Original Article

Tracking the Emergence of Pandemic Influenza A/H1N1/2009 and its Interaction with Seasonal Influenza Viruses in Singapore

Julian WT Tang,[1] *MRCP, FRCPath, PhD*, Chun Kiat Lee,[1] *BSc*, Hong Kai Lee,[1] *BSc*, Tze Ping Loh,[1] *MB BCh BAO*, Lily Chiu,[1] *MSc*, Paul A Tambyah,[2] *MBBS, DipABIM, FAMS*, Evelyn SC Koay,[1,3] *FRCPath, PhD*

Abstract

Introduction: Since the emergence of the pandemic influenza A/H1N1/2009 virus in April 2009, diagnostic testing in many countries has revealed the rapid displacement and then replacement of circulating seasonal influenza viruses by this novel virus. Materials and Methods: In-house seasonal and pandemic influenza-specific polymerase chain reaction assays were introduced and/or developed at the Molecular Diagnosis Centre (MDC) at the National University Hospital (NUH), Singapore. These assays have been used to test all samples received from in-patients, out-patients, staff and visitors for suspected pandemic influenza A/H1N1/2009 infection. Results: Prior to the arrival of the pandemic A/H1N1/2009 virus in Singapore at the end of May 2009, seasonal influenza A/H3N2 predominated in this population, with very little seasonal influenza A/H1N1 and B viruses detected. Within about 1 month of its arrival in Singapore (mainly during June to July 2009), this pandemic virus rapidly displaced seasonal influenza A/H3N2 to become the predominant strain in the Singaporean population served by MDC/NUH. Conclusions: Real-time molecular techniques have allowed the prompt detection of different influenza subtypes during this current pandemic, which has revealed the displacement/replacement of previously circulating seasonal subtypes with A/H1N1/2009. Although some of this may be explained by immunological cross-reactivity between influenza subtypes, more studies are required.

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Key words: Diagnostic, H1N1, Polymerase chain reaction

Introduction

The global preparedness that followed the severe acute respiratory syndrome (SARS) outbreaks of 2003 and the re-emergence of a potentially pandemic avian influenza A/H5N1, was still found to be inadequate to deal with the rapid spread of pandemic influenza A/H1N1/2009 as it emerged from the Americas in March/April 2009.[1-5]

One of the earliest, noticeable deficiencies was the lack of laboratory diagnostic capacity, which led to delays in true case ascertainment for H1N1/2009-infected individuals and therefore inaccurate estimates of case fatality rates (CFRs) early in the pandemic during April/May 2009.[6,7] The absence of any specific diagnostic assay for detecting this novel virus contributed to this delay, although a number of in-house assays were developed once the viral genome had been sequenced, which was achieved very early in the pandemic.[8-11] Even when such assays became available, suppliers were overwhelmed by the demands from diagnostic laboratories for reagents for these diagnostic polymerase chain reaction (PCR) and sequencing assays.

This article will examine the laboratory testing outcomes of this pandemic as experienced at the Molecular Diagnosis Centre (MDC), National University Hospital (NUH) in Singapore.

Materials and Methods

Details of the patients and samples received for testing as well as the diagnostic methodologies have been described elsewhere,[12] so only a brief summary is given here.

Patient Samples

From the emergence of the pandemic influenza A/H1N1/2009 strain in April/May 2009, samples were received from in-patients and out-patients, as well as staff who had travelled to and returned from A/H1N1/2009-endemic areas.

[1] Molecular Diagnosis Centre, Department of Laboratory Medicine, National University Hospital, Singapore
[2] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[3] Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
Address for Correspondence: Dr Julian W Tang, Department of Laboratory Medicine, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074.
Email: jwtang49@hotmail.com, julian_wt_tang@nuh.com.sg

Each of these samples consisted of a flocked nasopharyngeal swab (NPS)[13] that was collected in 3 mL of virus transport medium (VTM), using standard collection techniques.[14] These constituted the bulk of the samples received. Others included bronchioalveolar lavages (BALs), endotracheal aspirates (ETAs) and occasional cerebrospinal fluid (CSF) samples. Only the results from the respiratory samples are presented in the Results section below.

Diagnostic Testing

The diagnostic testing strategy consisted of separate in-house, reverse transcription-polymerase chain reaction (RT-PCR) tests for: i) all influenza A (human, avian and swine species – targeting the matrix protein gene) and B (human) viruses, ii) seasonal influenza A subtyping (into H1 or H3 targeting the haemagglutinin gene) and iii) pandemic influenza A/H1N1/2009 (targeting both the haemagglutinin and nucleoprotein genes). Each of these assays could be performed sequentially or simultaneously, depending on the demand for specific results. Hence, for each diagnostic specimen received, influenza subtyping into seasonal H1 (presumed N1), H3 (presumed N2) and pandemic A/H1N1/2009 was performed. During periods of high demand in the earlier stages of the pandemic, this assay was run 3 times per day. For timely reporting of A/H1N1/2009 positive results, the screening assay (i) was run in parallel, simultaneously with the A/H1N1/2009-specific assay, and subtyping for seasonal influenza viruses (A/H1N1 and A/H3N2) was performed later, when time permitted. The overall sensitivity and specificity of the A/H1N1/2009 assay was approximately 97.7% and 100% in validation studies using cloned plasmid of the PCR target regions (more details are available from the authors on request). The sensitivity/specificity of this assay with actual clinical samples may vary from these optimal values obtained with cloned plasmids, depending on the quality of the samples and the presence of other biological inhibitors to PCR.

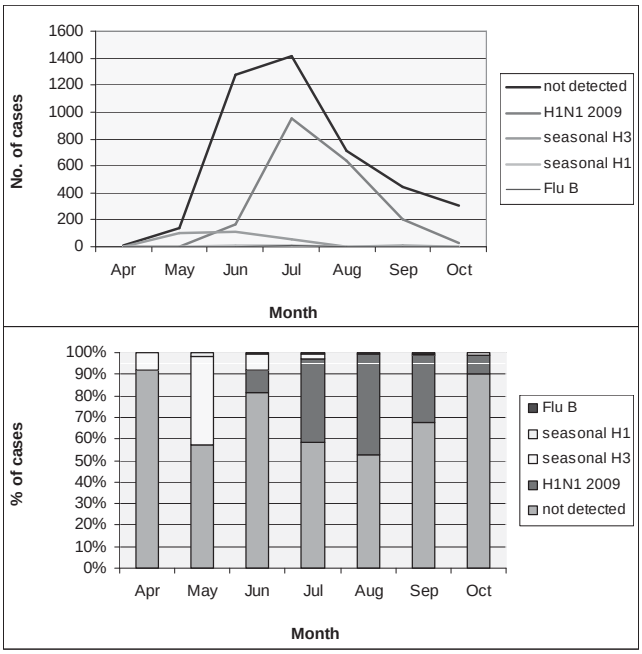

Fig. 1. Comparison of diagnostic testing results for clinical samples received for influenza testing since the beginning of the influenza A/H1N1/2009 ("H1N1 2009" in the figure) pandemic period at MDC/NUH.

Results

Table 1 and Figure 1 show the outcome of the influenza testing protocol, as performed on all clinical samples received to date. Influenza screening started in April 2009 with the emergence of the pandemic in the Americas at that time, but this novel virus did not reach Singapore and cause significant numbers of infections until the end of May 2009.

Just prior to the emergence of the pandemic strain A/H1N1/2009 from April 2009, it can be seen that seasonal influenza A/H3N2 predominated in this population, with very low background levels of influenza A/H1N1 and B. Plotting the results by percentage positives over time clearly reveals the initial apparent displacement and then replacement of seasonal influenza A/H3N2 as the continuing, predominant circulating subtype in Singapore by the novel pandemic virus A/H1N1/2009 (Fig. 2).

Although the novel virus is still the predominant strain in the number of samples testing positive for influenza,

Table 1. Number/Percentage of Cases of Influenza of Different Subtypes Diagnosed Since the Beginning of the Influenza A/H1N1/2009 Pandemic Period at Molecular Diagnosis Centre (MDC), National University Hospital (NUH)

	April		May		June		July		August		September		October	
No. not detected	11	91.67%	140	57.38%	1275	81.57%	1417	58.26%	714	52.58%	446	67.47%	308	90.32%
No. of A/H1N1/2009	0	0.00%	0	0.00%	165	10.56%	948	38.98%	638	46.98%	206	31.16%	30	8.80%
No. of H3	1	8.33%	100	40.98%	110	7.04%	56	2.30%	4	0.29%	7	1.06%	3	0.88%
No. of H1	0	0.00%	4	1.64%	11	0.70%	6	0.25%	0	0.00%	2	0.30%	0	0.00%
No. of flu B	0	0.00%	0	0.00%	2	0.13%	5	0.21%	2	0.15%	0	0.00%	0	0.00%
Total	12	100.00%	244	100.00%	1563	100.00%	2432	100.00%	1358	100.00%	661	100.00%	341	100.00%

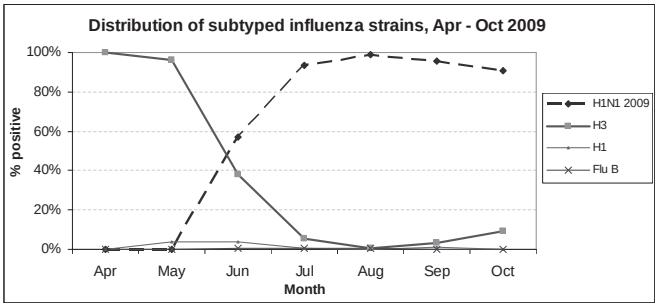

Fig. 2. Comparison of percentage positive by influenza subtypes, including the new pandemic strain H1N1/2009, since the beginning of the influenza A/H1N1/2009 ("H1N1 2009" in the figure) pandemic period at MDC/NUH.

since the end of July, the number of samples received for testing has dropped by about 50% from a peak of 2432 samples received during July to about 341 in October 2009. This may be due to a change in surveillance and therefore sampling strategies in this population. This overall trend at MDC/NUH is generally reflective of the national pattern in Singapore, which showed a sharp rise in cases of pandemic A/H1N1/2009 towards the end of July followed by a steady decline.[15]

Conclusions

This article summarises the results of diagnostic testing for the novel influenza A/H1N1/2009 as well as seasonal influenza viruses during the first 6 months of this pandemic at the MDC/NUH, Singapore. It is an example of a rapid, molecular diagnostic response to a new emerging virus, which has tracked the emergence and gradual dominance of the pandemic influenza A/H1N1/2009 virus in this population. This phenomenon has been observed with past influenza pandemics and warrants some further discussion here.

The displacement and then subsequent replacement of previously circulating "seasonal" influenza viruses by a new pandemic virus has been described following previous pandemics, e.g. in 1957, the A/H2N2 virus eventually replaced the previous 1918 A/H1N1 subtype, and the 1968 A/H3N2 virus subsequently replaced the 1957 A/H2N2 subtype. Yet most of the studies to date have examined the displacement and replacement of different inter-pandemic, seasonally epidemic influenza A strains via antigenic *drift*, revealing how individual subtypes of seasonal influenza viruses, e.g. A/H3N2 and A/H1N1 change from year to year. Many of these investigations have used phylogenetic and antigenic methods to study influenza A/H3N2 because it has been the predominant subtype over at least the last 10 years (except for the 2001/2002 season).[16-21]

There have been far fewer studies focusing on the much longer timescale, involving the larger antigenic *shift*, that is, the phenomenon of displacement and replacement of currently circulating influenza subtypes by incoming *pandemic* viruses. Perhaps because such events are much rarer, studies examining this pandemic influenza virus displacement/replacement of contemporary circulating seasonal influenza strains have tended to be largely theoretical, and are explored using mathematical modelling.[22-25] However, the underlying mechanisms may also be similar to those that govern the antigenic drift variation of the seasonal influenzas from year-to-year, that is, partial short- and long-term host cross-immunity between these strains, with varying degrees of viral immune escape.

The immunological cross-reactivity mechanism postulated for the eventual displacement/ replacement of the previously circulating seasonal influenza subtypes may involve the partial cross-reactivity of the more conserved internal viral proteins, such as the matrix protein and the nucleoprotein. These proteins have been targeted recently for a more "universal" influenza vaccine.[26-28]

However, such partial cross-immunity does not adequately explain why previous circulating strains have been completely replaced, yet at the same time, explain the reintroduction in 1977 (perhaps by a laboratory accident) and subsequent persistent and successful co-circulation of the seasonal A/H1N1 virus to this day – a period of over 30 years.[22,29] Also, it is important to note that whilst most studies refer to antibody cross-reactivity, this may be due to the predominance of serological testing using haemagglutination-inhibition (HAI) and microneutralisation (MN) assays, and long-term T cell-mediated immunity may exist in older populations exposed to previous viruses similar to any contemporary pandemic strain. This has been suggested recently for the current A/H1N1/2009 virus in those born before 1957.[30]

Therefore, whilst this study contributes additional data from a tropical region, providing another example of this influenza displacement/replacement process, more studies are required to better understand the mechanisms underlying this phenomenon. At the least, the availability of modern molecular diagnostic techniques will allow us to track the evolution of this new pandemic influenza A/H1N1/2009 virus in the context of the co-circulating seasonal influenza viruses, A/H3N2 and A/H1N1, as the current pandemic continues to unfold. This may help to answer some of these questions.

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