Extended Abstract

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Analysis of Viral bacterial Co infection in Respiratory sampling Using Multiplex actual time PCR

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Abstract

Influenza and non-Influenza virus related respiratory tract infections are amongst the most common reasons reported for physician visits and hospitalizations, globally. The economic burden associated with these viral infections, within the United States, are upwards of \$50 billion annually. Concomitant, or subsequent, co-infections of the respiratory tract by pneumonia-causing pathogenic bacteria, like Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Staphylococcus aureus, are often observed in patients suffering from these respiratory viral infections and contribute to significant co-morbidity and mortality. In the present study, respiratory swabs from patients were tested for the presence of viral and bacterial pathogens, by Real-Time PCR, employing the Thermo-Fisher Open Array® platform. Amongst the 5793 samples tested (Dec 2017-Dec 2018), 2357 (40.68%) tested positive for the presence of Influenza and Non-Influenza viral infections. Out of these positive patient samples, 1175 (49.85%) also tested positive for the presence of one or more pneumonia-causing bacterial species.

The co-infection data obtained was distributed on the basis of age. Interestingly, nearly 30% of the cases from the younger patient subset (<1 year old and 1-15 years old) were found to be co-infected with S. pneumoniae, H. influenzae and M. catarrhalis within the same sample. In addition, infant (<1 year old) patient viral positive samples, displayed higher than previously reported levels of M. catarrhalis co-infections (72.08%). With the ever-increasing popularity of point of care testing, this study demonstrates the importance and efficacy

of a rapid, comprehensive multiplex-PCR, syndromic-panel approach to respiratory disease diagnostics, assisting clinicians to make better, and timely, decisions, potentially leading to improved patient care and outcomes.

Keywords: Influenza; Co-infections; Influenza like illness; Respiratory virus; Respiratory infection

Introduction

Respiratory viral disease caused by Influenza A, B (Influenza) and other non-influenza viruses [Respiratory Syncytial Virus (RSV), Rhinovirus (RV), Coronavirus (CV) Parainfluenza virus (PIV), Human metapneumovirus (HMPV), Adenovirus (AV)], can be complicated by secondary bacterial infection, which has significant impact on disease presentation and the associated morbidity and mortality rates. Bacterial coinfections assert important implications on clinical and patient care decisions. Influenza and non- influenza viral infections of the respiratory tract renders the very young, and people over the age of 65 years, highly susceptible to bacterial pneumonia. The total economic burden of Influenza, calculated as both direct medical care and indirect costs, in the United States, is \$11.2 billion. Non-influenza viral respiratory tract infections have an even larger economic impact, with the economic burden of these infections, within the United States, estimated at approximately \$40 billion annually. The comorbidity associated with concurrent bacterial pneumonia and influenza infection, has been well documented in the four Influenza pandemics of the last century. It is now believed that the high rate of mortality and disease severity reported during these pandemics, was due in part to concurrent or secondary bacterial co-infections with Streptococcus pneumoniae, Hemophilus influenzae and Staphylococcus aureus. These same bacterial pathogens are also responsible for the increased morbidity of other noninfluenza respiratory viral infections. A number of cellular and molecular mechanisms have been elucidated, that demonstrate how viral infections of the respiratory tract predispose to bacterial infection. This necessitates a rapid and

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reliable diagnosis of the causative organism [17,18]. The growing popularity of low complexity point of care (POC)/near POC testing for respiratory disease diagnosis, is based on the rapid turnaround time and ease of operation, with minimal required technical expertise [19]. However, these tests can suffer from lower sensitivity and a limited test menu of organisms, especially with respect to respiratory tract bacterial pathogens. In many cases, these POC tests are restricted to one or only a few respiratory viruses [20-22]. Polymerase Chain Reaction (PCR) based molecular diagnosis is the new "gold standard" of laboratory-based testing for infectious diseases [23]. It has been asserted that employment of a comprehensive multiplex-PCR, syndromic-panel approach to the diagnosis of respiratory tract infectious diseases, can help achieve reliable results in a quick time frame, thus reducing the overall direct and indirect medical costs associated with these infections [24]. There is a large body of work detailing the association of bacterial coinfections during Influenza pandemics. However, the available data for seasonal variations of Influenza and other respiratory viruses, and the associated bacterial co-infections, is sparse [25]. Recommendations by the Center for Disease Control and Prevention (CDC) and other regulating bodies focus on good antibiotic stewardship, warn against treating these known pneumonia causing bacteria in out-patient settings, making clinical decisions on treatment tougher for physicians.

Real-Time PCR analysis

Real-Time PCR analysis was performed using the QuantStudioTM 12K Flex Real-Time PCR system (ThermoFisher, California, USA). Diluted pre-amplified nucleic acid, isolated from patient samples, was placed on Open Array TM plates containing the target primers and probes, using the AccuFillTM system.

Data analysis

All statistical analysis was performed using Microsoft Excel. While collating patient test results, no personal identifying data was accessed. Each patient sample was assigned a randomly generated sample identification number and only the gender and age of the sample were used for data analysis.

Results

In the present study, spanning a period of one year (December 2017 to December 2018), a total of 5793 respiratory samples were tested for the presence of viruses and bacteria, causing influenza like illness (ILI). Amongst these, 2357 (40.68%) tested positive for the presence of Influenza and Non-Influenza viral respiratory infections. Out of the patient samples that tested positive for the presence of these respiratory viruses, 1175 (49.85%) also tested positive for the presence of causing bacterial pneumonia. The main viral genera detected were Rhinovirus (24.17%), RSV (19.65%), Coronavirus (18.97%), Influenza virus (13.87%), HMPV (8.42%), Adenovirus (7.91%) and PIV (6.97%)