Journal of Infection (2014) 68, 125–130

BIA
British Infection Association

www.elsevierhealth.com/journals/jinf

Detection of viral and bacterial pathogens in acute respiratory infections

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Accepted 31 October 2013
Available online 7 November 2013

KEYWORDS
Upper respiratory infections;
Bacteria;
Common cold;
Inflammation

Abstract *Objectives:* The role of bacteria in acute respiratory illnesses (ARI) of adults and interactions with viral infections is incompletely understood. This study tested the hypothesis that bacterial co-infection during ARI adds to airway inflammation and illness severity.
Methods: Two groups of 97 specimens each were randomly selected from multiplex-PCR identified virus-positive and virus-negative nasal specimens obtained from adults with new onset ARI, and 40 control specimens were collected from healthy adults. All specimens were analyzed for *Haemophilus influenzae*(*HI*), *Moraxella catarrhalis*(*MC*) and *Streptococcus pneumoniae*(*SP*) by quantitative-PCR. General linear models tested for relationships between respiratory pathogens, biomarkers (nasal wash neutrophils and CXCL8), and ARI-severity.
Results: Nasal specimens from adults with ARIs were more likely to contain bacteria (37% overall; *HI* = 28%, *MC* = 14%, *SP* = 7%) compared to specimens from healthy adults (5% overall; *HI* = 0%, *MC* = 2.5%, *SP* = 2.5%; $p < 0.001$). Among ARI specimens, bacteria were more likely to be detected among virus-negative specimens compared to virus-positive specimens (46% vs. 27%; $p = 0.0046$). The presence of bacteria was significantly associated with increased CXCL8 and neutrophils, but not increased symptoms.
Conclusion: Pathogenic bacteria were more often detected in virus-negative ARI, and also associated with increased inflammatory biomarkers. These findings suggest the possibility that bacteria may augment virus-induced ARI and contribute to airway inflammation.

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0163-4453/$36
http://dx.doi.org/10.1016/j.jinf.2013.10.013

Introduction

Viruses are the major cause of acute respiratory infections (ARI) in both adults and children.[1] ARI, including both influenza and the common cold, is a worldwide problem that accounts for significant loss of productivity and financial burden on the healthcare system.[2–4] Although various experimental[5] and epidemiological[6,7] studies have identified viruses as the pathogens for most ARI, a significant number of ARI episodes have unknown etiologies despite improved diagnostic procedures.[8–10]

It has been suggested that bacterial pathogens such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* contribute to ARI, however; results from studies have been inconclusive.[11,12] Detection of bacteria is increased in symptomatic children[13,14] and adults[11] compared to healthy controls.[15,16] In a study involving 507 ARI sufferers[11] Heald et al. (1993) reported 56% positive bacteria cultures from nasopharyngeal secretions of adults with ARI illness, but found no bacteria in healthy controls. However, Winther et al. (1984) found no difference in nasal bacterial between healthy and ARI ill conditions.[12] Even so, antibiotics are often prescribed for uncomplicated ARI,[17] and widespread inappropriate use of antibiotics contributes to the emergence of antibiotic resistance and therefore to increased health care costs.[18]

There is evidence that virus-induced inflammation contributes to respiratory symptoms. During the course of viral illnesses, there are significant correlations between interleukin-8 (CXCL8)[7] levels and neutrophil counts[19] in nasal secretions and cold symptom severity. There is some evidence that detection of bacterial pathogens during ARI may be associated with increased inflammatory biomarkers.[11]

Given these findings, we hypothesized that during viral ARI, detection of specific bacterial pathogens would be associated with increased levels of inflammatory biomarkers and greater measures of severity of illness. A secondary goal was to examine nasal secretions for pathogenic bacteria in ARI adult sufferers with and without detectable viruses. The rationale for this stratified analysis is to determine if bacterial co-infection would lead to greater ARI illness severity compared to viral only or no pathogen detection. Stratification enabled us to determine whether symptoms were greater for "bacteria plus virus" vs. "virus alone", and also to determine whether symptoms were greater for "bacteria alone" compared to "no pathogen detected".

As a control group, we also evaluated the frequency of the same bacteria in nasal wash specimens from healthy adults. Finally, we assessed the relationship between these respiratory pathogens, inflammatory biomarkers and self-reported severity of illness.

Design and methods

Study populations

The study protocol was approved by the University of Wisconsin—Madison Institutional Review Board. The ARI specimens were obtained from a subset of participants in the NIH-sponsored randomized clinical trial, the "Physician, Echinacea, Placebo (PEP)" study.[20] A total of 712 nasal wash specimens were obtained from adults at the beginning of an ARI and were tested for viral nucleic acid by multiplex PCR multiplex.[21] Of these, 395 were found to be positive for virus and 317 found to be negative. For this study, 97 specimens per group were randomly selected (www.randomizer.org) from each of 2 groups: those with detectable respiratory viruses and those without detectable respiratory viruses. This sample size was selected based on 2-sided testing, with $\alpha = 0.05$, power = 80%, and hypothesized 20% difference in bacterial detection rates (effect size). The PEP trial spanned from January 2004 to August 2008 and enrolled 719 participants of whom 713 completed the study (one participant had missing viral nucleic acid result).[22] The study rationale and methods have been described previously.[23]

Briefly, the pill arm of the PEP trial examined placebo and Echinacea. Participants were eligible if they acknowledged having a cold, had $\geq$2 points on the Jackson symptom scale[24] and included $\geq$1 of the following symptoms within 36 h of enrollment: nasal discharge, obstruction, sneezing or sore-throat. Reasons for exclusion included active symptoms of allergy and asthma observed at enrollment, or use of antibiotics or other excluded medications.

Additional specimens were obtained from adults ($n = 40$) with no evidence of cold symptoms.

Outcome assessments

Global ARI severity was calculated using area-under-the-curve trapezoidal approximations with duration on the *x*-axis and symptom scores on the *y*-axis. Duration of illness was defined as time from symptom onset until the participant responded with "No" to the question "Do you think you still have a cold?" Symptom scores were self-reported on the Wisconsin Upper Respiratory Symptom Survey (WURSS-21).[25] The WURSS-21 consists of 10 symptom and 9-quality of life items used in severity estimations. Two remaining items assessing global severity "(How sick do you feel today?") and daily change of illness ("Compared to yesterday, I think my cold is…") were assessed separately. PEP findings showed no significant between-group differences in severity and duration of illness between treatment groups.

Nasal wash specimens were also analyzed for interleukin-8 (CXCL8) and neutrophils as described previously.[23,26]

Pathogen detection

Viral pathogens from nasal secretions collected on day-1 were identified using multiplex PCR (Respiratory MultiCode-PLx Assay, EraGen Biosciences, Madison WI). This assay detects all common respiratory viruses including; rhinovirus, coronavirus, influenza, respiratory syncytial virus, parainfluenza virus, adenovirus, bocavirus, metapneumovirus, and enterovirus.[21]

Nasal wash specimens were analyzed for specific bacterial pathogens. DNA was extracted from 300 μl of nasal wash specimens (BiOstic Bacteremia DNA isolation kit, MO