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Major article

Seasonal variation of respiratory pathogen colonization in asymptomatic health care professionals: A single-center, cross-sectional, 2-season observational study

Ali Hassoun MD [a], Matthew D. Huff BA [b,*], David Weisman PhD [c], Khushdeep Chahal MD [d], Esmeralda Asis MD [d], Don Stalons PhD, D(ABMM), MPH [b], Elena Grigorenko PhD [b], Jessica Green BS [b], Leslie L. Malone MS, MB(ASCP)^{CM} [b], Scott Clemmons BS [b], Stanley Lu MS [b]

[a] *Alabama Infectious Disease Center, Huntsville, AL*
[b] *Diatherix Laboratories Inc, Huntsville, AL*
[c] *Department of Biology, University of Massachusetts Boston, Boston, MA*
[d] *University of Alabama Birmingham at Huntsville Internal Medicine Residency Program, Huntsville, AL*

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Background: The purpose of this study was to determine the seasonal variance of potentially pathogenic bacterial and viral organisms in nasopharyngeal specimens obtained from asymptomatic health care professionals (HCPs) during the 2014 winter and summer months.

Methods: Nasopharyngeal specimens from 100 HCPs were collected from Huntsville Hospital (Huntsville, AL) during the winter and from 100 HCPs during the summer. All subjects were tested for 22 viruses and 19 bacteria using Target Enriched Multiplex Polymerase Chain Reaction. Both seasonal cohorts were composed of students, nurses, physicians, and residents.

Results: Of the 100 HCPs tested during the winter, 34 subjects were colonized with at least 1 bacterium, and 11 tested positive for at least 1 virus. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Moraxella catarrhalis*, and coronavirus were the most frequently detected potentially infectious agents. Of the 100 HCPs tested during the summer, 37 tested positive for at least 1 bacterium, and 4 tested positive for a viral agent. The most prevalent bacteria were MRSA and *Klebsiella pneumonia*.

Conclusion: Nasopharyngeal carriage among asymptomatic HCPs was common, but the frequency and presence of potential pathogens varied with each season. Understanding the colonization and infection potential of upper respiratory organisms is important, particularly for viruses. Although asymptomatic HCPs certainly harbor a number of different potentially infectious agents, future studies are needed to determine whether colonized pathogens are transmitted or initiate infection in at-risk patient populations.

* Address correspondence to Matthew D. Huff, BA, 601 Genome Way, Ste 2100, Huntsville, AL 35806.

E-mail address: matthew.huff@diatherix.com (M.D. Huff).

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Author Contributions: A.H. formed the hypothesis for this study, assisted in manuscript revisions, and oversaw study at Huntsville Hospital. M.D.H. wrote the manuscript, did most of the research, and created most of the figures and tables. K.C. and E.A. collected specimens, gathered and organized health care professional data into spreadsheets, did the initial data analysis, and presented a poster of these results at ID Week 2014. D.W. created Figure 3 and along with D.S. and E.G. provided scientific insight throughout manuscript revisions. J.G. oversaw all clinical laboratory employees at Diatherix that performed TEM-PCR testing. L.L.M. oversaw all research and development employees during the development of the TEM-PCR respiratory panel. S.C. developed all of Diatherix internal computer applications that allowed for the organization of all health care professional TEM-PCR results. S.L. supervised all of the steps in manuscript preparation and oversaw the study at Diatherix.

Other Information: This study was approved by the Huntsville Hospital Institutional Review Board in December 2013. All health care professional identities were kept confidential. The only personal information provided to Diatherix in this study were age, sex, and health care professional profession. Throughout this study, the guidelines set forth by the Declaration of Helsinki and the Health Insurance Portability and Accountability Act were strictly followed.

Conflicts of Interest: A.H. uses TEM-PCR testing at Huntsville Hospital on his patients. M.D.H., D.S., E.G., J.G., L.L.M., S.C., and S.L. are all full-time employees of Diatherix Laboratories Inc. D.W. is a paid consultant of Diatherix. K.C. and E.A. have no conflicting interests.

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Within healthy subjects, the upper respiratory tract fosters a complex, commensal microbiome.[1,2] However, introduction of a foreign bacterial or viral organism into the microbiologic community may disrupt normal intercellular relationships resulting in an imbalanced ecosystem.[1-3] The detailed mechanisms that drive these complex interactions are largely unknown; consequently, it is difficult to predict the behavior of this highly nonlinear system. Downstream consequences can be influenced by activities of other organisms, use of antimicrobials, or the host's immune response.[1] In immunocompromised subjects, such as neonates, elderly adults, and patients undergoing chemotherapy, the addition of an unfamiliar pathogen can offset the delicate equilibrium and increase the likelihood of pathogenic invasion.[2] In individuals with functional immune systems, internalized microbes are eliminated, whereas noninternalized pathogens can thrive within the respiratory microbiome, thereby using their hosts as vectors. However, these asymptomatic individuals can still pose a threat of transmitting the potentially pathogenic agent to at-risk subjects.

Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR; Diatherix Laboratories, Huntsville, AL) simultaneously detects 22 viral serotypes and 19 bacterial species that often inhabit the upper respiratory tract. This multiplex assay is performed from a single patient specimen and has high levels of specificity and sensitivity (Table 1). Little is known about the colonization rates of respiratory pathogens in asymptomatic adult subjects, especially for viruses. Here, we describe the frequency and seasonal variation of bacterial and viral detections in asymptomatic health care professionals (HCPs) during the winter and summer months of 2014. Seasonal variation of infectious disease rates is a common phenomenon that is clearly manifested when temperatures are at extremes; consequently, there may be seasonality of colonization levels and commensal relationships of pathogens in asymptomatic individuals.[4] By elucidating changes in pathogen colonization rates in asymptomatic HCPs during different time periods in the year, health care organizations can monitor which potentially pathogenic agents are most prevalent in carriers in a health care setting and observe correlations with infection levels in at-risk hospitalized patients.[5] New mechanisms of pathogen transmission can be hypothesized and tested, which could be potentially important in a clinical setting and have a positive effect on infection control practices.

Table 1
Limits of detection (LoD_{95}) for respiratory targets

Viral organism	LoD_{95} (pfu/mL)
Adenovirus type 3	79
Adenovirus type 4	71
Human bocavirus	86 (cop/μL)
Coxsackievirus A	1×10^3
Coxsackievirus B	1×10^2
Echovirus	1
Influenza A H1N1-09	7
Coronavirus 229E	6
Coronavirus HKU1	87 (cop/μL)
Coronavirus NL63	1
Coronavirus OC43	7
Influenza A H3N2	1
Influenza B	1
Parainfluenza virus type 1	10
Parainfluenza virus type 2	6
Parainfluenza virus type 3	100
Parainfluenza virus type 4A	88
Rhinovirus	1
Respiratory syncytial virus A	1
Respiratory syncytial virus B	1
Human metapneumovirus A	562
Human metapneumovirus B	316
Bacterial organism	**LoD_{95} (cfu/mL)**
Acinetobacter baumannii	7.5×10^4
Chlamydophila pneumoniae	100
Haemophilus influenzae (nonspecific: types A-F)	1×10^3-1×10^4
H influenzae type B	1×10^3-1×10^4
Klebsiella pneumoniae	1×10^3
Legionella pneumophila	750
Mycoplasma pneumoniae	10
Moraxella catarrhalis	100
Neisseria meningitidis	72
Pseudomonas aeruginosa	1×10^3
Bordetella pertussis	6.8×10^4
Staphylococcus aureus	100
Streptococcus pneumoniae	464
Str pyogenes	1×10^4
Panton-Valentine leukocidin (cytotoxin)	3.20×10^4
Methicillin resistance (antibiotic resistance)	100

NOTE. Analytical sensitivities were determined following Clinical and Laboratory Standards Institute guidelines. At the concentrations listed, the corresponding genetic target is a true positive result 95% of the time. The Supplemental Data describes the analytical validation procedures, including the measurement of >95% test specificity.
cfu, colony forming units; *cop*, copies; *pfu*, plaque forming units; *LoD95*, 95% Limit of Detection.

MATERIALS AND METHODS

HCP sample description

Two hundred HCPs, consisting of 100 during the winter and 100 during the summer, from Huntsville Hospital (Huntsville, AL) were tested for the presence of respiratory pathogens via TEM-PCR. HCP categories included medical students, nurses (intensive care unit and general patient care), physicians, and residents. Pertinent histories, including recent upper respiratory symptoms and antibiotic use, were obtained. All 200 HCPs were asymptomatic for a minimum of 4 weeks before samples were taken. Each HCP completed a questionnaire that evaluated their respiratory status. Questionnaires were reviewed by physicians at Huntsville Hospital, and it was determined that none of the subjects likely had current infections during specimen collection intervals. None of the physicians who participated in the study reviewed the surveys. Eligibility criteria required HCPs be free of respiratory symptoms for at least 4 weeks prior to specimen collection and agree to provide a nasopharyngeal specimen for testing. Nasopharyngeal specimens were collected in 2014 during January-February and June-July, representing the winter and summer observations, respectively. Specimens were sent via courier to Diatherix Laboratories. Results were sent to the study coordinator at Huntsville Hospital within 24 hours of sample receipt, and study participants were notified of the results.

TEM-PCR

Nucleic acid extraction for TEM-PCR was performed using a KingFisher system (Thermo Fisher Scientific, Waltham, MA). The amplification steps were completed using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Probe fluorescence was read using a SensoSpot FLAIR system (Sensovation, Radolfzell am Bodensee, Germany). A more detailed description of specimen collection and transport conditions, TEM-PCR technology, and analytical validation of the assay are included in the Supplemental Data.

Statistical and data analysis

All TEM-PCR results were organized and extracted from a reporting database at Diatherix Laboratories using Microsoft SQL