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LETTER TO THE EDITOR

A paucity of co-infecting respiratory viral pathogens in nasopharyngeal specimens from patients infected with H274Y-positive influenza A (H1N1) strains

The H274Y mutation in the neuraminidase gene of influenza A (H1N1) has been associated with oseltamivir resistance.¹ Initial evaluations of this mutation in animal models suggested that this mutation had reduced fitness and reduced pathogenicity when compared to corresponding wild-type viruses.^{2,3} Earlier reports of clinical infections supported this view and H274Y was rarely reported.⁴ However, in the last year, there have been reports of dramatic increases in the proportion of H274Y mutations identified in clinical isolates throughout the world.⁵ If pathogenicity was indeed compromised in these most recent mutants, then it should be determined whether patients infected with oseltamivir-resistant strains are presenting with illness associated with another co-infecting respiratory viral pathogen. The purpose of this brief investigation was to determine whether nasopharyngeal specimens from patients infected with strains of influenza A (H1N1) carrying the H274Y mutation were more likely than not to contain other commonly circulating respiratory viral pathogens.

Nasopharyngeal specimens from patients with influenza-like illness were sent to the Ontario Public Health Laboratories. Isolates of influenza A collected from Toronto, Ontario, Canada (estimated population 2.7 million) during the period November 14, 2007 to February 14, 2008 were screened by reverse transcriptase (RT)-PCR for the H1N1 subtype using primers described previously.⁶ Strains of isolates were confirmed by Sanger sequencing and sequence alignment. Neuraminidase gene sequencing was undertaken and sequences were aligned using CLUSTALX. Isolate sequences were compared to sequences with described H274Y mutations: GenBank accession No. [EU516123](#) influenza A virus (A/Hawaii/28/2007(H1N1)); GenBank accession No. [CY027037](#) influenza A virus (A/Kansas/UR06-0104/2007(H1N1)); GenBank accession No. [EU516027](#) influenza A virus (A/Texas/31/2007(H1N1)).⁷ Specimens corresponding to H274Y mutant and H274 wild-type isolates were blinded and chosen at random for a retrospective investigation for other respiratory viral pathogens (adenovirus, coronavirus 229E/NL63, coronavirus OC43, influenza A/B, parainfluenza virus 1/2/3, respiratory syncytial virus A/B, rhinovirus A) using the Seeplex[®] RV detection kit protocol (Seegene, Inc., Rockville, MD).⁸ Data analysis was carried out

using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

All influenza A (H1N1) isolates resembled most closely influenza A/Solomon Islands/03/06 (H1N1). Of the 19 specimens containing the H274Y mutation, none contained any other respiratory viral pathogen that could be detected by the Seeplex RV detection kit protocol. However, two of 40 specimens (5%) containing wild-type H274 influenza A (H1N1) were also positive for parainfluenza virus 2 and one specimen (2.5%) was positive for respiratory syncytial virus B. Statistical analysis indicated that there was no difference between H274Y mutant and H274 wild-type groups in the proportion of specimens carrying other respiratory viral pathogens (Fisher's exact test, $p = 0.5$).

Until the pathogenicity of oseltamivir-resistant influenza A viruses is understood better, infections with these viruses should be taken seriously by both clinicians and public health workers.⁹ The detection of influenza and the lack of other respiratory viral pathogens detected in the H274Y mutant group suggest that illness in these patients could only be associated with influenza and not any other of the more common respiratory viral pathogens. However, it should be noted that the interaction of influenza A and bacterial pathogens could not be determined in these specimens given issues of specimen collection and data collection. Given the lack of data supporting a co-infection model, it is considered that further work must be undertaken to determine the fitness and virulence of the H274Y mutant influenza A strains circulating currently.

Conflict of interest: No competing interests declared.

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A. Eshaghi^a
J. Blair^a
L. Burton^a
E. Lombos^a
K. Choi^a
C. De Lima^a
S.J. Drews^{a,b,c,*}

^aOntario Public Health Laboratories, Toronto, ON, Canada

^bDepartment of Laboratory Medicine and Pathobiology,
University of Toronto, Toronto, ON, Canada

^cMount Sinai Hospital, Toronto, ON, Canada

*Corresponding author. Tel.: +1 416 235 5703
E-mail address: steven.drews@ontario.ca
(S.J. Drews)

Corresponding Editor: Jane Zuckerman, London, UK

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