Use of the Seeplex RV Detection Kit for Surveillance of Respiratory Viral Outbreaks in Toronto, Ontario, Canada

Steven J. Drews,1,2,3 Joanne Blair,1 Ernesto Lombos,1 Cedric DeLima,1 Laura Burton,1 Tony Mazzulli,1,2,3 and Donald E. Low1,2,3
1Ministry of Health and Long-Term Care, Public Health Laboratories Branch, Etobicoke, Ontario; 2Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario; 3Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Ontario, Canada

Abstract. The Seeplex® RV Detection kit was used to identify specific respiratory viruses from specimens collected during respiratory outbreaks in the Greater Toronto Area from 1 September 2007 to 1 February 2008. Two hundred-thirty-one patient samples (nasopharyngeal swabs) were collected from 63 respiratory outbreaks. The distribution of outbreaks characterized by molecular means was: 30% (n = 19) no identification; 52.5% (n = 33) one pathogen; 14.5% (n = 9) two pathogens; and 3% (n = 2) three pathogens. In contrast, culture-based protocols identified pathogens in fewer outbreaks: 63% (n = 40) no identification; 35% (n = 22) 1 pathogen; and 2% (n = 1) 2 pathogens (p < 0.05). Compared to virus isolation, molecular testing identified a greater proportion of positive specimens for rhinovirus: 22% (n = 51/231) vs 5% (n = 12/231) (p = 0.01); and RSV A/B: 12% (n = 27/231) vs 5% (n = 11/231) (p < 0.05). Superiority of the molecular assay to detect rhinovirus and RSV outbreaks compared to culture is evident from this study.

Keywords: respiratory viruses, multiplex RT-PCR, viral culture, infection surveillance, rhinovirus, RSV

Introduction

Culture-based methods have traditionally been the reference method for detection of viral pathogens in respiratory specimens from both sporadic and outbreak cases of respiratory virus infection. Attempts to provide rapid turn-around times for the diagnosis of respiratory infections with antigen detection kits have been hampered by the poor test characteristics of these assays [1]. In contrast, molecular techniques offer greater analytical sensitivity, quicker turn-around-times, and allow identification of strains that are difficult to culture using standard methods in clinical laboratories [2]. Enhancement of molecular techniques by means of multiplexed technologies enables laboratorians to test small volumes of patient specimens for multiple pathogens, using a limited number of tests while maintaining excellent sensitivity and specificity [3,4]. The purpose of this manuscript is to describe the impact of a multiplexed molecular methodology, the Seeplex® RV Detection kit [5], on surveillance of respiratory virus pathogens in outbreak settings.

Materials and Methods

Respiratory outbreaks in the Greater Toronto Area (GTA) were declared by public health workers and each outbreak was given a unique identifier. Respiratory infection outbreaks in long-term care faculties were defined as two cases of acute respiratory tract illness, at least one of which must be laboratory confirmed, or three cases of acute respiratory tract illness occurring within 48 hr in a geographic area (eg, unit, floor), or more than two units having a case of acute respiratory tract illness within 48 hr. For acute care facilities, respiratory outbreaks were defined as two or more cases of nosocomially
acquired influenza-like illness occurring within 48 hr on a specific hospital unit, with at least one case being laboratory confirmed.

All nasopharyngeal (NP) swabs from declared outbreaks in the GTA sent to the Central Public Health Laboratory from 1 September 2007 to 1 February 2008 were included in this analysis. Specimens were collected using the Flocculated Starswab® Multitrans collection and transport system (Starplex, Bolton, ON, Canada). Where possible, specimens were also tested by the Remel Xpect® RSV (Remel, Lenexa, KS) and BD Directigen Flu A/B (Oakville, ON, Canada) methods. All specimens were cultured on rhesus monkey kidney and WI-38 cells (Diagnostic Hybrids, Athens, OH) followed by post-culture direct fluorescence antibody (DFA) staining for influenza A, influenza B, respiratory syncytial virus (RSV) A/B, parainfluenza (PIV) 1, PIV2, PIV3, and adenovirus (Diagnostic Hybrids, Athens, OH). Enterovirus/rhinovirus was detected by visual determination of cytopathic effect (CPE).

Total nucleic acid was extracted from each specimen using the easyMag automated extraction system (bioMérieux, Montreal, QC) according to the manufacturer's protocol. To control for extraction, all specimens were tested for human target gapdh by using the gapdh RT-PCR kit (ABI, Foster City, CA) according to the manufacturer's instructions. Total nucleic acid was tested by the Seeplex® RV Detection kit protocol (Seegene, Inc., Rockville, MD) for the following pathogens: influenza A, influenza B, respiratory syncytial virus A (RSVA), RSVB, parainfluenza (PIV) 1, PIV2, PIV3, human rhinovirus A, human metapneumovirus, adenovirus, coronavirus OC43, and combined coronavirus 229E/NL63. Multiplexed molecular testing was carried out according to the manufacturer's instructions. Specimens were batched and tested by the molecular method weekly each Thursday.

Statistical analyses was carried out using GraphPad Prism 5.01 (Graphad Software Inc., El Camino Real, CA).

Results

Between 1 September 2007 and 1 February 2008, 231 patient specimens (NP swabs) were collected from 63 declared respiratory outbreaks in the GTA. One specimen was collected from each patient. Of the outbreaks identified, 58 (92%) were from long-term care facilities, 3 (5%) were from acute care facilities, and 2 (3%) were from an unknown setting. The average number of specimens per outbreak was 4 for long-term care facilities, 4 for acute care facilities, and 4 for unknown settings. The median age of patients from whom a specimen was collected was 85 yr.

Rapid antigen detection assays detected 1 influenza A outbreak compared to 3 outbreaks identified by culture and molecular methods. Rapid antigen detection assays detected 2 influenza B outbreaks compared to 2 outbreaks by culture and molecular methods. Rapid antigen tests detected 3 RSV outbreaks compared to 5 outbreaks identified by culture and 9 outbreaks identified by molecular methods.

The distribution of outbreaks positive for single and multiple respiratory viral pathogens is indicated in Table 1. One group of agents had the possibility of being detected by both molecular and culture-based methods: influenza A, influenza B, respiratory syncytial virus A (RSVA), RSVB, parainfluenza (PIV) 1, PIV2, PIV3, human rhinovirus A, adenovirus, a human metapneumovirus, coronavirus OC43, and combined coronavirus 229E/NL63. Table 1 indicates that there were no culture-positive and molecular-negative outbreaks involving specific pathogens. Table 1 also indicates that the molecular

<table>
<thead>
<tr>
<th>Viral agent</th>
<th>Culture-positive outbreaks</th>
<th>Molecular-positive outbreaks</th>
<th>Total outbreaks for each virus tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Influenza B</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>PIV1</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>PIV2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PIV3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Human rhinovirus</td>
<td>6</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>RSV</td>
<td>6</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>One or more viral agent cannot be detected by current culture methods</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

aagents not detected using current culture methods include human metapneumovirus, coronavirus 229E/NL63, and coronavirus OC43. bincludes outbreaks positive for single or multiple etiologies.

Table 2. The distribution of outbreaks associated with respiratory viral pathogens.

<table>
<thead>
<tr>
<th>No pathogens identified</th>
<th>Culture n (%)</th>
<th>Molecular n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 (63)</td>
<td>19 (30)</td>
</tr>
<tr>
<td>Single pathogen</td>
<td>22 (35)</td>
<td>33 (52.5)</td>
</tr>
<tr>
<td>Dual pathogens</td>
<td>1 (2)</td>
<td>9 (14.5)</td>
</tr>
<tr>
<td>Three pathoghs</td>
<td>0</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>
The method was able to detect agents that previously could not be detected by the culture-based methods used in our laboratory: human metapneumovirus, coronavirus 229E/NL63, and coronavirus OC43.

Table 2 indicates that molecular methods were more likely than culture-based methods to identify a respiratory viral pathogen in the majority of outbreaks (Fisher's exact test, p <0.05). Comparison of the molecular assay vs culture for the detection of pathogens in specimens alone also favored the molecular assay. Compared to virus isolation, the molecular assay identified a greater proportion of positive specimens for rhinovirus: 22% (n = 51/231) vs 5% (n = 12/231) (p <0.05), and RSV A/B: 12% (n = 27/231) vs 5% (n = 11/231) (p <0.05).

Discussion

Respiratory infections are a major cause of morbidity and mortality in long-term care facilities [6-8] and they cause a significant economic burden on the health-care system [9]. This study indicates that the introduction of multiplexed molecular methods allows for the identification of a respiratory viral pathogen in the majority of respiratory outbreaks. The increased ability to identify specific viral pathogens in outbreak settings may allow clinicians to utilize appropriate measures to control these infections and to avoid the inappropriate utilization of anti-influenza agents where no influenza is present [10]. This concern regarding inappropriate use of anti-viral agents is justified by the increasing levels of resistance of some influenza A (H1N1) strains during the 2007-2008 respiratory season and the yet unknown forces driving this resistance (http://www.who.int/csr/disease/influenza/h1n1_table/en/index.html).

The benefit of molecular assays for the detection of rhinovirus and RSV outbreaks compared to culture is evident in this study. Although rhinovirus has been identified as a possible underestimated pathogen in long-term care settings [11], RSV is an important pathogen responsible for significant morbidity and mortality in older adults who make up a major component of the residents of long-term care facilities and this particular study population [12,13]. Although specimens were batch processed in this study, previous workers have indicated that molecular assays for RSV detection can be implemented in same- or next-day testing algorithms [2]. Molecular tests for RSV detection may be the most viable approach to rapid detection in long-term care facility outbreaks due to the relatively poor sensitivity of rapid antigen tests [1]. It should be noted that even with the relatively low numbers of RSV and influenza outbreaks identified in this study, the rapid antigen detection assay detected only one-third of the RSV outbreaks detected by molecular testing. The previously published poor sensitivity of rapid influenza tests was not evident in this study, which may be due to the low prevalence of influenza during the start of this study period [14].

One limitation of this study is that it did not utilize other molecular tests for the analysis of outbreak specimens. Therefore, the comparison of molecular and culture techniques for the surveillance of respiratory viral outbreaks in this study is dependent on the previously published test characteristics of the Seeplex assay [5] as well as the culture-based methodology used in the authors’ laboratory. Other groups may see different patterns of pathogen detection depending on the types of assays used in their facilities. Another issue is that even with the use of a molecular method, no respiratory viral pathogens were detected in 30% of the outbreaks. The lack of detectable viral pathogen in these outbreaks may have been due to several factors. It is possible that the patients were infected by a respiratory virus that was not included in this commercial panel. Candidates could include coronavirus or parainfluenza virus-4. Patients may have also been infected with a common bacterial pathogen or an atypical agent such as Chlamydia pneumoniae or Mycoplasma pneumoniae that was not detected by this panel [15]. A final possibility is that some patients with non-infectious respiratory illnesses may have been included as part of outbreak investigations within some institutions [16].

Although culture methods have rarely identified more than one pathogen in a respiratory outbreak, molecular methods are able to detect multiple pathogens in nearly 20% of outbreaks. This implies that infection control practitioners and clinicians may have in the past unknowingly lumped patients with infections of multiple viral etiologies into
common outbreak groups. The new ability to subdivide respiratory illness clusters by molecular means may allow for an increased ability to discern epidemiologic linkages between patients. Molecular techniques may also have a positive benefit in the management of patients who may have previously had an undefined respiratory illness during a concurrent influenza outbreak in a long-term or acute care facility. In conclusion, molecular testing may be able to identify a non-influenza respiratory pathogen in specific outbreaks, which should result in more rational utilization of infection control and clinical laboratory resources.

References


