Abstract 41066:

Age-Specific Prevalence of Influenza A Positive with Bacterial Co-detection during the 2012-2013 Influenza Season

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Abstract

Background: Multiplex PCR approach is a proven tool in molecular diagnostics for the surveillance of complex respiratory tract infections in the human population. Bacterial colonization and/or infection associated with Influenza A is typically overlooked because of the limitations of existing technology that does not allow simultaneous detection of viruses and bacteria in the same sample. Using TEM-PCR™ (Target Enriched Multiplex Polymerase Chain Reaction) technology, the respiratory panel illustrated in this study highlighted the potential implication of bacteria in the development of complex pneumonia in patients who are Influenza A positive.

Methods: Nasopharyngeal specimens collected from symptomatic patients were submitted for testing using TEM-PCR panel that contains 12 viral and 15 bacterial targets. DNA and RNA were obtained from a single specimen and the extraction and amplification steps incorporated 27 target-specific primers. Pathogen specific targets were detected on a Luminex® platform and results were reported as "detected" or "non-detected" based on established cut-off criteria.

Results: Overall, the Influenza detection rate in tested clinical specimens submitted for analysis (n= 22,300) was 7.4%, which is comparable with the Influenza detection rate of 9.3% reported by CDC. However, during the peak season encompassing mid-December to mid-February, the number of positive tests for Influenza increased to 15.1%. Nearly 50% of all detected Influenza cases were distributed within the pediatric population of ages 2 to 13. Half of the cases positive with Influenza within the 2-13 year age group had co-detection or infection with *S. pneumoniae*. Significant co-detection or co-infection was seen with *H. influenza* (33%) and *M. catarrhalis* (29%) as well. The highest percentage of influenza positive cases fell within the 5-6 year age group (30%) and this prevalence steadily declined; reaching 10% in the 12-13 year age group. Bacterial co-detection was seen more frequently in the 3-6 year group; 40% to 60% depending upon the bacterial species.

Conclusions: This study brings the importance of consideration of bacterial co-detection associated with seasonal influenza infection. The multiplex detection of respiratory viruses and bacteria using Diatherix TEM-PCR panels could warrant more effective disease treatment in pediatric population.

Introduction

For most respiratory tract infections, clinical presentations are often not specific enough to allow definitive determination of pathogens. For example, coughing and fever are symptoms during the flu season that may be caused by different bacterial and viral pathogens or a combination of both. The notion that respiratory viruses contribute to and even predispose patients to bacterial infections in the lung has been suspected and carefully studied for over a century. Estimates from a variety of sources suggest that the number of bacterial pneumonias that develop following influenza viral infection may be as high as 90%[1].

Several multiplex PCR-based devices are commercially available for detection and differentiation of a panel of viral pathogens and they had been used in the clinical setting. Diatherix's Respiratory Panel, that contains a combination of 12 viral and 15 bacterial targets for detection in a single clinical sample, is a unique tool which can be used for identification and detection of viral and bacterial pathogens and help clinicians to provide accurate patient management. Clinical accuracy of TEM-PCR based multiplex panels and the correlation of sensitivity with TaqMan® PCR assays was described earlier[2]. After receiving a substantial amount of feedback from healthcare professionals and clinicians during the 2012-2013 influenza season who voiced their concern about the 'kids' who seemed to be having a more problematic course of 'flu' during this interval, we began to look closely at the patient demographics of patients who had been screened for influenza on a multiplex molecular platform that identifies both viral and bacterial pathogens. Here we report the data analysis of 31,320 total specimens that were screened for 12 viral and 15 bacterial targets using a TEM-PCR Respiratory Panel for identification of association between Influenza A and most prevalent bacterial targets during 2012-2013 flu season.

Methods

Nasopharyngeal specimens (n=31,320) were collected and submitted to Diatherix from symptomatic patients for testing using a multiplex TEM-PCR Respiratory Panel from November 1, 2012 to April 26, 2013. DNA and RNA extractions were performed on the KingFisher™ system using kits from Omega Biosciences (Marietta, GA) and MO-BIO (Carlsbad, CA). Using 27 target-specific primer sets, gene targets from the extracted samples were amplified using the TEM-PCR Respiratory Panel. The amplified targets were then detected using a bead-based platform and results were reported as "detected" or "not detected" based on established cut-off criteria.

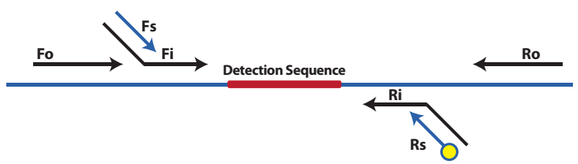

Figure 1. TEM-PCR Scheme.
Low concentration of nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) are designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal Super Primers (Fs and Rs) is used to amplify all targets. Reverse Super Primer is labeled with biotin. Labeled PCR products are detected with a complimentary detection probe which is covalently coupled to a detection platform.

Results

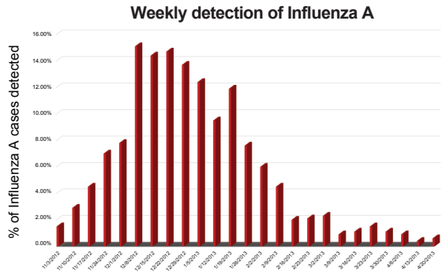

Figure 2. Weekly detection of Influenza A-positive samples during 2012-2013 Flu season (n=31,320)

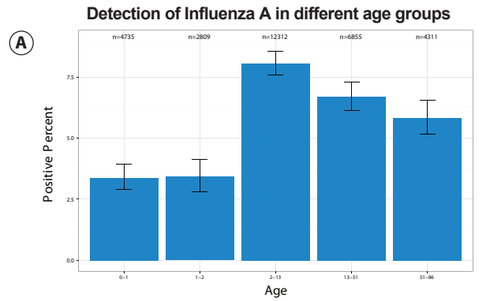

Figure 3. Detection of Influenza A in different age groups **(A)**. Co-detection rate with other targets present in Diatherix Respiratory Panel during 2012-2013 flu season **(B)**. The 95% confidence intervals were computed based on algorithm described by Brown at al, Statistical Science, 2001, 16, 101-133. Note significant co-detection of 3 bacterial targets, *H. influenzae, M.catarrhalis* and *S. pneumonia* (red boxes) in pediatric population

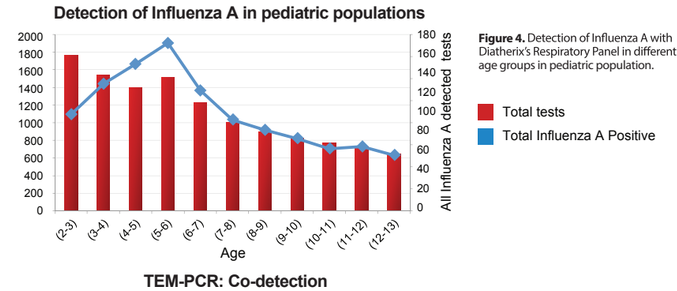

Figure 4. Detection of Influenza A with Diatherix's Respiratory Panel in different age groups in pediatric population.

Total tests
Total Influenza A Positive

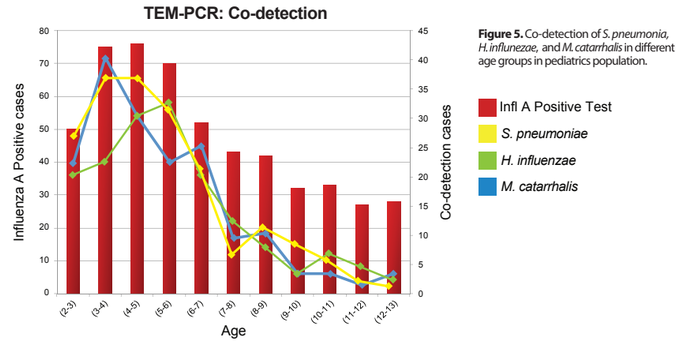

Figure 5. Co-detection of *S. pneumonia, H. influenzae,* and *M. catarrhalis* in different age groups in pediatrics population.

Infl A Positive Test
S. pneumoniae
H. influenzae
M. catarrhalis

Discussion

The specimens that were positive for Influenza A (presumed to be H3N2) showed a detection rate of 9.92% of 31,320 samples tested. Within the group of specimens that were positive for Influenza A (n=3,108), co-detection of *S. pneumonia* was detected at a rate of 11%, *H. influenza* at a rate of 6.8%, and *M. catarrhalis* 8.7%. Of particular interest was the increased rate of detection of these bacterial pathogens in the 2 to 12 age group.

Noteworthy is the fact that this study can only suggest a relationship between the co-detection data presented and the worsening of respiratory symptoms in the younger population groups identified in this data-mining exercise. There was a significant difference in the frequency of encountering bacterial co-detection in Influenza A positive specimens for the age group of 2-12; e.g. ($p<0.01$).

Differentiation of viral and bacterial pneumonia in children can be particularly difficult. With some of the emerging diagnostic techniques that use molecular amplification technologies, we have learned that there may be a large number of both established and newer pathogens, acting by themselves or synergistically, that can cause or contribute to a more problematic course of respiratory illness. The availability of suitable amplification technologies together with the challenges of obtaining representative specimens that reflect the ecosystem of the lower airway, add additional obstacles to obtaining a meaningful diagnosis in the clinical environment. This study did not address the challenges of respiratory specimen collection. Furthermore, there was not an attempt to retrospectively gather clinical correlation data that may have made the implied associations more meaningful. A properly designed prospective study is planned that utilizes this multiplex diagnostic platform along with a systematic approach to the collection of representative lower respiratory secretions.

1. Babuik, LA et al, Avd in Virus Research, 1988, v.38, p. 219-249
2. Deng, J et al., Vir. Sinica, 2013, v.28, p.97-102

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