseltamivir is generally 5 days, but longer treatment (10 days, then guided by clinical and virological testing as recommended by the WHO) is advisable for critically ill patients with respiratory failure with persistent viral replication in the LRT.<sup>51,58,69</sup> Whether higher than standard dose of NAI would provide greater antiviral effects in such patients requires further investigation. One RCT of hospitalized patients (76% being children) revealed no clinical or

**Table 2** Laboratory diagnosis of influenza infections in the clinical setting<sup>55,57</sup>

Testing method	Advantages	Disadvantages
Influenza virus RIDT (immunoassay for antigens)	<ul style="list-style-type: none"> <li>• Results available in &lt;30 min</li> <li>• Applicable to a range of upper respiratory samples</li> <li>• Differentiates between influenza A and B</li> <li>• High specificity (90–95%)</li> </ul>	<ul style="list-style-type: none"> <li>• Low sensitivity (40–70%); performance affected by virus subtype/strain, sampling time, specimen type and pre-test probability</li> <li>• Cannot distinguish between virus subtypes</li> </ul>
Immunofluorescence, direct (DFA) or indirect (IFA) antibody staining	<ul style="list-style-type: none"> <li>• Results available within hours</li> <li>• Applicable to upper and lower respiratory samples</li> <li>• Detects influenza A, B and other viruses simultaneously (e.g. RSV) if included in the panel</li> <li>• High specificity (90–95%)</li> </ul>	<ul style="list-style-type: none"> <li>• Moderate sensitivity (50–85%)</li> <li>• Quality specimen containing adequate epithelial cells (e.g. NP aspirate and flocced swab) and laboratory expertise is essential</li> <li>• Cannot distinguish between virus subtypes</li> </ul>
Viral cell culture (conventional or shell vial)	<ul style="list-style-type: none"> <li>• High specificity</li> <li>• Allows virus subtyping, strain identification, titre assay/quantification and resistance testing</li> <li>• Provides information on infectiousness</li> </ul>	<ul style="list-style-type: none"> <li>• Moderate sensitivity (7–20% lower than PCR)</li> <li>• Requires optimal sampling (e.g. prior to antiviral use) and post-sampling processing conditions</li> <li>• Results too slow to guide patient care (conventional: &gt;3 to 10 days; shell vial: &gt;2 to 3 days)</li> </ul>
Reverse transcription PCR (rRT-PCR)	<ul style="list-style-type: none"> <li>• Results available within hours</li> <li>• High sensitivity and specificity (gold standard)</li> <li>• Applicable to a wider range of specimen types</li> <li>• Able to distinguish between virus subtypes (e.g. H1, H3, H5 and H7) and detect genotypic resistance (e.g. H275Y)</li> <li>• Multiplex PCR methods may allow simultaneous detection of other respiratory pathogens</li> </ul>	<ul style="list-style-type: none"> <li>• Accessibility, technical demands and cost are the practical concerns</li> <li>• Unable to distinguish non-viable from viable viruses</li> <li>• ‘False-negative’ upper tract samples (~10%) may result from site differential viral kinetic changes in pneumonia cases</li> </ul>

Most RIDT are chromatographic immunoassays (some are fluorescence-based immunoassays); applicable to NP swabs, nasal and/or throat swabs and NP aspirates/washes (training and protection equipment are required). Performance is best if applied within 48–72 h from onset before a significant drop in viral load (up to 4–5 days in selected populations). Lower sensitivity for A(H1N1)pdm09 virus has been reported.

Viral cell culture detects viable viruses, including those contained in the live-attenuated influenza vaccines (LAIV). Isolates can be subjected to phenotypic resistance assays (e.g. neuraminidase enzyme inhibition assay). Viral load, specimen quality, transport, storage and processing techniques may affect test performance.

PCR assays can either provide universal detection of influenza A virus by targeting the matrix (M) gene or subtype-specific virus detection (e.g. H1N1pdm09, H3N2, H5N1 and H7N9) by targeting the haemagglutinin (HA) gene. Viruses that cannot be subtyped may indicate a novel strain. Newer molecular-based point-of-care tests may improve accessibility and reduce processing time and technical demands; some may allow detection of multiple viruses. Cost-effectiveness of PCR is variable, depending on the circumstances.

Some multiplex PCR platforms may provide detection of >14 respiratory viruses (e.g. RSV, human metapneumovirus, parainfluenza virus, rhinovirus and coronavirus) and atypical pathogens (e.g. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*).

DFA, direct fluorescent antibody test; IFA, immunofluorescence assay; NP, nasopharyngeal; RIDT, rapid influenza diagnostic test; RSV, respiratory syncytial virus.

virological advantages when comparing double dose of oseltamivir against standard dose.<sup>70</sup> No additional benefit of higher dose oseltamivir treatment was observed in adults hospitalized with influenza A, although a faster virological response was noted in influenza B.<sup>71</sup> However, an RCT of 18 critically ill patients with A(H1N1)pdm09 in Canada found that therapy with a triple dose of oseltamivir was associated with higher proportions of viral clearance at 5 days than standard therapy (78% vs 11%;  $P = 0.015$ ).<sup>72</sup> Intravenous (i.v.) peramivir treatment was associated with viral RNA decline as well as culture and

RNA negativity, which occurred at similar rates to those on oral oseltamivir by Day 5 among adult patients hospitalized for influenza-associated LRT complications.<sup>73</sup> The various NAI dosages for adults with adjustment for renal failure are shown in Table 3.<sup>58</sup>

Zanamivir and laninamivir have quite similar profiles of drug susceptibility. One example is that H275Y mutation, which confers high level resistance to oseltamivir carboxylate and reduced susceptibility to peramivir in N1-containing viruses, does not reduce susceptibility to zanamivir and laninamivir

**Table 3** Dosage adjustment of oral oseltamivir, i.v. peramivir and i.v. zanamivir for adults with and without renal impairment<sup>58</sup>

	CrCl (mL/min)				Dialysis
	>60 to 90	>30 to 60	>10 to 30	<10	
Oseltamivir	75 mg bd	30 mg bd	30 mg qd	<10 Data limited; may consider single dose at 30 mg	CAPD/HD CAPD: 30 mg single dose HD: 30 mg after every HD cycle
Peramivir	600 mg qd	200 mg qd	100 mg qd	<10 Data limited; may consider 100 mg on Day 1, then 15 mg qd thereafter	HD 100 mg on Day 1, then 100 mg given 2 h after each HD session on dialysis days only
Zanamivir	50 to <80	30 to <50	15 to <30	<15	—
Initial dose 600 mg; maintenance dose for CrCl > 80 mL/min is 600 mg bd <sup>†</sup>	400 mg bd	250 mg bd	150 mg bd	60 mg bd	—

<sup>†</sup>Time interval between initial dose of zanamivir and the maintenance dose in patients with CrCl > 80 mL/min = 12 h; CrCl 15 to <30 = 24 h; CrCl <15 mL/min = 48 h.

CAPD, continuous ambulatory peritoneal dialysis; CrCl, creatinine clearance; HD, haemodialysis; qd, once a day.

significantly.<sup>74</sup> Intravenous zanamivir was used widely on a compassionate ground for late treatment of critically ill adults with influenza A(H1N1)pdm09 and those with suspected or proven oseltamivir resistance.<sup>75</sup> There were no drug-related trends in safety parameters and in a subset of 93 patients with positive PCR tests at baseline for influenza, showed a median decrease in nasopharyngeal viral RNA load of 1.42 log<sub>10</sub> copies/mL after 2 days of treatment.<sup>76</sup> Intravenous zanamivir was used with a favourable outcome in a patient with severe A(H7N9) infection complicated by pneumonia which did not respond to oseltamivir initially.<sup>77</sup> Favipiravir, which is an inhibitor of a viral RNA-dependent RNA polymerase, has strong antiviral activity against NAI-resistant and sensitive influenza viruses.<sup>67,78</sup>

### Systemic corticosteroids and other immunomodulating agents for severe influenza

Systemic corticosteroid has been used frequently for the treatment of influenza-related ARDS. However, a meta-analysis of data predominantly related to treatment of severe influenza A(H1N1)pdm09 has shown that systemic corticosteroid was associated with an increase in mortality (OR: 3.06, 95% CI: 1.58–5.92). Pooled subgroup analysis of adjusted estimates of mortality from four studies found OR of 2.82 and 95% CI of 1.61–4.92.<sup>79</sup> In comparison to controls, high-dose corticosteroids (>150 mg/day methylprednisolone eqv) was associated with increased risks in 30-day mortality (38.5% vs 7.7%,  $P = 0.021$ ) and 60-day mortality (50% vs 15.4%,  $P = 0.022$ ) and longer viral shedding (15 days vs 13 days,  $P = 0.039$ ) in patients with influenza A (H7N9) viral pneumonia while there was no difference between low dose (25–150 mg/day methylprednisolone) and controls.<sup>80</sup> In a study of 2649 adults hospitalized with influenza in Hong Kong, Singapore and

Beijing during 2008–2011, 23.1% had received systemic corticosteroids. Bacterial superinfections increased risk of death (adjusted hazard ratio (HR): 2.2, 95% CI: 1.5–3.1). Systemic corticosteroids increased risks of superinfections (from 2.7% to 9.7%) and deaths when controlled for indications (adjusted HR: 1.7, 95% CI: 1.1–2.6).<sup>81</sup> Among adults with severe sepsis not in septic shock, use of low-dose hydrocortisone versus placebo did not reduce the risk of septic shock within 14 days. The findings do not support the use of hydrocortisone in these patients without septic shock.<sup>82</sup> The role of low-dose systemic corticosteroid needs further investigation by a properly planned RCT.<sup>83</sup>

In a study of critically ill patients infected with influenza A (H1N1)pdm 09 requiring IMV, addition of a mammalian target of rapamycin inhibitor, sirolimus 2 mg/day for 14 days to oseltamivir and prednisolone ( $n = 19$ ), was associated with a higher frequency of liberation from IMV (84.2% vs 47.4%,  $P = 0.04$ ), a shorter duration of IMV (13.8 days vs 33 days,  $P = 0.03$ ) and a higher chance of achieving LRT viral RNA negativity by Day 7 (75% vs 33%,  $P < 0.05$ ) than without addition of sirolimus ( $n = 19$ ).<sup>84</sup> The role of sirolimus plus oseltamivir versus oseltamivir alone in a larger sample size should be examined without systemic corticosteroid.

A recent study of adults hospitalized for A(H3N2) has shown that a triple combination treatment of 2 days of clarithromycin 500 mg, naproxen 200 mg and oseltamivir 75 mg twice daily (bd), followed by 3 days of oseltamivir reduced both 30- and 90-day mortalities and length of hospital stay versus oseltamivir 75 mg bd without placebos for 5 days as control.<sup>85</sup> Confirmatory studies of the role of this triple combination would be of interest.

Exploratory post hoc meta-analysis of studies of severe acute respiratory syndrome (SARS) and severe influenza showed a significant reduction in the pooled odds of mortality following convalescent plasma versus placebo or no treatment (OR: 0.25; 95% CI: 0.14–0.45).<sup>86</sup> Thus, convalescent plasma and hyperimmune globulin may



provide useful alternatives for the treatment of severe novel influenza infections provided there are suitable donors with significant neutralizing antibody. Other immunomodulating agents such as acute use of statins, N-acetylcysteine, nitazoxanide, macrolides, peroxisome proliferator activated receptor (PPAR) agonists, i.v. gammaglobulin (IVIG), celecoxib, mesalazine and the role of plasmapheresis and haemoperfusion as rescue therapy require further investigation.<sup>87</sup>

### Infection control and prevention

A survey of acute care hospitals in the Canadian Nosocomial Infection Surveillance Program from 2006 to 2012 has shown that 570 (17.3%) of 3299 influenza cases were healthcare associated; 345 (60.5%) acquired in a long-term care facility and 225 (39.5%) acquired in an acute care facility.<sup>88</sup> Nosocomial opportunistic airborne transmission of A(H3N2) has been implicated in a medical ward with imbalanced airflow and use of non-invasive ventilation (NIV) for an index patient with acute exacerbation of chronic obstructive pulmonary disease (COPD).<sup>89</sup>

Standard, contact and droplet precautions are recommended for routine management of patients hospitalized for influenza.<sup>90</sup> The main infection prevention and control measures for managing influenza are droplet precaution (by wearing a surgical mask within 1 m of the patient) and contact precaution (by wearing gown and gloves on entering the room and removing them on leaving). Droplet precautions should be added to the standard precautions when providing care to all patients with symptoms of acute respiratory infection. Contact precautions and eye protection should be added when caring for probable or confirmed cases of avian influenza infection (Table 4).<sup>90</sup>

A study that measured the amount of influenza A(H1N1)pdm09 RNA in aerosols in the vicinity of H1N1-positive patients undergoing aerosol-generating procedures (AGP) has shown that bronchoscopy and

respiratory/airway suctioning were the most definite procedures to produce aerosols above background baseline values.<sup>92</sup> Thus, it is important for healthcare workers to take airborne precautions and perform AGP preferably in an isolation room with negative pressure. In order to reduce room contamination in the hospital setting, major health organizations have recommended the application of a minimum room ventilation rate of 6 air changes per hour (ACH) in existing facility whereas a higher ventilation of 12 ACH is recommended for new or renovated construction, especially when caring for patients receiving mechanical ventilation and during AGP.<sup>91,93</sup>

As single circuit NIV may lead to room contamination by exhaled aerosols,<sup>94,95</sup> it has been recommended to apply NIV via a helmet and double circuit tubes for patients with mild to moderate respiratory failure during the influenza A(H1N1) pandemic in 2009.<sup>96</sup> However, it is important to ensure a good seal at the neck-helmet interface to prevent nosocomial transmission through environmental contamination by exhaled aerosols.<sup>97</sup>

### Antiviral prophylaxis

Post-exposure prophylaxis with a daily dose of NAI (e.g. oseltamivir 75 mg daily for adults) for 10 days is recommended for unprotected close contacts of patients with seasonal influenza who are at risk of complicated influenza.<sup>98</sup> WHO does not recommend routine post-exposure antiviral chemoprophylaxis for A(H7N9) virus. However, for some asymptomatic persons in which a substantial unprotected or prolonged exposure to an ill patient with A(H7N9) infection has occurred, initiation of empiric post-exposure antiviral treatment (e.g. oseltamivir 75 mg orally bd for 5 days), on the presumption that influenza virus infection has occurred, may be considered. This is likely to be limited to healthcare or other settings involving substantial exposure of *those at higher risk for complications* from

**Table 4** Infection prevention and control measures when assessing patients with complicated influenza<sup>51,90,91</sup>

1. Clinicians should pay attention to cases of community-acquired pneumonia and patients' travel history for early case detection
2. Clinicians should remain vigilant against avian influenza and elicit any relevant clinical and epidemiological information from patients (fever, travel history, occupation, contact history and clustering)
3. Standard, contact and droplet precautions are recommended for routine management of patients hospitalized for influenza. Droplet precaution (by wearing a surgical mask within 1 m of the patient) and contact precaution (by wearing gown and gloves on entering the room and removing them on leaving) when providing care to all patients with symptoms of acute respiratory infection. Contact precautions and eye protection should be added when caring for probable or confirmed cases of avian influenza infection
4. Risk assessment should be conducted before performing AGP. For cases with severe influenza, it is advisable to perform AGP in an airborne isolation room. When an airborne isolation room is not available, the following minimum hourly averaged ventilation rates should be provided for natural ventilation:
  - a. 160 L/s/patient (hourly average ventilation rate) for airborne precaution rooms (with a minimum of 80 L/s/patient) (note that this only applies to new healthcare facilities and major renovations);
  - b. 60 L/s/patient for general wards and outpatient departments; and
  - c. 2.5 L/s/m<sup>3</sup> for corridors and other transient spaces without a fixed number of patients
5. In view of the increasing influenza activity during winter season and the emerging threat of avian influenza, all healthcare workers and visitors are recommended to wear surgical masks when entering patient care areas and strengthen hand hygiene

AGP, aerosol-generating procedure.

influenza virus infection, including patients with severe immunosuppression, neonates and infants, pregnant and early post-partum women, elderly adults, persons with co-morbidities and other highly vulnerable patients; or, *unprotected healthcare workers*, especially those involved in AGP.<sup>99</sup>

## VACCINES

Annual seasonal influenza vaccinations are recommended for the high-risk groups, including residents of institutions for elderly and disabled, any age with chronic illness, age > 65 years; 6 months to 6 years, poultry workers in countries affected by avian influenza, healthcare workers especially those frequently in contact with high-risk persons and household contacts of high-risk persons. While vaccination rates among healthcare workers are highly variable in different countries,<sup>100</sup> there is no conclusive evidence that seasonal influenza vaccination of healthcare workers may protect their patients in terms of reduction in risks of mortality and influenza-like illness.<sup>101</sup> A survey in Hong Kong assessing the acceptability of an additional ad hoc influenza vaccination among the healthcare professionals following seasons with significant antigenic drift has shown that the strongest factors associated with accepting an additional influenza vaccine included immunization with influenza vaccines in the past 3 years, higher perceived risk of contracting influenza and higher perceived severity of the disease impact.<sup>102</sup>

The current influenza vaccines induce a strain-specific immunity that is not ideal for this rapidly mutating virus. A universal influenza vaccine capable of inducing a broad cross-protection across strains is needed. To this end, the M2e antigen, a linear peptide that is conserved across influenza A strains, is being evaluated a vaccine target.<sup>103</sup> The other approach is to prepare virus-like-particles based on HA, NA and M1 proteins.<sup>104,105</sup> Virus-like particles with a cocktail of seasonal and potential pandemic strains have been prepared and demonstrated promising results.<sup>106</sup>

## PUBLIC HEALTH MEASURES TO REDUCE ZONOTIC AND PANDEMIC RISKS FROM AVIAN INFLUENZA IN ASIA

As A(H7N9) is now endemic in over half of the provinces in Mainland China and will continue to cause recurrent zoonotic disease in the winter months, public health measures to control the source such as some rest days every month for the poultry workers to thoroughly clean the LPM environment, and banning the stock of live poultry in markets overnight are important. Separation of live ducks and geese from land-based (i.e. non-aquatic) poultry in LPM systems can reduce the risk of emergence of zoonotic and epizootic viruses at source. In the long run, it is advisable to adopt a complete transition from sale of live poultry in wholesale and retail LPM to centralized slaughter and sale of chilled or frozen poultry.<sup>107</sup>

## SUMMARY

Seasonal influenza epidemics and periodic pandemics are important causes of morbidity and mortality. Apart from influenza A(H5N1) virus and sporadic human cases of A(H5N6), A(H10N8) and A(H6N1) infections, infection due to A(H7N9) virus, especially the recent upsurge in the number of human infections in Mainland China, is of great concern. The widespread poultry outbreaks due to A(H5N8) in many countries increases the risk of human infection. Early isolation and treatment with an NAI for adults hospitalized with severe influenza is associated with lower mortality and better clinical outcomes. Whether higher than standard doses of NAI may provide greater antiviral effects in such patients would require further investigation. High-dose systemic corticosteroids were associated with worse outcomes in patients with severe influenza complicated by ARDS. There is an urgent need for developing more effective antiviral therapies for the treatment of influenza infections.

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### Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

**Figure S1** Phylogenetic tree of haemagglutinin (HA) genes of H7N9 isolates collected from human infections.