

# Influenza A viruses dual and multiple infections with other respiratory viruses and risk of hospitalisation and mortality

Edward Goka,<sup>a</sup> Pamela Vallely,<sup>b</sup> Kenneth Mutton,<sup>a,b</sup> Paul Klapper<sup>a,b</sup>

<sup>a</sup>Institute of Inflammation and Repair, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK. <sup>b</sup>Department of Clinical Virology, Central Manchester University Hospitals – NHS Foundation Trust, Manchester, UK.

*Correspondence:* Edward Goka, Institute of Inflammation and Repair, Faculty of Medical and Human Sciences, 1st Floor Stopford building, University of Manchester, Oxford Road, Manchester, M13 9PL, UK.

E-mail: edward.goka@postgrad.manchester.ac.uk

Accepted 22 August 2012. Published online 19 October 2012.

**Introduction** Recent literature suggests that dual or multiple virus infections may affect disease severity. However, few studies have investigated the effect of co-infection with influenza A viruses.

**Objectives** To identify the association between influenza A and respiratory viruses co-infections with disease outcome.

**Methodology** Data for samples from North West England tested between January 2007 and June 2011 was analysed for patterns of co-infection between influenza A viruses and eight respiratory viruses. Risk of hospitalisation to ICU or general ward in single versus co-infections was assessed using logistic regression.

**Results** Of the 25 596 samples analysed for respiratory viruses 40.7% (10 501) were positive for any virus. Co-infections were detected in 4.7% (137/2879) of all patients with influenza A(H1N1)pdm09, and 7.3% (57/779) of those with other influenza A virus infections. Co-infection between seasonal influenza A viruses and influenza B virus was associated with a significant

increase in the risk of admission to ICU/death (OR: 22.0, 95% CI: 2.21–219.8,  $P = 0.008$ ). Respiratory syncytial virus/influenza A (RSV/Flu A) co-infection also increased this risk but was not statistically significant. For influenza A(H1N1)pdm09, RSV and AdV co-infection increased risk of hospitalisation to general ward whereas Flu B increased risk of admission to ICU, but none of these were statistically significant.

**Conclusion** Co-infection is a significant predictor of disease outcome; combined treatment, introduction of an integrated vaccine for all respiratory viruses and development of multi-target rapid diagnostic tests is recommended. Integration of respiratory viruses' co-infections into public health reports could also contribute to the accumulation of evidence.

**Keywords** Co-infection, dual or multiple infections, influenza A viruses, Influenza B virus, respiratory virus infections.

Please cite this paper as: Goka et al. (2013) Influenza A viruses dual and multiple infections with other respiratory viruses and risk of hospitalisation and mortality. *Influenza and Other Respiratory Viruses* 7(6), 1079–1087.

## Introduction

Influenza virus and other acute respiratory tract infections (ARTIs) cause considerable mortality and morbidity worldwide. In each winter season between 10% and 20% of populations suffering from influenza<sup>1</sup> and at least 2.2 million deaths occurring from ARTI throughout the world.<sup>2</sup> In the United States of America (USA), influenza is responsible for 20–40 million outpatient visits, 330 000 hospitalisations and 40 000 deaths annually.<sup>3</sup> In the United Kingdom, a study on GP consultations and hospitalisation for influenza estimated that influenza causes about 779 000 (95% CI  $\pm$  258 000) to 1 164 000 (95% CI  $\pm$  425 000) general practice consultations, 19 000 (95% CI  $\pm$  5000) to 31 200 (95% CI  $\pm$  11 000) hospital admissions and 18 500 (95% CI  $\pm$  2500) to 24 800 (95% CI  $\pm$  2500) deaths annually.<sup>4</sup>

Although respiratory virus infections have traditionally been thought to be caused by single viruses, an increasing number of reports have reported respiratory diseases occurring as dual or multiple virus infections.<sup>5–14</sup> There are suggestions that respiratory viral co-infections affect disease severity with some studies suggesting that dual and multiple infections increase severity of respiratory disease,<sup>6,7,10,12,14</sup> while others have found either no association<sup>15–19</sup> or that dual or multiple infections may actually be protective.<sup>8</sup> Influenza A viruses cause varying disease severity in different persons. For example, the pandemic influenza A(H1N1)pdm09 virus caused widely differing outcomes ranging from mild respiratory illness to severe disease or death in some cases.<sup>20,21</sup> Thus, while there is currently conflicting data on the subject, most studies have been in infants <1 year old where the infection pattern is different from

adults (due to pre-existing passive immunity in adults), and very few studies have investigated the effect of co-infection in influenza A viruses on disease outcome. This study aimed to identify the association between the pandemic influenza A(H1N1)pdm09 and seasonal influenza A viruses, dual or multiple infections with other respiratory viruses and severity of influenza disease.

## Methodology

### Study design and setting

Electronic data on samples that were sent to the Manchester Microbiology Partnership Laboratory (MMPL) in North West England, for viral detection, between 1 January 2007 and 23 June 2011, were interrogated to determine the association between a positive diagnosis of influenza A(H1N1)pdm09 or other influenza A viruses, and influenza B virus (Flu B) or other respiratory viruses including respiratory syncytial virus (RSV), rhinoviruses (RV), adenoviruses (AdV), human metapneumovirus (hMPV) and parainfluenza viruses 1–3 (PIV 1–3), and severity of influenza disease. The MMPL is a reference virology laboratory for the North West of England receiving respiratory viral samples from hospitals, medical centres and surgeries located in the region and serves a population of 6.9 million people.<sup>22</sup>

The database records only results for samples from patients whose reason for admission or medical consultation was due to respiratory virus infection. Apart from indicating types of all tests that were requested and results of testing, the database also contains information on patients' age (in years), date sample was collected, date received, medical facility that submitted the sample or the secondary locations and the type of sample that was submitted. A one-step reverse transcriptase real-time polymerase chain reaction (RT-PCR) protocol was used in the identification of respiratory viruses. Nucleic acid was extracted from samples using the Qiagen total nucleic acid extraction kit run on the Qiagen Biorobot MDX (Qiagen, Crawley, UK). Reverse transcription was accomplished using the Invitrogen Superscript III platinum one-step RT-PCR kit (Invitrogen, Paisley, UK). Influenza A and B viruses were tested using a duplex assay and additionally for influenza A H1N1 using the Health Protection Agency (HPA (H1)v) assay.<sup>23</sup> Duplex assays of well-characterised 'in-house' RT-PCR assays (unpublished) were used for the identification of respiratory syncytial virus, metapneumoviruses, adenoviruses, rhinoviruses and triplex assays for parainfluenza viruses 1–3. All the PCRs were run on the ABI7500 real-time PCR instrument (Applied Biosystems, Warrington, UK). Positive amplification was determined using amplicon-specific probes labelled with MGB or TAMRA (Applied Biosystems). Samples that were submit-

ted by attending physicians for respiratory virus identification included the following: nose and throat swabs (VNT), throat swabs (VTS), nasopharyngeal aspirates (VNPA) and other types of swabs taken from the mouth and nose area (VSW).

All patients with a positive PCR test for influenza A(H1N1)pdm09 virus or seasonal influenza A viruses who were hospitalised, seen as outpatients or who died within the study period were eligible for inclusion. All entries where outcome data were missing, that is, either because PCR test was not performed or where there was insufficient sample, were excluded from the analysis. In addition, for the calculation of measures of association, entries that did not have information as to whether the patient was seen as an outpatient, admitted to general ward or the ICU were omitted.

### Statistical analysis

Descriptive statistics were computed to describe co-infection patterns among different age and sex, and the differences calculated using Pearson's chi-square ( $\chi^2$ ) statistic. The measure of outcome was the risk of hospitalisation to a general ward or the ICU or death, and the exposure of interest was whether or not the patient had dual or multiple respiratory viral infections. Association between influenza A viruses and respiratory viruses co-infection and risk of hospitalisation and admission to ICU or death was assessed using simple logistic regression models. Further, multivariate logistic regression models, including age and interaction term as covariates, were employed, and the significance of the covariate was assessed using the log rank test. For each of the co-infections with respiratory viruses, separate multivariate logistic models, controlling for age and an interaction term between age and co-infections, were applied to assess the risk of hospitalisation using other respiratory virus single infections as the baseline. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs) with significance level of  $P = 0.05$ . All analyses were performed using the STATA software, version 11.0. (STATA Corp, College Station, TX, USA).

The study received ethical approval from the National Health Service – Research Ethics Committee (NHS-REC), reference number 11/NW/0698 and from the University of Manchester Research Ethics Office. In addition, the Central Manchester Universities Hospitals NHS Foundation Trust also granted Research and Development approval for this project (reference number R01835).

## Results

### Identification of influenza and respiratory viral infections from Jan 2007–June 2011

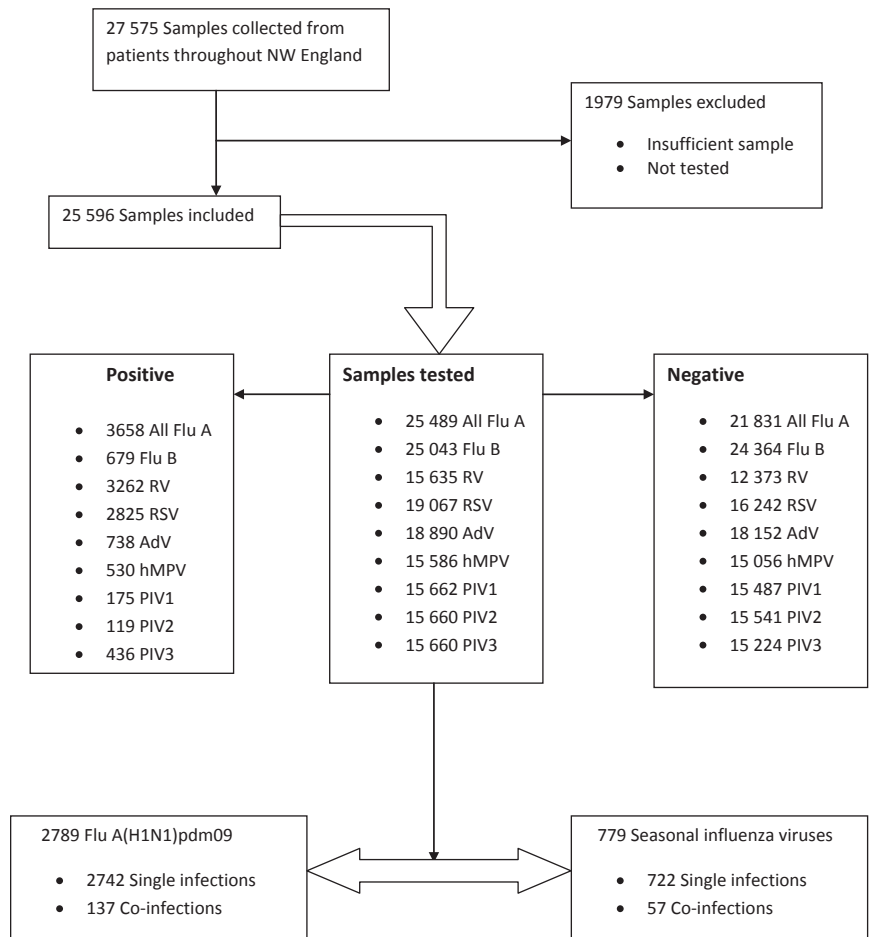
Of the 27 575 samples that were received at the Manchester Microbiology Partnership Laboratory (MMPL) between the

10 January 2007 and 23 June 2011, a large proportion, that is, 25 596 (92.8%), had complete information on the RT-PCR results for respiratory viral infections and were included in the analysis and 1979 (7.2%) had this information missing and were excluded. Of the 25 596 samples included in this study, 40.7% (10 501) were positive for any of the respiratory viruses of which 14.4% (3658/25 489) were positive using a generic influenza A PCR with 2879 typed as influenza A(H1N1)pdm09 virus and 779 as seasonal influenza A viruses. The positivity rates for the other respiratory infections were as follows: influenza B 2.7% (679/25 043), RV 20.9% (3262/15 635), RSV 14.8% (2825/19 067), AdV 3.9% (738/18 890), hMPV 3.4% (530/15 586), PIV1 1.1% (175/15 662), PIV2 – V 0.8% (119/15 660) and PIV3 2.8% (436/15 660) (Figure 1).

**Characteristics of included and excluded patients**

There was a significant difference in age between included and excluded patients such that young patients were more

likely to be included than adults. For example, 35.0% (8959/25 595) of included patients were infants <1 year old versus 19.7% (389/1979) among excluded patients. The age differences for included and excluded patients for the other age groups were 6.5% versus 4.0 in 1- to 5-year-olds, 8.3% versus 5.2% in 5- to 17-year-olds, 7.8% versus 8.9% in 17- to 25-year-olds, 12.6% versus 15.7% in 25- to 40-year-olds, 17.6% versus 29.1% in 40- to 65-year-olds, 6.5% versus 15.0% in 65- to 85-year-olds and 5.6% versus 2.5% among those >85-year-old, and these differences were statistically significant ( $P < 0.0001$ ). However, there were no significant gender differences between the two groups (% female included: excluded = 48.7% versus 50.0%,  $P = 0.285$ ). The difference in number of samples that were tested for different viruses, that is, 25 489 for all influenza A viruses and 15 660 for PIV 2 and 3, was because, in compliance with ethical issues, only tests requested by the physicians who submitted the samples were conducted.



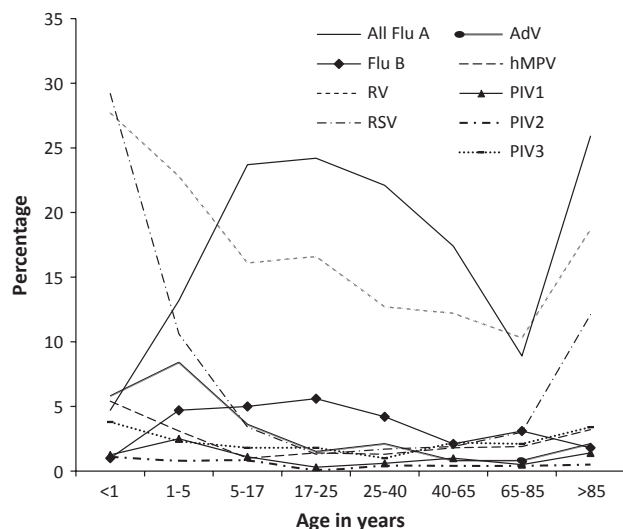
Notes: All Flu A = All influenza A viruses i.e. both pandemic influenza A(H1N1)pdm09 and seasonal influenza A viruses, RV = rhinovirus, RSV = respiratory syncytial virus, AdV = adenovirus, MPV = human metapneumovirus, PIV1 – 3 = human parainfluenza virus types 1 to 3

**Figure 1.** Schematic diagram of patient inclusion and exclusion.

## Respiratory virus positivity rates and patients demographics

The positivity of influenza A and B virus infection was significantly lower in infants <1 year of age but peaked in 5- to 17-year-olds and in people aged 17–65 years: (positivity proportions 4.7% versus 17.4%–23.7% for influenza A virus infection and 1.0% versus 4.7%–5.6% for influenza B virus infection, respectively). It was lower among those aged 65–85 years, presumably because of immunity derived from previous infections with strains antigenically related to the influenza strains that circulated between 2009 and 2011, but increased in those older than 85 years. On the other hand, other respiratory viruses were found predominantly more in children <5 years old (Figure 2). Consequently, the majority of study participants who were positive for RV, RSV, AdV, hMPV and PIV 1–3 were aged ≤5 years, whereas participants who were positive for influenza A, and B were mainly adults. These differences were statistically significant –  $P = <0.0001$  (Table 1).

Regarding positivity rates by gender, males were more likely to have a positive diagnosis of other respiratory viruses than females, and apart from PIV1 and PIV3, these differences were statistically significant (Flu A  $P = <0.0001$ , Flu B  $P = 0.007$ , RV  $P = 0.001$ , RSV  $P = 0.02$  and AdV  $P = 0.003$  and hMPV  $P = 0.004$ , PIV2  $P = 0.04$ ). However, such differences were not observed in influenza A and B virus infections (data not shown). The gender-related differences in positive rates for other respiratory viruses may relate to more men than women consulting medical services, for example, 8286 male samples were submitted for identification of rhinovirus compared with 7153 female



Notes: Age groups were demarcated in such a way as to make the best depiction of the distribution of respiratory viruses taking into account previously published literature indicating that identification of respiratory viruses is higher among children <1 year old and among school going children. People aged 25–40 were grouped together as they are generally considered fit, those aged 40–65 starting to age, 65–85 aged and >85 very old and very susceptible to respiratory infections

Figure 2. Respiratory virus' positivity rates by age group.

Table 1. Demographic characteristics of patients positive for any respiratory virus

Characteristics (n = 10 501)	n	%	P
Age ≤5			
All Flu A	639	17.5	<0.0001
Flu B	173	25.5	<0.0001
RV	2307	70.7	<0.0001
RSV	2593	91.8	<0.0001
AdV	586	79.4	<0.0001
hMPV	410	77.4	<0.0001
PIV1	120	68.6	<0.0001
PIV2	89	74.8	<0.0001
PIV3	312	71.6	<0.0001
Male sex			
All Flu A	1561	47.6	<0.0001
Flu B	306	46.2	0.007
RV	1819	56.3	0.001
RSV	1517	54.7	0.021
AdV	409	56.8	0.03
hMPV	315	59.4	0.004
PIV1	104	59.8	0.104
PIV2	75	63.0	0.04
PIV3	251	57.7	0.086

$\chi^2$ , Pearson's chi-square test, significance was at  $\alpha = 0.05$ ; All flu A, influenza A (H1N1)pdm09 and seasonal influenza A viruses; RV, rhinovirus; RSV, respiratory syncytial virus; AdV, adenovirus; hMPV, human metapneumovirus; PIV1–3, human parainfluenza types 1–3.

samples, and the male/female ratios for the other samples were as follows: influenza B 12 145/11 492, RSV 9899/8909, AdV 9805/8827, hMPV 8191/7176, PIV1 8301/7167, PIV2 8299/7167 and PIV3 8299/7167. An alternative explanation is that gender is a risk factor for respiratory virus infection. Because positivity was biased towards males, it is not surprising that the majority of study subjects, positive for any of the other respiratory viruses were males (Table 1). The gender differences were statistically significant.

## Respiratory viral infections yield by type of sample

Influenza viruses were mainly isolated from nose and throat swabs (VNT), with half (50.3%) of the Flu A viruses and 44.3% of Flu B viruses identified from VNT. The majority of respiratory viruses, that is, rhinoviruses (59.3%), RSV (74.9%), AdV (53.9%), hMPV (66.0%) and PIV 1–3 (47.4%–72.3%), were identified from nasopharyngeal aspirates (VNPA). Swabs where the site of swabbing was not given (VSW) and throat swabs (VTS) yielded almost equal proportions of influenza and respiratory viruses. This trend is probably because more VNTs were obtained from adults. The physiological changes of the

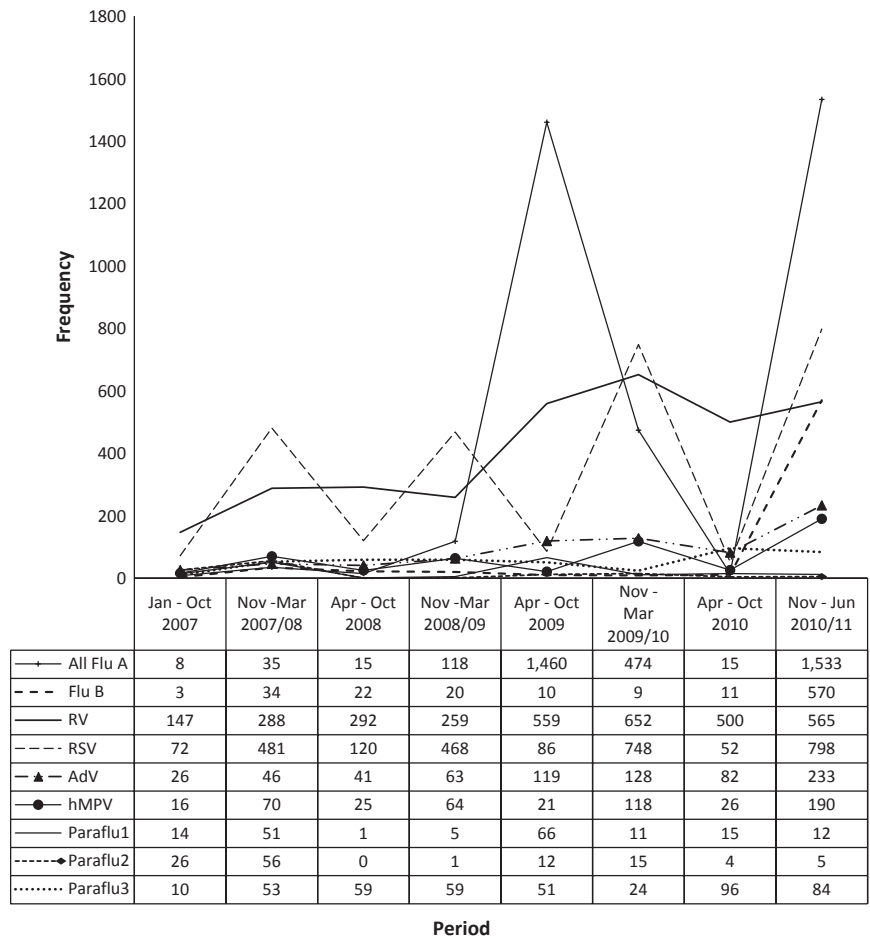
nasopharynx (narrowing of the orifice) and other morphological changes beginning at 6 months and continuing through adolescence make it painful to draw adequate nasopharyngeal aspirates in adults.<sup>24</sup>

**Seasonal distribution of respiratory viral infections**

Influenza A viruses, rhinoviruses, adenoviruses and parainfluenza type 3 viruses showed no distinct seasonal patterns. Specifically, there was little influenza A activity during the 2007/2008 summer season and 2008/2009 winter seasons. However, from April, 2009, a rise in the number of influenza A viruses was noted coinciding with the WHO declaration of the influenza A(H1N1)pdm09 pandemic. There was little influenza activity between April and October 2010, but the virus returned in the 2010/2011 winter season. Influenza B virus activity, however, was low during the study period, although the virus co-circulated with the influenza A(H1N1)pdm09 virus in winter 2010/2011. On the other hand, RSV and hMPV had a clear seasonal pattern, predominating during winter and subsiding during summer; and this trend was the same throughout the study period (Figure 3).

**Flu A(H1N1)pdm09 and seasonal Flu A, pattern of co-infections and patients demographics**

Co-infection was identified in 4.7% (137/2879) of patients who had influenza A (H1N1)pdm09 with rhinovirus as the most common, accounting for 39.4% (54/137) of all co-infections, followed by respiratory syncytial virus (27.7%; 38/137), adenovirus (10.2%; 14/137), human metapneumovirus (2.9%; 4/137), parainfluenza type 1 viruses (2.2%; 3/137) and parainfluenza type 3 viruses (0.7%; 1/137). Among patients who were positive for seasonal influenza A viruses, co-infections occurred in 7.3% (57/779). In this group, RSV was the most predominant virus, occurring in 36.8% (21/779) of all co-infections, followed by influenza B viruses (28.1%; 16/779), rhinoviruses (12.3%; 7/779), adenoviruses (8.8%; 5/779) and human metapneumovirus (7.0%; 4/779) (Tables 2 and 3). Thus, RSV co-infection was the highest among seasonal influenza A viruses, whereas rhinovirus was more frequent in influenza A(H1N1)pdm09 co-infections. The difference is likely due to the circulation of RSV only during winter seasons, while rhinoviruses circulate throughout the year. Few patients were infected by more than three respiratory viruses.



**Figure 3.** Seasonal distribution of respiratory viral infections January 2007–June 2011.

**Table 2.** Influenza A(H1N1)pdm09 co-infections, demographics and risk of hospitalisation, admission to ICU and death

Demographic & clinical characteristics	A/H1N1 only (n = 2742)	A/H1N1 + Flu B (n = 16)	A/H1N1 + RV (n = 54)	A/H1N1 + RSV (n = 38)	A/H1N1 + AdV (n = 14)
Age ≤ 5 years – n (%)	383 (15.2)	11 (68.8)	45 (83.3)*	35 (92.1)	14 (100)
Sex male – n (%)	1406 (53.6)	5 (31.1)	31 (57.4)	19 (50.0)	8 (57.1)
Hospitalised					
All – n (%)	1910 (76.2)	9 (75.0)	36 (73.5)	31 (86.1)	13 (92.2)
≤5 years – n (%)	315 (82.3)	4 (100)	17 (94.4)	24 (85.7)	10 (90.9)
>5 years – n (%)	1595 (75.1)	5 (62.5)	19 (61.3)	7 (87.7)	3 (100)
ICU/Dead					
All – n (%)	236 (28.4)	4 (57.1)	5 (27.8)	2 (28.6)	
≤5 years – n (%)	26 (27.6)	2 (100)	1 (50.0)	2 (33.3)	
>5 years – n (%)	210 (28.4)	2 (40.0)	4 (25.0)	0 (0)	

A/H1N1, influenza A(H1N1)pdm09 virus; Flu B, influenza B virus; RV, rhinovirus; RSV, respiratory syncytial virus; AdV, adenovirus. Difference in number of single respiratory virus and co-infections by age and sex was assessed using the chi-square test, \*means that the statistic was significant at  $\alpha = 0.05$ , single influenza A virus infection was used as a baseline for calculation of odds ratios, multivariate model adjusted for age and co-infection\*age mixed variable.

**Table 3.** Other influenza A virus co-infections, demographics and risk of hospitalisation, admission to ICU or death

Demographic & clinical features	NDFlu A only (n = 772)	NDFlu A + Flu B (n = 16)	NDFlu A + RSV (n = 21)	NDFlu A + AdV (n = 5)	NDFlu A + hMPV (n = 4)
Age ≤ 5 years, n (%)	120 (16.6)	14 (87.5)*	19 (90.5)*	5 (100)	2 (50.0)
Sex = Male, n (%)	221 (47.8)	8 (50.0)	6 (28.6)	1 (25.0)	3 (75.0)*
Hospitalised					
All – n (%)	607/705 (86.1)	11/12 (91.7)	16/20 (80.0)	4/5 (80.0)	3/4 (75.0)
≤5 years – n (%)	94 (81.0)	4 (100)	14 (82.4)	3 (100)	1 (50.0)
>5 years – n (%)	513 (87.1)	7 (87.5)	2 (66.7)	1 (50.0)	2 (100)
ICU/Dead					
All – n (%)	17/115 (14.8)	4/5 (80.0)*	1/5 (20.0)		
≤5 years – n (%)	4 (15.4)	1 (100)	0 (0)		
>5 years – n (%)	13 (14.6)	3 (75.0)*	1 (50.0)		

NDFlu A, seasonal influenza A viruses; Flu B, influenza B virus; RSV, respiratory syncytial virus; AdV, adenovirus; hMPV, human metapneumovirus. \*The statistic was significant at  $\alpha = 0.05$ , single seasonal influenza A virus infection was used as a baseline for calculation of all odds ratios, multivariate model adjusted for age and co-infection\*age mixed variable.

Co-infections were predominantly identified in children <5 years old. Thus, 83.3% (45/54) of patients with RV/A(H1N1)pdm09 co-infection, 92.1% (35/38) RSV/A(H1N1)pdm09, 100% (14/14) AdV/A(H1N1)pdm09, 75% (3/4) hMPV/A(H1N1)pdm09 and 68% (11/16) Flu B/A(H1N1)pdm09 were under 5 years of age, and these differences were statistically significant ( $P = 0.002$  to  $P = <0.0001$ ) (Table 2). A similar age distribution was observed among patients who were diagnosed with seasonal influenza A virus co-infections (Table 3). As for gender, apart from hMPV co-infection, there were no significant differences in the proportion of males and females diagnosed with single versus dual or multiple respiratory viral infections. The male to female ratios among different types of

co-infections were 57.4%/43.6% for RV/A(H1N1)pdm09 and 50%/50% for RSV/A(H1N1)pdm09 (Table 2). Similar proportions were observed among seasonal influenza A virus co-infections (Table 3).

### Risk of hospitalisation, admission to ICU and death associated with influenza A(H1N1)pdm09 co-infections

Of patients who had single influenza A (H1N1)pdm09, 76.2% were hospitalised in a general ward. Comparatively, a higher proportion of patients with RSV and AdV co-infection were hospitalised in a general ward (86.1%, 31/36, and 92.9%, 13/14; respectively), but the general ward hospitalisation among patients with RV and influenza



B co-infection was lower than that in single influenza A(H1N1)pdm09 (75.0%; 9/12 and 73.5%; 36/49, respectively) (Table 2). The majority of co-infections were in children <5 years old, so when patients were stratified by age group, rates of admission to a general ward were still high among patients co-infected with either Flu B, RV, RSV or AdV than those who had single Flu (H1N1)pdm09 infection. On the other hand, only RSV and AdV co-infection had higher hospitalisation among patients aged 5 years of age or older (Table 2). However, in a simple and multiple logistic regression, controlling for age and age\*co-infection interaction factor, these increases in risk were not statistically significant.

Regarding risk of admission to the ICU or death, 28.4% of patients with single influenza A(H1N1)pdm09 infection were admitted to the ICU/died compared with rates of 57.1% among those with influenza B co-infection and 27.8% and 28.6% among those with RV and RSV co-infection, respectively. Young age was again also associated with higher proportions of hospitalisations to ICU/death due to co-infection, but again, in simple and multivariate logistic regression models, this increase in risk was not statistically significant.

### Risk of hospitalisation, admission to ICU and death associated with seasonal influenza A virus co-infection

Seasonal influenza A viruses were in circulation throughout the study period, and they also co-circulated with the pandemic influenza A(H1N1)pdm09 virus between April 2009 and June 2011. Among patients who had single seasonal influenza virus infection, 86.1% were admitted to the general ward compared with 91.7% of those with influenza B co-infection and 80% and 75% of those who had RSV, AdV and hMPV co-infection, respectively (Table 3). The percentage of co-infected patients who were hospitalised differed with age with younger patients having higher proportions than those aged 5 years or older. In simple and multiple logistic regression models, the increased risk of hospitalisation due to co-infection between seasonal influenza A viruses and Flu B, RSV or AdV was not statistically significant.

Regarding co-infection and risk of admission to the ICU/death among patients infected with seasonal influenza A viruses, only the influenza B co-infection with seasonal influenza A viruses was associated with a significant increase in the risk of hospitalisation to the ICU/death, in simple and multivariate logistic model. Specifically, of five patients who had FluB/FluA co-infection, 80% (4/5) were admitted to ICU or died, compared with the rate of 14.8% (17/115) among those who had single seasonal influenza A viruses infection (OR: 23.10, 95% CI: 2.4–219.0,  $P = 0.006$ ). Age adjustment slightly altered this estimate, but a positive association was still maintained (OR: 22.0, 95% CI: 2.21–219.8,  $P = 0.008$ ). RSV co-infection also

increased risk of admission to ICU/death, but this increase was not statistically significant.

## Discussion and conclusion

The overall positive rate for any respiratory virus of 40.7% (10 501/25 596) in this study is generally lower than positive rates of between 60% and 96% identified by most studies that recruited hospitalised patients,<sup>6,10,17,19,25</sup> but is generally higher than rates reported by studies that recruited patients from the emergency department or recruited patients presenting with influenza-like illnesses to the general practitioners (GPs) (range 15%–31.0%).<sup>7,12,15</sup> Co-infections occurred in 4.7% (137/2689) of patients who had a positive diagnosis of influenza A(H1N1)pdm09 and 7.3% (57/779) of samples with seasonal influenza A virus infections. The rates of co-infections observed in this study are similar to those observed in studies that recruited similar study populations; however, they are generally lower than those that recruited infants and children. For example, among studies of both adults and children<sup>5,12,26</sup> in USA, France and Madagascar, co-infection rates were reported to be 5.0% (66/1347), 13.1% (30/226) and 29.4% (53/389), respectively. In contrast to studies conducted in infants and children,<sup>10,14,16,27,28</sup> rates of 16.8%–36.1% have been reported.

Co-infection between seasonal influenza A viruses and influenza B virus was associated with a significant increase in risk of admission to ICU or death, whereas co-infection between influenza A(H1N1)pdm09 and AdV and RSV were associated with insignificant increases in risk of admission to a general ward. The findings of this study are in agreement with previous studies that implicated AdV, RSV and flu B virus co-infection in aggravating respiratory disease outcome.<sup>10,12,14,25,28,29</sup> Further, in this study, RV co-infection was associated with an insignificant increase in risk of hospitalisation to a general ward, ICU or death among children <5 years old, but no such increases among adults. Reports from other studies on role of RV on disease outcome have been contradictory with some studies indicating that they decreased severity<sup>5,11</sup> and others indicating they increase the risk.<sup>30,31</sup> More recently, studies from Australia, Sweden and USA have suggested that rhinoviruses reduce or interfere with circulation of influenza viruses.<sup>8,9,32,33</sup> The mechanisms driving virus virulence in co-infections are not clearly understood. However, some authors have suggested that it could either be due to virus–virus interactions in the form of protein–protein or protein–RNA interactions such as the direct or indirect heterologous transactivation<sup>34</sup> that aid in their coexistence and replication.

In this study, co-infections were predominantly among children <5 years old, which could introduce confounding into the calculated measures of risk. Therefore, in the logistic regression models, age was adjusted for so as to

eliminate any change in such confounding. Also, in this study, the positivity of most of the respiratory viruses was higher in males than in females, and, apart from PIV1 and PIV3, these differences were statistically significant. This could be either because of differences in the health-seeking behaviours of men and women or because gender is a risk factor for respiratory virus infection. Some previous studies have also reported higher positivity rates in males,<sup>15,35,36</sup> while others have reported higher rates in females,<sup>12,26,37–39</sup> but only in our study are gender differences statistically significant. A chi-square analysis comparing the gender of included and excluded patients found no statistically significant gender difference between the two groups ( $\chi^2 = 1.14$ ,  $P = 0.29$ ), ruling out any possibility that this finding could have been due to recruitment or diagnostic bias.

The weaknesses of this study are that it was retrospective and, because the database included limited information on disease outcome, therefore we could not measure the impact of other covariates such as disease comorbidities. Most of the co-infections (range: 68.8%–100%) occurred in children <5 years old Tables 2 and 3. Children could suffer from chronic conditions like leukaemia, cystic fibrosis asthma and immune-suppressing infections such as HIV; therefore, these factors should be borne in mind when interpreting our results. Also, as genetic mutations, that is, antigenic drift and reassortment, can play a major role in the virulence of influenza A viruses, it would have been useful to sequence the identified influenza A viruses. However, as no major mutation to influence disease virulence was observed during the period of study, it is unlikely that major change occurred as exemplified by similar mutations (39/40) occurring in almost all fatal and non-fatal influenza A(H1N1)pdm09 infections in 2010/11 winter season in the UK.<sup>40</sup> The population-attributable fraction (AFp) indicates on how much of the observed risk could have happened in the absence of the exposure in question. A good estimation of AFp requires thorough knowledge of the prevalence of the exposure in question in the general community. Due to the limited number of studies investigating the prevalence of dual infections between influenza and respiratory viral infections in the community, the true prevalence of dual infections is not known, and AFp could not therefore be estimated.

Further, excluded patients significantly differed by age from included patients such that infants and children were more likely to be included than adults (e.g. inclusion: exclusion rates 35.0%/19.7% versus 29.1%/17.62% among those aged 40–65 years), and these differences were statistically significant  $P = <0.0001$ . If physicians requested tests for respiratory virus infections more frequently in younger children than older patients, it might have introduced diagnostic bias leading to an exaggeration of the observed number of co-infections among young children. However, this

study investigated co-infections among patients primarily infected with influenza virus infection. As influenza is an acute respiratory illness; the nature of the sickness would drastically reduce the possibility of patients not seeking medical treatment or physicians not requesting viral testing. We therefore believe that the information contained in the database closely estimates the epidemiology of influenza A and respiratory viral infections in North West England. However, the possibility of diagnostic bias should be borne in mind when interpreting our results.

In conclusion, despite some shortcomings, this study found that influenza B co-infection, among patients infected with seasonal influenza A virus, significantly increased the risk of admission to ICU or death. It also observed positive associations between influenza A (H1N1)pdm09 and RSV, AdV and risk of hospitalisation to the general ward, although these associations were not statistically significant. We recommend comprehensive testing and a careful integration of the patterns of co-infections among respiratory viruses into public health reports so as to help accumulate up more evidence on the role of co-infections on patient outcome in respiratory infections. Such information would be vital in shaping policy on an integrated investigation and treatment of respiratory viral infections, introduction of an integrated vaccine for multiple respiratory viruses and development of multi-target rapid diagnostic tests.

## References

- 1 WHO. Acute respiratory infections (update september 2009). Available at [http://www.who.int/vaccine\\_research/diseases/ari/en/index.html](http://www.who.int/vaccine_research/diseases/ari/en/index.html) (Accessed 12 December 2011).
- 2 Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2002; 2:25–32.
- 3 Molinari NA, Ortega-Sanchez IR, Messonnier ML *et al.* The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine* 2007; 25:5086–5096.
- 4 Pitman RJ, Melegaro A, Gelb D, Siddiqui MR, Gay NJ, Edmunds WJ. Assessing the burden of influenza and other respiratory infections in England and Wales. *J Infect* 2007; 54:530–538.
- 5 Esper FP, Spahlinger T, Zhou L. Rate and influence of respiratory virus co-infection on pandemic (H1N1) influenza disease. *J Infect* 2011; 63:260–266.
- 6 Ali SA, Williams JV, Chen Q *et al.* Human metapneumovirus in hospitalized children in Amman, Jordan. *J Med Virol* 2010; 82:1012–1016.
- 7 Cilla G, Onate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community acquired pneumonia in children aged less than 3 years old: high rate of viral coinfection. *J Med Virol* 2008; 80:1843–1849.
- 8 Martin ET, Kuypers J, Wald A, Englund JA. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respir Viruses* 2012; 6:71–77.
- 9 Greer RM, McErlean P, Arden KE *et al.* Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol* 2009; 45:10–15.



- 10 Richard N, Komurian-Pradel F, Javouhey E *et al.* The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. *Pediatr Infect Dis J* 2008; 27:213–217.
- 11 Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Krupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-[gamma] response. *Pediatr Infect Dis J* 2005; 24:605–610.
- 12 Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB. Dual respiratory virus infections. *Clin Infect Dis* 1997; 25:1421–1429.
- 13 Portnoy BE, Eckert HL, Hanes B, Salvatore MA. Multiple respiratory virus infections in hospitalised children. *Am J Epidemiol* 1965; 82:262–272.
- 14 Semple MG, Cowell A, Dove W *et al.* Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 2005; 191:382–386.
- 15 Renois F, Talmud D, Huguenin A *et al.* Rapid detection of respiratory tract viral infections and coinfections in patients with influenza-like illnesses by use of reverse transcription-PCR DNA microarray systems. *J Clin Microbiol* 2010; 48:3836–3842.
- 16 Huang JJ, Huang TY, Huang MY *et al.* Simultaneous multiple viral infections in childhood acute lower respiratory tract infections in southern Taiwan. *J Trop Pediatr* 1998; 44:308–311.
- 17 De PM, Gilio AE, Ferraro AA *et al.* Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011; 87:307–313.
- 18 Subbarao EK, Griffis J, Waner JL. Detection of multiple viral agents in nasopharyngeal specimens yielding respiratory syncytial virus (RSV). An assessment of diagnostic strategy and clinical significance. *Diagn Microbiol Infect Dis* 1989; 12:327–332.
- 19 Huguenin A, Moutte L, Renois F *et al.* Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. *J Med Virol* 2012; 84:979–985.
- 20 WHO. Weekly epidemiological record (No. 8, 2011, 86, 61–72). WHO 2011 February 18;86(8):61–72. Available at <http://www.who.int/wer/2011/wer8608.pdf> (Accessed 8 October 2011).
- 21 WHO. Global Alert and Response (GAR): clinical features of severe cases of pandemic influenza (Pandemic (H1N1) 2009 briefing note 13). WHO 2009 October 16. Available at [http://www.who.int/csr/disease/swineflu/notes/h1n1\\_clinical\\_features\\_20091016/en/index.html](http://www.who.int/csr/disease/swineflu/notes/h1n1_clinical_features_20091016/en/index.html) (Accessed 15 September 2009).
- 22 Office of the National Statistics (Newport). Regional profiles – population and migration – north west – October 2011. Available at <http://www.ons.gov.uk/ons/rel/regional-trends/region-and-country-profiles/population-and-migration/population-and-migration—north-west.html> (Accessed 2 July 2012).
- 23 Ellis J, Iturriza M, Allen R *et al.* Evaluation of four real-time PCR assays for detection of influenza A(H1N1)v viruses. *Euro Surveill* 2009; 14(22); pii: 19230.
- 24 Lieberman DE, McCarthy RC, Hiimeae KM, Palmer JB. Ontogeny of postnatal hyoid and larynx descent in humans. *Arch Oral Biol* 2001; 46:117–128.
- 25 Do AHL, van Doorn HR, Nghiem MN *et al.* Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004–2008. *PLoS ONE* 2011; 6:e18176.
- 26 Razanajatovo NH, Richard V, Hoffmann J *et al.* Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS ONE* 2011; 6:e17579.
- 27 Legg JP, Warner JA, Johnston SL, Warner JO. Frequency of detection of picornaviruses and seven other respiratory pathogens in infants. *Pediatr Infect Dis J* 2005; 24:611–616.
- 28 Peng D, Zhao D, Liu J *et al.* Multipathogen infections in hospitalized children with acute respiratory infections. *Virology J* 2009; 6:155.
- 29 Aebi T, Weisser M, Bucher E, Hirsch HH, Marsch S, Siegemund M. Co-infection of Influenza B and Streptococci causing severe pneumonia and septic shock in healthy women. *BMC Infect Dis* 2010; 10:308.
- 30 Cheuk DK, Tang IW, Chan KH, Woo PC, Peiris MJ, Chiu SS. Rhinovirus infection in hospitalized children in Hong Kong: a prospective study. *Pediatr Infect Dis J* 2007; 26:995–1000.
- 31 Miller EK, Khuri-Bulos N, Williams JV *et al.* Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. *J Clin Virol* 2009; 46:85–89.
- 32 Linde A, Rotzen-Ostlund M, Zweyberg-Wirgart B, Rubinova S, Brytting M. Does viral interference affect spread of influenza? *Euro Surveill* 2009; 14(40); pii: 19354.
- 33 Casalegno JS, Ottmann M, Duchamp MB *et al.* Rhinoviruses delayed the circulation of the pandemic influenza A (H1N1) 2009 virus in France. *Clin Microbiol Infect* 2010; 16:326–329.
- 34 DaPalma T, Doonan BP, Trager NM, Kasman LM. A systematic approach to virus-virus interactions. [Review]. *Virus Res* 2010; 149:1–9.
- 35 Gencay M, Roth M, Christ-Crain M, Mueller B, Tamm M, Stolz D. Single and multiple viral infections in lower respiratory tract infection. *Respiration* 2010; 80:560–567.
- 36 Venter M, Visser A, Lassauniere R. Human polyomaviruses, WU and KI in HIV exposed children with acute lower respiratory tract infections in hospitals in South Africa. *J Clin Virol* 2009; 44:230–234.
- 37 Al-Turab M, Chehadeh W, Al-Mulla F, Al-Nakib W. Human metapneumovirus in patients with respiratory tract infection in Kuwait. *J Med Virol* 2011; 83:1811–1817.
- 38 Malato L, Llavador V, Marmier E *et al.* Pandemic influenza A(H1N1) 2009: molecular characterisation and duration of viral shedding in intensive care patients in Bordeaux, south-west France, May 2009 to January 2010. *Euro Surveill* 2011; 16(4); pii: 19776.
- 39 Druce J, Tran T, Kelly H *et al.* Laboratory diagnosis and surveillance of human respiratory viruses by PCR in Victoria, Australia, 2002–2003. *J Med Virol* 2005; 75:122–129.
- 40 Ellis J, Galiano M, Pebody R *et al.* Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11. *Euro Surveill* 2011; 16(1); pii: 19760.