Influenza Surveillance: 2014–2015 H1N1 “Swine”-Derived Influenza Viruses from India

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The 2014–2015 H1N1 outbreak in India has reportedly led to 800 fatalities. The reported influenza hemagglutinin sequences from India indicate that these viruses contain amino acid changes linked to enhanced virulence and are potentially antigenically distinct from the current vaccine containing 2009 (Cal0709) H1N1 viral hemagglutinin.

Between 2009 and 2010, the 2009 pandemic influenza A H1N1 (2009pdmH1N1) virus is estimated to have caused over 18,300 deaths across 74 countries worldwide (Cheng et al., 2012). Since the initial pandemic outbreak, the 2009pdmH1N1 has replaced the prior seasonal H1N1 and established itself in the human population. This is largely due to sequence evolution of the hemagglutinin (HA) protein, whose activity critically governs the receptor binding, fusion, and transmission properties of the virus (Smith et al., 2009).

Influenza activity in the northern hemisphere has been high this year, predominantly due to influenza A(H3N2). Further, antigenic analyses of the recent circulating A(H3N2) viruses have shown differences from the A(H3N2) virus used in the influenza vaccines for 2014–2015. However, some countries in Northern Africa, Middle East, and notably India reported an increase in influenza A(H1N1) pdm09 activity. It has been reported that the recent 2014–2015 H1N1 outbreak in India has resulted in >8,000 cases with over 800 deaths, although it remains unclear if there is an underestimation of the number of real cases. Anecdotal reports indicate that the majority of these cases involve young adults—a trend that is similar to the 1918 Spanish Flu pandemic, when 50–100 million people died worldwide. One recent news report from India indicates that influenza genes sequenced from patient swab samples revealed no new mutations in the virus (http://indianexpress.com/article/cities/pune/silver-lining-no-mutation-of-h1n1-says-study/). Additionally, it was suggested the 2009pdmH1N1 pandemic strain—A/California/04/2009—was responsible for the outbreak in India.

Continuous surveillance of influenza viruses enables researchers to track viral evolution, identify amino acid mutations in key viral proteins governing their circulation, predict potential “outbreaks,” and assist in the development of various “outreach” approaches to treat as well as prevent further spread. Typically, influenza-genome data collected from field studies or research efforts are sequenced and submitted to GenBank and/or one or more specialized open-access databases. Open-access databases such as National Center for Biotechnology Information (NCBI) Influenza Virus Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html), Influenza Research Database (http://www.fludb.org/), and EpiFlu (http://platform.gisaid.org/epi3/start) facilitate sharing of viral genome sequences and encourage collaborative research world over. In addition to providing access to nucleotide and amino acid sequences, these specialized databases provide researchers additional information such as genetic markers (e.g., drug resistance, increased virulence, etc.) and form the basis of epidemiological and clinical data and tools for analyzing the genomic sequences, e.g., sequence comparison and alignment, phylogeny tree construction, epitope prediction, and mapping. An analysis of the publicly available influenza databases suggests that influenza monitoring has not yet reached sufficient levels to enable real-time surveillance.

Overall, there exist 15,173 H1N1 pdm HA sequences in the public sequence databases, out of which 4,213 represent full-length, nonredundant entries. Examination of the geography of the isolated strains shows that the majority of the deposited influenza sequences come from the United States (38.4%), China (7.2%), United Kingdom (6.5%), and Singapore (6%). Unfortunately, India ranks low (14th) in this list, contributing less than 1.5% of sequences. Furthermore, despite the vastness of the Indian subcontinent, only two sequences have been deposited during 2014–2015 from India, suggesting poor surveillance and potentially limiting the response to a deadly outbreak. Additionally, swine can also contribute to the emergence of novel H1N1 variants through the process of reverse zoonoses and thus should also be monitored. Despite the risk posed by these animals, the number of swine influenza sequences collected in the 2009–2015 period is insignificant, with notably no swine influenza strains deposited from India. These numbers highlight the irregular, reactive nature of the influenza surveillance response.

Although there are limited Indian-origin influenza sequences available in the public database to make any causal inference on the perceived increased fatalities in India, examination of the 2014 Indian H1N1 HA sequences shows traits with potential cause for concern. Amino acid changes in specific positions in the receptor binding site (RBS) of 2009pdmH1N1 have been shown to impact glycan RBS specificity and have been linked to increased virulence and...
disease severity. Among these changes, the Indian-origin strain A/India/6427/2014 contains amino acid changes T200A and D225N compared to the 2009pdmH1N1 pandemic strain. The T200A amino acid change has been shown to improve human glycan receptor-binding of 2009pdmH1N1 HA (Xu et al., 2012b). The D225N mutation has been linked to increased virulence and disease severity in patients infected by the 2009 pdm virus (Ruggiero et al., 2013). Importantly, a previous study showed that the D225N mutation in the context of H1 HA affected receptor binding and also decreased susceptibility to NA inhibitors (McKimm-Breschkin et al., 2013). It should be noted that the D225N mutation was previously linked to serious illness resulting in hospitalization or death (L’vov et al., 2010). The high population density in India, ease of person-to-person transmission, and lack of effective treatment options create ample opportunities for this variant to sustain and become dominant. It is unknown if the strain A/India/6427/2014 is still in circulation; however, the apparent severity of the current outbreak seems to suggest that it could be.

Gene reassortment wherein segments of the genome are exchanged between different strains is another mechanism that drives rapid influenza evolution. Indeed, the previous three pandemics emerged as a result of gene reassortment. The 1957 H2N2 (Asian flu) pandemic emerged through reassortment between human H1N1 and avian H2N2. Similarly, the H3N2 (Hong Kong) pandemic was caused by a human-adapted H2N2 virus as it obtained avian H3 and PB1 genes through reassortment. The 2009 swine-origin H1N1 pandemic emerged as a result of reassortment between avian, human, and swine influenza viruses. India houses billions of farmed birds and swine animals across the country. Combined with this, export of animals and challenges to farming infrastructure augment the risk of reassortment events. Although the 2014 Indian-origin strains appear to have not undergone reassortment, the involvement of gene reassortment in the current outbreak in India cannot be determined without full genome sequence information.

Since 2009, HAs of the 2009pdmH1N1 lineage have gradually evolved (Figure 1) and acquired mutations in the H1 antigenic sites (Sa, Sb, Ca, and Cb) (Caton et al., 1982). Notably, strains carrying seven antigenic-site mutations appeared in 2013, which has implications for reevaluation of the H1N1 vaccine component (A/California/04/2009). Importantly, the mutation K166Q at the “Sa” antigenic site discriminated strains that circulated before 2013 from those that circulated during 2014–2015. While the majority of strains that circulated before 2013 possessed Lys at 169, >80% of the strains that circulated after 2013, including the two 2014 Indian isolates, possessed a Gln. Importantly, a previous study showed that a variation at 166 could lead to escape from neutralizing antibodies elicited by the current H1N1 vaccine component A/California/07/2009 (Linderman et al., 2014). Additionally, while the antigenic residues N129 (Sa), G158 (Sa), and N159 (Sb)
In the context of various outreach toward slowing or halting an epidemic outbreak, access to both antivirals and vaccines plays a large part; thus a priority should be development of infrastructure to make, store, and distribute appropriate antivirals for targeted administration can buy time to allow for a widespread vaccination campaign. Additionally, the development of a universal vaccine can go a long way in mitigating the risk of high morality.

In summary, the influenza outbreak in India should be further examined to determine the virulence and potential threat of the virus. Improved surveillance and monitoring of the influenza outbreak will significantly enhance the options of how best we can manage outreach to both treat as well as prevent spread of the virus.

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