Influenza disease burden in adults by subtypes following the initial epidemic of pandemic H1N1 in Singapore

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Peaks of influenza activity in July 2009 and January 2010 were >90% pandemic H1N1 (pH1N1), but by May 2010, H3N2 predominated in hospital attendances (46.5%, versus 38.9% pH1N1); H3N2 hospital attendances were older (72.9% aged ≥60 years versus 13.5% for pH1N1), but the age-stratified proportions admitted for pneumonia]were similar. As at the end

of the third epidemic wave in Singapore, pH1N1 cases in hospital attendances were still markedly younger than cases of H3N2 or influenza B, with little evidence for any changes in severity.

Keywords Adults, influenza subtypes, pandemic H1N1, Singapore.

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Introduction

In April 2009, pandemic H1N1 (pH1N1), a virus of swine origin, surfaced in Mexico and the United States. It then spread rapidly around the world.^{1–3} Singapore identified its first imported case of pH1N1 on 26 May 2009.⁴ Local transmission was detected in mid-June 2009, followed by a single epidemic wave, which peaked in late July and subsided by September. During this period, only 18 deaths were identified in a population of about 4 million,⁵ although this only includes confirmed cases and may underestimate mortality.⁶

Given the experience of previous pandemics,^{7,8} there are concerns as to whether subsequent pH1N1 epidemic waves of greater severity will occur. In addition, the re-appearance of new influenza subtypes has historically been associated with the replacement of the previously dominant influenza A subtype,⁹ and the evolving relationship between pH1N1 and H3N2 is hence of some interest. Tropical Singapore is receptive to influenza year round with traditional bimodal peaks around May and January¹⁰ and is a key travel hub in South-East Asia, a significant region in the global ecology of influenza subtypes.¹¹ Following the 2009 autumn and winter epidemics of pH1N1 in the Northern hemisphere, there has been little data published on influenza circulation

in tropical South-East Asia. We describe influenza activity in tropical Singapore after the initial epidemic wave, which may provide clues on any changes in the epidemiology and severity of pH1N1-related disease, as well as the relative future contribution of pH1N1 and H3N2 to the burden of disease from influenza virus infections.

Methods

Study population & data sources

Communicable Disease Centre (CDC) is the national clinical management arm in outbreak response. It is administratively under Tan Tock Seng Hospital (TTSH), a 1200-bed acute tertiary care general hospital for adults in Singapore, and was the designated screening center for treatment and isolation of adult pH1N1 cases identified during the containment phase of the pandemic.

Testing for influenza virus, including subtyping, was performed with multiplex reverse transcription polymerase chain reaction (RT-PCR) with the “Multiplex Swine H1N1, Flu A, Flu B and H3N2” kit (Cat. No. AITB4PSWQ-200; AitBiotech, Singapore), a 4-plex real time RT-PCR assay that simultaneously tests for Influenza A and B with H3N2 and H1N1 (2009) specific subtyping. Unpublished data show it to be more sensitive compared with CDC and

Roche assays marketed for H1N1 (2009). Samples for influenza virus testing came from several sources. Routine Influenza samples were combined nasal and throat-flocked swabs (Copan, Brescia, Italy) in liquid transport media (RT-UTM; Copan). The enhanced surveillance described in our prior study screened all patients presenting at the Emergency Department with an influenza-like illness until February 2010.¹² Inpatient hospital surveillance activities continued throughout the study period, whereby any respiratory samples (sputa, bronchial lavage, and tracheal aspirates) submitted for bacterial culture within 48 hours of admission were screened for influenza by RT-PCR if the patient had not already been tested for Influenza. Nucleic acids were extracted with a NucliSENS[®] easyMAG[®] instrument (Biomérieux, Singapore) or with the EZ1 virus mini-kit v2.0 on an EZ1 Advanced XL instrument (Qiagen, Singapore). Finally, the study also included samples submitted by clinicians from the Emergency Department and all TTSH specialist outpatient clinics based on clinical suspicion of influenza.

The Chairman of TTSH Medical Board approved the surveillance program and our study.

Data analysis

For the purpose of comparison, we defined three periods in our study: Period 1, mitigation phase of initial pH1N1 epidemic wave from weeks 27 to 37 in 2009; period 2, initial post-pandemic period from week 38 in 2009 to week 11 in 2010; and period 3, 2010 influenza epidemic season from weeks 12 to 37 in 2010. We analyzed data on all samples positive for influenza virus by RT-PCR from TTSH hospital attendances (including all tests performed on outpatients and inpatients), discarding repeat samples from the same patient within 14 days. In addition, we also analyzed the subset of attendances requiring admission for pneumonia as a reasonably objective indicator of severity; notably, 70.2% of these admissions had RT-PCR tests for influenza virus in the study period. Trends in influenza positive cases for a 1-year period (20 September 2009 to 18 September 2010, epidemiological weeks 38–37) subsequent to the initial epidemic of pH1N1 were also compared with data from the National Public Health Laboratory, Singapore. These were based on attendances for influenza-like illness (acute respiratory symptoms with documented temperature $>38^{\circ}\text{C}$) at sentinel general practice (GP) clinics, restricted to data from adults (age ≥ 16 years). We also performed age-stratified analysis on TTSH cases testing positive for different influenza subtypes in the three different time periods, with both the three time periods and age categories analyzed as ordinal variables using chi-squared test for trend in SPSS software version 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Figure 1 presents the number of influenza cases in GP attendances, hospital attendances, and pneumonia admissions. Following the initial pH1N1 epidemic ending in September 2009, we saw typical seasonal patterns¹⁰ with a minor pH1N1 epidemic peaking in January 2010 (week 1) and a larger epidemic of mixed pH1N1 and H3N2 activity peaking in May 2010 (week 19); influenza B circulated at low levels throughout except for a small peak in June 2010 (week 24) and in May 2010 (week 22) in GP (Figure 1A) and hospital attendances, respectively (Figure 1B). Interestingly, in the influenza epidemic season of 2010 (period 3: weeks 12–37 in 2010), there was more pH1N1 than H3N2 in GP samples: 45.7% of the typeable samples were pH1N1, with 32.7% being H3N2 and the remaining 21.6% being B subtypes. Influenza activity in hospital samples lagged behind that in GP samples.

Criteria for cases seen at and admitted to hospital switched from public health to medical indications in week 27 in 2009, in line with the transition from pandemic containment to mitigation measures in Singapore.⁵ From week 27 in 2009 to the end of our study period (September 18, 2010, week 37), of 11 513 attendances tested, 1092 were positive for pH1N1 (9.5%), 377 for H3N2 (3.3%), and 145 for B influenza (1.3%) viruses; and of 1998 pneumonia admissions tested, 158 were positive for pH1N1 (7.9%), 80 for H3N2 (4.0%), and 14 for B influenza viruses (0.7%). Figure 2 stratifies the hospital data by age and subtypes and further analyzes pH1N1 over three periods. Tests for linear trend suggests a statistically significant shift in the distribution of pH1N1 attendances over time toward older ages ($P < 0.001$, see Figure 2A); a similar (but non-significant) trend was also observed for pneumonia admissions ($P = 0.245$, see Figure 2B). Hospital attendances for pH1N1 were significantly younger than those for H3N2 and influenza B ($P < 0.001$ for both pH1N1 versus H3N2 and pH1N1 versus B, chi-squared test for trend); likewise, pneumonia admissions for pH1N1 were significantly younger than those for H3N2 and influenza B ($P < 0.001$ for pH1N1 versus H3N2, $P = 0.005$ for pH1N1 versus B, chi-squared test for trend). Without accounting for differences in age, H3N2 appears significantly more severe (Figure 2C), with 21.5% of attendances requiring admission for pneumonia compared with 14.5% for pH1N1 and 8.9% for influenza B ($P = 0.002$ for H3N2 versus pH1N1, $P < 0.001$ for H3N2 versus B, chi-squared test). However, stratified analysis reveals that age-specific pneumonia admission ratios were not significantly different between subtypes, except in those aged 30–59, where pH1N1 attendances were significantly more likely to require admission for pneumonia than H3N2 ($P = 0.021$, chi-squared test).

Figure 1. Temporal trends in influenza activity from reverse transcription polymerase chain reaction testing for respiratory samples. (A) General practice attendances, (B) Hospital attendances, and (C) Pneumonia admissions. Number of samples positive for pH1N1, H3N2, and influenza B subtypes are given as blue, red, and green lines, respectively.

Discussion

Ongoing surveillance revealed mixed activity in the 2010 season, with pH1N1 and H3N2 both contributing substantially to the burden of hospitalization among adults in Singapore. There was a subtle shift toward an older age distribution over three successive epidemic waves of pH1N1, but no marked differences in severity by time period or influenza subtype.

While over-representation of H3N2 in hospital compared with GP samples may give the impression that H3N2 is more severe than pH1N1, age-stratified analysis suggests this was largely because of the marked differences in the age distribution of infections. More robust estimates for case hospitalization and case fatality ratios for the two subtypes will require better estimates of incidence, either using age-stratified incidence from symptomatic disease¹³ or serological surveys.¹⁴

Figure 2. Distribution of influenza cases in Tan Tock Seng Hospital attendances (A) and pneumonia admissions (B), and the proportion of attendances requiring admission for pneumonia (C). Data are stratified by age (as colored bars), subtype, and for pandemic H1N1, the period of diagnosis (on X-axis). Period 1 is mitigation phase of initial pandemic H1N1 epidemic wave from weeks 27 to 37 in 2009; period 2 is the initial post-pandemic period from week 38 in 2009 to week 11 in 2010; period 3 is the 2010 influenza epidemic season from weeks 12 to 37 in 2010. Error bars in (C) give the 95% confidence intervals for the proportions requiring admission for pneumonia, as computed by the Wilson-score method, while *P*-values in (A) and (B) are computed using chi-squared test for trend, with age category and sequential time periods as ordinal variables when comparing distribution across time, and the influenza subtype as a categorical variable in pair-wise comparisons between subtypes.

Other limitations of our study that must be acknowledged include our choice of study population, because our study did not include any data from pediatric populations, being restricted to adults attending an acute tertiary care general hospital. Also, the lack of standard case

definitions meant that there might be biases in the selection of patients to be tested for influenza; this may be an issue, particularly with regards to the pH1N1 data because there may have been subtle shifts in clinical practices across the study period. However, we did also subanalyze our data restricting to pH1N1 infections identified in period 3 and arrived at essentially the same conclusions that the age distribution of pH1N1 infections was significantly younger than that for H3N2 and B influenza virus subtypes, and that the fraction of hospital attendances requiring admission for pneumonia was not significantly different, except for some suggestion that in younger age groups, pH1N1 infections were more likely to be admitted for pneumonia.

A shift in disease burden toward older ages was observed following the introduction of previous pandemic influenza subtypes,¹⁵ and our data may be an early empirical demonstration of this phenomenon. However, as at the end of the third epidemic wave in Singapore, pH1N1 cases in hospital attendances were still markedly younger than cases of H3N2 or influenza B, with little evidence for any changes in severity.

Conclusion

To conclude, continued surveillance and research into the diverse behavior and global circulation of influenza subtypes are needed, even as pH1N1 evolves to take its place among seasonal influenza subtypes in the post-pandemic period.

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