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Influenza and rhinovirus viral load and disease severity in upper respiratory tract infections

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ABSTRACT

Background: The role of viral load in respiratory viral infection is unclear. It is proposed that the viral load of some, but not all respiratory viruses correlate with disease severity.
Objectives: We aimed to determine if an association exists between viral loads among patients in ambulatory settings, compared to those requiring hospitalization/intensive care unit (ICU) admission with influenza A/H3N2, influenza B, or human rhinovirus (HRV); we also explored the impact of age, gender and co-detection of *Streptococcus pneumoniae* on patient setting. We hypothesized that hospitalized/ICU patients have higher respiratory virus viral loads compared to ambulatory (e.g. walk-in clinics, family practices)/ER patients.
Study design: We quantified viral load by in-house real-time RT-PCR in 774 nasopharyngeal swabs with influenza A/H3N2, or B or HRV viruses from various patient settings in Ontario, Canada.
Results: Mean viral load (log_{10} copies/ml) of influenza A/H3N2 (6.94) was higher than influenza B (4.96) and HRV (5.58) ($p < 0.0001$). Influenza A/H3N2 viral loads were highest in infants and the elderly; however, increased A/H3N2 viral loads were not associated with hospitalization/ICU admission compared to swabs collected in ambulatory/ER settings. Influenza B viral loads were higher in patients in hospital/ICU settings compared to those in ambulatory settings (OR 1.28, 95% CI 1.11–1.47). HRV viral loads did not differ by age ($p = 0.67$) or setting ($p = 0.54$); there was no association between *S. pneumoniae* colonization and setting for any virus.
Conclusion: When compared to ambulatory/ER patients, viral load was higher in hospitalized/ICU patients with influenza B, but not influenza A or HRV.

1. Background

Real-time, reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) has become a useful and important technology in the detection of disease-specific markers and to determine the dynamics of viral infections. It is routinely used for hepatitis B and human immunodeficiency virus (HIV) to monitor responses to antiviral treatment and transmission potential [1,2]. However, for respiratory viruses, the association between viral load and disease severity is not as clear. Bont (2013) hypothesized that the viral loads of some respiratory viruses but not all will correlate with disease severity.

Influenza A and B viruses are the leading infectious cause of morbidity and mortality amongst the elderly and children [4]. Human rhinovirus (HRV) is the leading cause of mild upper respiratory tract infections in all age groups. It is also responsible for 60% of viral induced asthma exacerbations, and HRV-induced wheezing during the first three years of life has been associated with an increased odds (9.8) of developing asthma [5,6]. While certain reports have indicated that increased viral loads in influenza and HRV correlate with severe symptoms in the elderly or the need for mechanical ventilation in children <5 years, others have found no correlation [3,7,8]. Differing accounts of the impact of viral load on disease severity in influenza and HRV could be the result of different populations tested (elderly vs. infants) in these studies as virus dynamics

Abbreviations: AMB, ambulatory; ER, emergency rooms; HOS, hospitalized; ICU, intensive care unit.

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differ with age. We hypothesized that hospitalized patients, particularly those requiring ICU care, would have higher viral loads in their nasopharyngeal swab (NPS) specimens as compared to ambulatory patients.

2. Objectives

In this proof-of-concept study we aimed to determine if there is any association between viral load in ambulatory/ER patients with acute respiratory infections (ARI), as compared to patients requiring hospitalization/ICU admission with influenza A, or B, or HRV. We also wanted to determine whether age, gender, and co-detection of *Streptococcus pneumoniae* were associated with patient settings in individuals infected with influenza A or B, or HRV.

3. Study design

3.1. Specimen collection

Specimens included in this study were tested as part of the routine clinical respiratory virus testing at the Public Health Ontario Laboratory (PHOL). PHOL performs approximately 50% of primary respiratory viral testing in Ontario, from patients cared for in various clinical settings including community physicians' offices, emergency rooms (ERs), and hospitals, including intensive care units (ICUs). In accordance with PHOL's clinical testing algorithm, there was no restriction placed on specimens submitted regarding patient setting during the study period. NPS (Copan Italia, Brescia, Italy) specimens testing positive for influenza A, influenza B, or HRV from September 2012 to May 2013 (influenza A and HRV) and September 2013 to May 2014 (influenza B) were selected (n = 811). Total nucleic acid was extracted from 200 μl of universal transport medium (UTM) (Copan Italia, Brescia, Italy) using the KingFisher Flex (ThermoScientific, Mississauga, ON) according to the manufacturer's instructions. Influenza A positives were subtyped as H1N1 pdm09 (pH1N1) or H3N2 by a previously described real-time PCR assay [9].

3.2. Influenza A/B and HRV RT-qPCR

Influenza A and B, and HRV viral load was determined by previously described real-time PCR assays [10–12] and adapted to the ABI 7500 FAST (ThermoScientific, Mississauga, ON). Quantified RNA controls for each virus were obtained through Vircell (Burlington, ON). All viral loads were normalized against RNase P and tested in triplicate; the mean viral load of each sample was used in further analysis [13].

3.3. Detection of Streptococcus pneumoniae

Concomitant qualitative detection of *S. pneumoniae* was determined by previously described real-time PCR assays detecting both the cpsA and lytA genes [14,15]. The assays were adapted to the ABI 7500 FAST (ThermoScientific, Mississauga, ON) using the TaqMan Universal real-time PCR mix (ThermoScientific, Mississauga, ON) under the following conditions: UNG activation at 50 °C for 2 min, polymerase activation 95 °C for 10 min, and 50 cycles at 95 °C for 15 s and 60 °C for 60 s. Positive identification of *S. pneumoniae* was made when both cpsA and lytA genes were detected in a sample with a cycle threshold (C_T) value of ≤ 40.

3.4. Data analysis

Age group (≤1 year, 1–4 years, 5–19 years, 20–64 years, and ≥65 years), gender, viral load, and patient setting data were recorded and entered into excel. Patient setting was determined by the location of specimen collection as indicated on the PHOL specimen requisition form. All statistical analyses were conducted on RStudio version 0.98.1091 (R-Group, Austria). Comparisons of viral load ($\log_{10}$ copies/ml) by virus type were conducted by ANOVA and pair-wise comparisons were conducted using the Tukey's Honest Squares Difference (HSD). Differences in viral load in different age groups and settings were assessed by the Kruskal Wallis Rank Sum test and pair-wise comparisons were conducted using the Mann-Whitney test. Multinomial logistic regression was used to determine whether viral load was associated with patient setting in individuals with influenza A or B or HRV after adjustment for age, gender and concomitant detection of *S. pneumoniae*. Ambulatory and ER settings were grouped together and compared to hospital/ICU for the multinomial logistic regression. In a secondary multivariable logistic regression, the ambulatory setting alone was compared to ER, and hospital/ICU. All statistical tests were two-sided with results considered statistically significant at $p < 0.05$.

3.5. Ethics

This study was approved by the PHOL Research Ethics Board. Specimens included in this study were analyzed as part of PHOL's respiratory viral molecular surveillance program that supports Ontario's Ministry of Health and Long-Term Care.

4. Results

4.1. Patient characteristics

Eight hundred and eleven specimens from individuals whose sample was obtained in ambulatory settings, ERs, hospitals, or ICUs across the province of Ontario, Canada and positive for influenza A (n = 37 pH1N1 and n = 267 H3N2), influenza B (n = 294), or HRV (n = 213) were selected (Table 1). Only influenza A/H3N2, influenza B, and HRV specimens were selected for qPCR analysis; the number of influenza A/pH1N1 specimens was too small to allow for further analysis.

4.2. Comparison of viral load by virus type

The mean viral loads (±SD) of influenza A/H3N2, influenza B and HRV were: 6.94 ± 2.5, 4.96 ± 1.9, and 5.58 ± 0.9 $\log_{10}$viral copies/ml respectively ($p < 0.0001$) (Fig. 1). Pair-wise comparisons determined that influenza A/H3N2 viral loads were higher than influenza B and HRV ($p < 0.01$) and HRV viral loads were higher than influenza B ($p < 0.01$).

4.3. Viral load by age and setting

Viral load was compared by age (Fig. 2A) and setting (Fig. 2B) for influenza A/H3N2, influenza B, and HRV. HRV viral loads did not differ in different age groups or patient care settings. For influenza A/H3N2 and influenza B, children 1–4 years had the lowest viral loads, and children ≤1 year and adults ≥65 years had the highest viral loads of the five age groups. Pair-wise comparisons identified significant differences for ages ≤1 vs ages 1–4 ($p < 0.01$), and ages ≥65 vs ages 1–4 ($p < 0.01$) for influenza A/H3N2. For influenza B, viral load was higher for all other age groups vs 1–4 year olds ($p = 0.01$). When patient settings were investigated, influenza A/H3N2 viral loads were highest in both the ER and hospital settings compared to either the ambulatory or ICU setting ($p < 0.001$), whereas for influenza B, the ICU was associated with the highest viral loads compared to all other settings ($p < 0.001$).