Does Virus–Bacteria Coinfection Increase the Clinical Severity of Acute Respiratory Infection?

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This retrospective cohort study investigated the presence of bacteria in respiratory secretions of patients hospitalized with acute respiratory infections and analyzed the impact of viral and bacterial coinfection on severity and the mortality rate. A total of 169 patients with acute respiratory infections were included, viruses and bacteria in respiratory samples were detected using molecular methods. Among all samples, 73.3% and 59.7% were positive for viruses and bacteria, respectively; 45% contained both virus and bacteria. Bacterial coinfection was more frequent in patients infected by community respiratory viruses than influenza A H1N1pdm (83.3% vs. 40.6%). The most frequently bacteria detected were Streptococcus pneumoniae and Haemophilus influenzae. Both species were co-detected in 54 patients and identified alone in 22 and 21 patients, respectively. Overall, there were no significant differences in the period of hospitalization, severity, or mortality rate between patients infected with respiratory viruses alone and those coinfected by viruses and bacteria. The detection of mixed respiratory pathogens is frequent in hospitalized patients with acute respiratory infections, but its impact on the clinical outcome does not appear substantial. However, it should be noted that most of the patients received broad-spectrum antibiotic therapy, which may have contributed to this favorable outcome. J. Med. Virol.

INTRODUCTION

Viral and bacterial respiratory tract infections are major causes of morbidity and mortality worldwide despite the development of vaccines and powerful antibiotics [Deng, 2013]. An estimated 3–5 million people die of these infections annually, which is greater than the number of deaths caused by HIV/AIDS and tuberculosis combined [WHO, 2011]. During the 2009 pandemic influenza (H1N1pdm), bacterial pneumonia was registered in 25–30% of cases requiring hospitalization. Furthermore, almost 50% of small autopsy series highlight the importance of viral–bacterial coinfections, suggesting that several lower respiratory tract infections are caused by multiple pathogens acting in synergy [Deng, 2013].

The outcomes of this pandemic influenza were especially severe in the southern region of Brazil, with a sample positivity rate of 39% and a mortality rate of 35% [Raboni et al., 2011]. The high mortality rate observed in these patients led to questions about the presence of other factors that could be related to the severity of these infections, including associations with bacterial infection.

Therefore, this study evaluated the detection frequency of common community bacteria in respiratory tract samples from hospitalized patients with acute respiratory infections by using molecular methods.
respiratory infections as well as the impacts of bacteria–virus coinfection in these patients during the H1N1 influenza A pandemic.

**MATERIALS AND METHODS**

**Patients**

Specimens collected from the respiratory tract, nasopharyngeal aspirates (n = 164, 97%), or bronchoalveolar lavage fluid (n = 5, 3%) from patients presenting with acute respiratory infection hospitalized from April to December 2009 were included in this study. Samples were analyzed for the detection of community respiratory viruses (CRVs) and to investigate H1N1pdm. All patient records were reviewed. Cases were reported by completing a specific notification form that included medical history, epidemiological information, laboratory findings, and clinical outcome. The Hospital de Clinicas/UFPR Institutional Ethics Review Board approved this study (IRB#: 2160.055/2010-03).

**Pandemic H1N1 Influenza A Virus Detection**

H1N1pdm was detected and characterized by real-time reverse transcription-PCR (rtRT-PCR) according to the Centers for Disease Control and Prevention (CDC) protocol [WHO, 2011], which detects seasonal influenza A and H1N1pdm viruses. Viral RNA was extracted using a nucliSENS easyMAG kit (bioMérieux, Marcy l'Etoile, France) according to the manufacturer’s instructions.

**CRV and Bacteria Detection**

CRVs were detected using a multiplex RT-PCR technique. In brief, the viral genome was extracted using a High Pure viral RNA kit (Roche, Inc., Mannheim, Germany) according to the manufacturer’s instructions. First-strand cDNA was synthesized using random primers and an Improm-II reverse transcription system (Promega, Inc., Madison, WI). The resultant cDNA was subsequently subjected to PCR by using a Seeplex RV12ACE detection kit (Seegene, Inc., Seoul, South Korea) according to the manufacturer’s protocol. This multiplex PCR technology enables the simultaneous detection of multiple viruses including adenovirus (AdV); coronavirus types (CoV) 229E/NL63, and OC43/HKU1; human metapneumovirus (HMPV); parainfluenza virus types 1, 2, and 3; seasonal influenza A (FLUA) and B (FLUB); respiratory syncytial virus types (RSV) A and B; and human rhinovirus (HRV) types A and B.

Bacterial infections were investigated using Seeplex 2 PneumoBacter ACE Detection kit (Seegene, Inc., Seoul, South Korea) according to the manufacturer’s protocol to detect the following pathogens: Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, and Bordetella pertussis.

**Statistical Analysis**

Data were compiled using JMP version 5.2.1 (SAS Institute, Inc., Cary, NC) and analyzed using GraphPad Prism version 5.03 (GraphPad Software, Inc., La Jolla, CA). Fisher’s exact test or the χ² test was used to assess differences between groups, and the Mann–Whitney U-test was used to analyze continuous variables where appropriate. Continuous data are expressed as median (interquartile range). The difference between the medians of onset of illness was calculated by the nonparametric Kruskal–Wallis test.

Univariate analysis was performed to identify independent predictors of mortality. Furthermore, multiple logistic regression analysis including all statistically significant variables identified in the univariate analysis (P < 0.05) was performed to determine the variables that were risk factors for death. All P-values are two-tailed, and the level of significance was set at P < 0.05.

**RESULTS**

A total of 169 hospitalized patients were investigated for viruses and bacteria in respiratory secretions; 51% were male, the median age was 24 years (interquartile range: 1–44 years), and 20% progressed to death. Patients were divided into six groups according to the laboratory results: (1) samples positive only for CRVs (n = 10, 6%); (2) samples positive for CRVs plus respiratory bacteria (n = 50, 29.5%); (3) samples positive only for H1N1pdm (n = 38, 22.5%); (4) samples positive for H1N1pdm plus respiratory bacteria (n = 26, 15%); (5) samples positive only for respiratory bacteria (n = 25, 15%); and (6) samples negative for all investigated pathogens (n = 20, 12%). Demographic data, clinical presentation, radiographic findings, and outcomes are shown in Table I.

A predominance of younger patients was observed in group 2. Meanwhile, hospitalization duration was longer among patients who had only bacterial infection (group 5). The frequency of myalgia was higher among patients with H1N1pdm infection (groups 3 and 4) and in those with negative results (group 6). A higher frequency of comorbidities was observed in patients with CRV and bacterial infections (groups 1 and 5). Furthermore, a higher rate of hospitalization in intensive care units (ICUs) and greater mortality rate were observed in patients with H1N1pdm infection (groups 3 and 4) than in those infected by CRVs.

The predominant radiologic finding in the investigated patients was interstitial infiltrate; this was observed in all groups, regardless of bacteria detection, including the group positive only for bacteria. Among CRV-positive samples, 83.3% (50/60) were coinfected with bacteria. However, among FLUA H1N1pdm-positive patients, only 40.6% (26/64) were also positive for bacteria (odds ratio [OR] = 7.3, 95% confidence interval [CI]: 3.1–16.9, P < 0.0001).

Respiratory viruses were detected in 129 (75%) cases, 67 (39%) were diagnosed with FLUA H1N1pdm,

and 62 (36%) were diagnosed with CRVs, 9% of which were seasonal FLUA. In addition to seasonal FLUA, the most frequent CRVs were RSV A/B (8%) and HRV A/B (8%). Viral coinfections were more common among CRV cases (8%) than FLUA H1N1pdm cases (1%) [Raboni et al., 2011].

There was a high frequency of bacterial co-detection among all analyzed cases: 1, 2, and 3 bacteria were found in 45%, 54%, and 1% of the positive samples, respectively. The bacteria most commonly detected were S. pneumoniae and H. influenzae (22 and 21 patients, respectively); both bacteria were co-detected in 54 patients. Rare combinations were H. influenzae + S. pneumoniae + Mycoplasma pneumoniae (n = 1), S. pneumoniae + M. pneumoniae (n = 1), and M. pneumoniae (n = 2) (Fig. 1).

The presence of bacteria in the respiratory tract was not associated with increased mortality \( (P = 0.135) \) or longer hospitalization \( (P = 0.36) \). The interval between symptom onset and sample collection was significantly shorter in patients positive for bacteria than those negative for bacteria \( (P = 0.0076) \).

Comorbidities were frequent and associated with severity in all groups. Among all female patients, 15% (13/83) were pregnant; however, the presence of bacteria infection was not associated with increased mortality among them \( (P = 0.28) \).

Logistic regression analysis was subsequently performed to determine the factors that were independent predictors of mortality. In univariate analysis, ICU admission, mechanical ventilation, and radiological pattern were significantly associated with the mortality. However, in multivariate analysis, only ICU admission \( \text{(OR} = 54.4, 95\% \text{CI:} 12.1–310.8) \) and mechanical ventilation \( \text{(OR} = 12.4, 95\% \text{CI:} 2.4–65.9) \) were significant independent predictors of mortality.

### TABLE I. Epidemiologic and Clinical Data, and Outcomes of Hospitalized Patients with Acute Respiratory Infection Due to Pneumonia

<table>
<thead>
<tr>
<th>GROUP 1</th>
<th>GROUP 2 CRV n = 10 (6%)</th>
<th>GROUP 3 FLUA H1N1p n = 38 (22.5%)</th>
<th>GROUP 4 FLUA H1N1p + bacteria n = 26 (15%)</th>
<th>GROUP 5 bacteria n = 25 (15%)</th>
<th>GROUP 6 negative n = 20 (12%)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age y (Median, IQR)</td>
<td>24.5 (8–62.25)</td>
<td>35 (22.75–44.25)</td>
<td>26 (3.25–43.25)</td>
<td>22 (2–46)</td>
<td>45.5 (33.25–56.75)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>Male 50 60</td>
<td>39 46</td>
<td>64 40</td>
<td>50 40</td>
<td>61 54</td>
<td>60 40</td>
</tr>
<tr>
<td>Pregnant or puerperal (%)</td>
<td>Yes 30 100</td>
<td>13.2 86.8</td>
<td>11.5 88.5</td>
<td>8 92</td>
<td>5 95</td>
<td>0.2566</td>
</tr>
<tr>
<td>Setting (%) ICU</td>
<td>20 14</td>
<td>31.5 19</td>
<td>35.8 20</td>
<td>16 20</td>
<td>15 20</td>
<td>0.0336</td>
</tr>
<tr>
<td>Emergency room</td>
<td>30 54</td>
<td>8 19</td>
<td>6.5 20</td>
<td>4.25 20</td>
<td>4.25 20</td>
<td>0.0891</td>
</tr>
<tr>
<td>Nursery</td>
<td>50 32</td>
<td>60.5 42.5</td>
<td>64 65</td>
<td>50 32</td>
<td>60.5 42.5</td>
<td>64 65</td>
</tr>
<tr>
<td>Symptom duration (days)</td>
<td>3.5 (1.75–6.5)</td>
<td>4.5 (3–7)</td>
<td>4 (2–5.25)</td>
<td>3.5 (1.5–4)</td>
<td>4 (2–6)</td>
<td>0.091</td>
</tr>
<tr>
<td>Hospitalization duration (days)</td>
<td>4 (2.75–8.5)</td>
<td>6 (2–11.5)</td>
<td>8.5 (4.5–15.5)</td>
<td>6 (4–8)</td>
<td>0.0129</td>
<td></td>
</tr>
<tr>
<td>Comorbidity (%)</td>
<td>70 38</td>
<td>39 35</td>
<td>64 60</td>
<td>60 38</td>
<td>39 35</td>
<td>0.0387</td>
</tr>
<tr>
<td>Most frequent comorbidity</td>
<td>Cardiopathy</td>
<td>Chronic pneumopathy</td>
<td>Cardiopathy; chronic pneumopathy</td>
<td>Chronic pneumopathy</td>
<td>Cardiopathy; chronic pneumopathy; smokers and alcoholics</td>
<td>0.0268</td>
</tr>
<tr>
<td>Time between onset of disease and sampling, days (Median, IQR)</td>
<td>3 (1–7)</td>
<td>5 (2.75–7)</td>
<td>3 (2–4)</td>
<td>4 (2.25–5.75)</td>
<td>0.0268</td>
<td></td>
</tr>
<tr>
<td>Cough (%)</td>
<td>100 96</td>
<td>89 90</td>
<td>95 95</td>
<td>95 95</td>
<td>95 95</td>
<td>0.7155</td>
</tr>
<tr>
<td>Myalgia (%)</td>
<td>20 12</td>
<td>47 38</td>
<td>16 28</td>
<td>5 45</td>
<td>0.0057</td>
<td></td>
</tr>
<tr>
<td>Fever (%)</td>
<td>80 92</td>
<td>95 88</td>
<td>88 88</td>
<td>90 90</td>
<td>0.6674</td>
<td></td>
</tr>
<tr>
<td>Sore throat (%)</td>
<td>20 4</td>
<td>16 19</td>
<td>16 16</td>
<td>5 5</td>
<td>0.2340</td>
<td></td>
</tr>
<tr>
<td>Headache (%)</td>
<td>10 2</td>
<td>11 3.8</td>
<td>8 8</td>
<td>5 5</td>
<td>0.7098</td>
<td></td>
</tr>
<tr>
<td>Dyspnea (%)</td>
<td>70 58</td>
<td>63 68</td>
<td>62 68</td>
<td>75 75</td>
<td>0.8157</td>
<td></td>
</tr>
<tr>
<td>Diarrhea (%)</td>
<td>0 16</td>
<td>5 12</td>
<td>16 5</td>
<td>NA 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain (%)</td>
<td>0 4</td>
<td>0 8</td>
<td>0 0</td>
<td>NA 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics (%)</td>
<td>70 30</td>
<td>92 80</td>
<td>95 80</td>
<td>90 90</td>
<td>0.1103</td>
<td></td>
</tr>
<tr>
<td>Corticoida (%)</td>
<td>0 17.1</td>
<td>12 8</td>
<td>12 8</td>
<td>25 25</td>
<td>NA 25</td>
<td></td>
</tr>
<tr>
<td>Death (%)</td>
<td>20 8</td>
<td>37 34</td>
<td>12 10</td>
<td>10 10</td>
<td>0.0047</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as numbers, percentages, or median (IQR—interquartile range). CRV, community respiratory virus; NA, not applicable; NI, not informed.
Overall, bacterial–viral coinfection was detected in 45% of patients hospitalized with severe respiratory infection. H1N1pdm-infected patients exhibited a significantly higher mortality rate than other groups. However, the presence of bacterial infection was not associated with higher mortality. Coinfection was more frequently found in hospitalized patients with CRV than in those with H1N1pdm. Regarding the pandemic virus, the severity of the infection appears to be related to the virulence of the pathogen because bacteria associated with community-acquired pneumonia were significantly less frequent in this group.

Acute respiratory infections are a critical disease of the 21st century, incurring high mortality rates and significantly impacting the frequency and duration of hospitalization as well as severity. Therefore, their etiology should be thoroughly investigated in order to offer patients appropriate treatment and provide community safety surveillance to detect emerging pathogens. The association between influenza and bacterial pneumonia has been recognized for a long time [Morens et al., 2014]. Epidemiologic studies in the 20th century demonstrate that the incidence of pneumonia peaks with influenza activity [Stuart-Harris et al., 1949; Tyrrell, 1952; Oswald et al., 1958]. The present findings of high rates of bacterial coinfection are corroborated by these previous results.

Studies comparing the impact of viral–bacterial coinfection on patient outcome report conflicting results. Viral–bacterial coinfection is an important trigger for the exacerbation of asthma and chronic obstructive pulmonary disease. In vitro analyses show that respiratory viruses can promote bacterial infection through epithelial disruption and that chronic H. influenzae infection potentiates the inflammatory response to respiratory viruses [Deng, 2013; Wark et al., 2013].

Although our understanding of the epidemiology of influenza viruses is changing rapidly, bacterial pneumonia remains an important contributor to the severity and lethality of influenza infections [Deng, 2013]. Bacterial coinfection was probably associated with the majority of pneumonia deaths in the 1918 influenza pandemic and has been reported in up to 34% of 2009 influenza A H1N1 pandemic cases managed in ICUs; the most common pathogens in these cases are Staphylococcus aureus, Streptococcus pneumoniae, and Streptococcus pyogenes [Chertow and Memoli, 2013]. However, even though bacterial pneumonia is a common contributor to poor outcomes, various epidemiological data suggest the incidence, severity, and the identified pathogens differ among epidemics and geographical locations. Few studies in Brazil have identified the bacteria species in patients hospitalized owing to community respiratory infections. Mauad et al. [2010] reported
21 necropsies of Brazilian patients infected by influenza A H1N1pdm; the only bacterial species isolated in three samples from nine investigated patients was S. pneumoniae. It has been suggested that there is a geographic variation of the bacterial expression of virulence factors, which could explain the observed regional differences in severity [McCullers, 2013].

Several authors reported a high frequency of community-acquired pneumonia during the influenza A H1N1 pandemic. However, despite these patients’ higher severe pneumonia index scores, their mortality rate was similar to that of patients without bacterial coinfection [Dhanao et al., 2011; Cillóniz et al., 2012; Blyth et al., 2013; Martín-Löeches et al., 2013]. It is likely that coinfection contributed to the need for ICU admission in patients without other risk factors for severe influenza disease [Blyth et al., 2013].

The American CDC recognizes the importance of early empirical antibiotics in H1N1pdm-infected patients who might have concurrent bacterial pneumonia [CDC, 2009; Shieh et al., 2010]. Therefore, a protocol has been established in our institution on the basis of the evidence of frequent bacterial coinfection. In this protocol, all patients admitted to the ICU with pneumonia, even those only suspected of viral infection, receive antibiotics, usually a combination of ceftriaxone and azithromycin or respiratory quinolone. This approach may be associated with the attenuated severity and consequently decreased mortality rate observed in this study.

Of note, Falsey et al. [2012] reported that only respiratory viruses were detected in 28.4% of patients for whom antibiotics were prescribed unnecessarily; this resulted in an increased risk of developing Clostridium difficile colitis. Therefore, early empirical antibiotic therapy for patients with an unstable condition is appropriate but is not without risk.

Despite the interesting findings, the present study has some limitations that should be kept in mind when interpreting the results. First, the diagnostic value of nasopharyngeal aspirates is questionable because pathogen detection in asymptomatic patients is relatively common [Pavia, 2013]. Nevertheless, the prevalence of coinfection differed significantly between the CRV and H1N1pdm infection groups, suggesting the bacterial positivity found in these cases could be clinically relevant. Moreover, Obasi et al. [2014] reported a significantly lower detection rate of bacteria in respiratory secretions from healthy adult than patients with acute respiratory infection. Second, only the results of molecular methods were analyzed. Thus, it is possible that bacterial and viral infections diagnosed on the basis of DNA and RNA detection overestimate the incidence of infection because it is often unfeasible to distinguish colonization from infection. Third, the presence of viral RNA could merely indicate prolonged viral detection following a previous severe respiratory tract infection. Therefore, other factors such as clinical parameters, radiographic patterns, and biomarkers should be used to predict the risk of bacterial infection [Falsey et al., 2012]. Fourth, although S. aureus is usually described in association with influenza infections, it was not evaluated in the present study because the kit used does not detect it and bacterial culture was not performed because of safety reasons. However, this pathogen is not usually identified in community respiratory infections in our region. A previous study of Brazilian patients who died from influenza A H1N1pdm pneumonia also failed to detect this species [Mauad et al., 2010].

In conclusion, during the 2009 influenza pandemic, bacterial–viral coinfection was frequent in hospitalized patients. However, the precise effects of this coinfection on ICU admission remain unclear. Furthermore, a higher mortality rate was observed among H1N1pdm-infected patients independent of the bacteria present. Finally, this study demonstrates that bacterial coinfection is commonly associated with viral respiratory infection, while coinfection appears not to influence the outcome.

DISCLAIMER AND AUTHOR’S CONTRIBUTIONS

All authors contributed to development of this manuscript, approved the final version of this report and declare have no competing financial interests.

ACKNOWLEDGMENT

We are thankful to Mr. Ricardo R. Patterle, Statistical, Universidade Federal do Paraná, Health Science Section, for his support in statistical analysis.

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