



Patterns of Viral and Bacterial Co-detection among Otherwise Healthy Adults with Influenza-like Illness: Utilization of a Multiplex Respiratory Pathogen Panel



Michelande Ridoré MS^{2,9}, Wei-Ju Chen PhD^{2,9}, Mary P. Fairchok MD^{2,3,9}, Christina Schofield MD³, Kristina St. Clair DO MTM&H⁴, Patrick J. Danaher MD⁵, Michael Rajnik MD⁶, Erin McDonough BS⁷, Leslie Malone MS, MB(ASCP)CM⁸, Elena Grigorenko PhD⁸, Donald Stalons PhD⁸, Deepika Mor MS^{2,9}, Timothy H. Burgess MD MPH⁶, John C. Arnold MD¹, Eugene V. Millar PhD^{2,9}

1 Infectious Disease Clinical Research Program, Bethesda MD; 2 Naval Medical Center San Diego CA; 3 Madigan Army Medical Center, Fort Lewis WA; 4 Naval Medical Center Portsmouth VA; 5 San Antonio Military Health System, San Antonio TX; 6 Walter Reed National Military Medical Center, Washington DC; 7 Naval Health Research Center, San Diego CA; 8 Diatherix Laboratories, Huntsville AL; 9Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda MD



Correspondence: emillar@idcrp.org

Introduction

- Bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* frequently colonize the nasopharynx of healthy individuals. During acute respiratory infection (ARI), the majority of which are viral in origin, these and other bacteria may worsen the severity of disease.
- To examine these associations, we used a multiplex assay to evaluate patterns of viral/bacterial co-detection among otherwise healthy individuals with influenza-like illness (ILI).

Methods

- Since 2009, we enrolled otherwise healthy military personnel and beneficiaries into an observational, longitudinal study of influenza-like illness (ILI) at five military treatment facilities across the continental United States (Figure 1).

Figure 1. Clinical sites participating in the ARIC Natural History Study

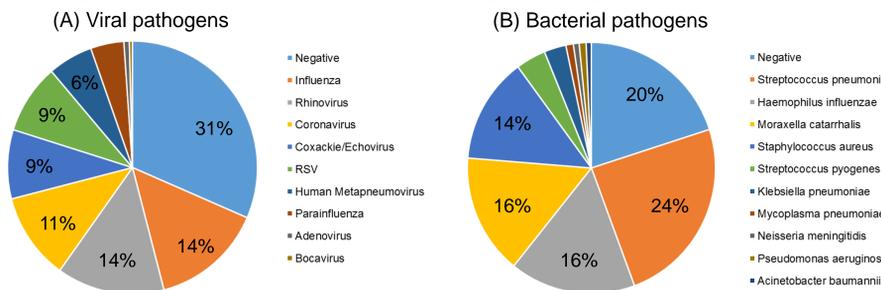


- Eligibility. Patients presenting for care <72h after the onset of ILI, defined as fever (temperature of 100.4° F or greater at the time of evaluation, or by self-report) and sore throat or one of the following respiratory symptoms: cough, sputum production, shortness of breath, or chest pain. Patients with underlying medical conditions were excluded.
- Clinical and demographic information, and a nasopharyngeal swab was collected at baseline (day 0). Participants returned on days 3±1, 7±2 and 28±7; a daily symptom diary was completed for the first seven days following ILI onset. Symptom presence and severity was recorded either by self-report (diary) or interview as: 0 (none); 1 (mild: not changing activity or requiring treatment); 2 (moderate: requiring some modification in activity and/or medication); and 3 (severe: incapacitating, unable to perform normal activities, requiring bed rest and/or medication). Participants were trained by research personnel on the definitions of each score. Swabs were tested for influenza by real-time reverse transcription polymerase chain reaction (rRT-PCR) at the Naval Health Research Center (San Diego, CA).
- A target-enriched multiplex PCR (TEM-PCR) panel for 13 bacterial and 10 viral respiratory pathogens was developed by Diatherix Laboratories, LLC. (Huntsville, AL). The platform relies upon nested multiplex PCR to provide the initial target enrichment and super primers to amplify and label the PCR products. The viral respiratory pathogens on the panel include: adenovirus, coxsackievirus/echovirus, bocavirus, coronavirus, human metapneumovirus, rhinovirus, influenza A/B, parainfluenza and respiratory syncytial virus. The bacterial respiratory pathogens on the panel include: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Neisseria meningitidis*.
- Statistical analyses were performed using SAS (Version 9.3; SAS Institute, Cary, NC). The study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences (IDCRP-045).

Results

- 895 specimens were evaluated by Diatherix TEM-PCR: 259 children (28.9%) and 636 adults (71.1%)
- 583 (65%) were positive for at least one virus, 643 (72%) were positive for at least one bacterium, and 399 (44%) were positive for both. The most common viruses were: rhinovirus, n=140 (16%); Influenza, n=144 (16%); Coxsackie/Echovirus (CVEV), n=89 (10%); Coronavirus, n=111 (12%); and RSV, n=89 (10%). The most common bacteria were: *Streptococcus pneumoniae*, n=330 (36%); *Haemophilus influenzae*, n=224 (25%); *Moraxella catarrhalis*, n=209 (23%); and *Staphylococcus aureus*, n=181 (20%). (Figure 2)

Figure 2. Detection of viral and bacterial pathogens among ILI patients (n=895)



- Children <5 years were more likely to have CVEV, RSV, *H. influenzae* and *M. catarrhalis*. Day care attendance was associated with increased detection of viral and bacterial pathogens, except for influenza virus which was more likely to be detected among non-attendance. (Table 1)

Table 1. Demographics and risk factors associated with viral and bacterial detection

| | Influenza N(%) | Rhinovirus N(%) | CVEV N(%) | Coronavirus N(%) | RSV N(%) | <i>S. pneumoniae</i> N(%) | <i>H. influenzae</i> N(%) | <i>M. catarrhalis</i> N(%) | <i>S. aureus</i> N(%) |
|---|----------------|-----------------|-----------|------------------|----------|---------------------------|---------------------------|----------------------------|-----------------------|
| Age (year) | | | | | | | | | |
| 0-4 | 7(4.0) | 31(17.6) | 34(19.3) | 13(7.4) | 54(30.7) | 95(54.0) | 63(35.8) | 97(55.1) | 33(18.8) |
| 5-11 | 22(33.8) | 7(10.8) | 3(4.6) | 2(3.1) | 5(7.7) | 28(43.1) | 20(30.8) | 19(29.2) | 13(20.0) |
| 12-17 | 4(22.2) | 0(0.0) | 0(0.0) | 5(27.8) | 0(0.0) | 4(22.2) | 4(22.2) | 3(16.7) | 2(11.1) |
| 18-44 | 93(16.3) | 94(16.4) | 49(8.6) | 85(14.9) | 26(4.5) | 178(31.1) | 117(20.5) | 78(13.6) | 121(21.2) |
| 45-65 | 18(28.1) | 7(10.9) | 2(3.1) | 6(9.4) | 4(6.3) | 18(28.1) | 14(21.9) | 9(14.1) | 12(18.8) |
| Year of enrollment | | | | | | | | | |
| 2010-11 | 30(11.2) | 46(17.2) | 36(13.5) | 19(7.1) | 27(10.1) | 104(39.0) | 71(26.6) | 71(26.6) | 56(21.0) |
| 2011-12 | 24(9.2) | 48(18.4) | 28(10.7) | 34(13.0) | 25(9.6) | 84(32.2) | 73(28.0) | 54(20.7) | 57(21.8) |
| 2012-13 | 47(29.0) | 13(8.0) | 6(3.7) | 29(17.9) | 18(11.1) | 61(37.7) | 31(19.1) | 38(23.5) | 28(17.3) |
| 2013-14 | 43(21.0) | 32(15.6) | 18(8.8) | 29(14.1) | 19(9.3) | 74(36.1) | 43(21.0) | 43(21.0) | 40(19.5) |
| Study site | | | | | | | | | |
| WRAMC | 3(10.7) | 1(3.6) | 0(0.0) | 1(3.6) | 1(3.6) | 10(35.7) | 5(17.9) | 4(14.3) | 6(21.4) |
| SAMMC | 32(21.9) | 28(19.2) | 11(7.5) | 12(8.2) | 4(2.7) | 50(34.2) | 38(26.0) | 24(16.4) | 43(29.5) |
| NMCS | 60(16.9) | 56(15.8) | 43(12.1) | 59(16.7) | 43(12.1) | 139(39.3) | 94(26.6) | 99(28.0) | 68(19.2) |
| NMCP | 27(11.3) | 30(12.6) | 19(8.0) | 24(10.1) | 18(7.6) | 73(30.7) | 45(18.9) | 39(16.4) | 43(18.1) |
| MAMC | 22(17.1) | 24(18.6) | 15(11.6) | 15(11.6) | 23(17.8) | 51(39.5) | 36(27.9) | 40(31.0) | 21(16.3) |
| Having under-5 children in the household | | | | | | | | | |
| Yes | 48(17.1) | 42(14.9) | 32(11.4) | 31(11.0) | 30(10.7) | 109(38.8) | 91(32.4) | 78(27.8) | 65(23.1) |
| No | 95(15.6) | 97(15.9) | 56(9.2) | 80(13.1) | 58(9.5) | 214(35.1) | 126(20.7) | 127(20.8) | 116(19.0) |
| Index attending daycare? (children only) | | | | | | | | | |
| Yes | 7(6.9) | 22(21.6) | 22(21.6) | 5(4.9) | 33(32.4) | 69(67.6) | 47(46.1) | 64(62.7) | 19(18.6) |
| No | 26(16.7) | 16(10.3) | 15(9.6) | 15(9.6) | 26(16.7) | 57(36.5) | 40(25.6) | 55(35.3) | 29(18.6) |
| Smoking Status (adults only) | | | | | | | | | |
| Current | 15(12.0) | 32(25.6) | 16(12.8) | 16(12.8) | 8(6.4) | 46(36.8) | 17(13.6) | 15(12.0) | 24(19.2) |
| Former | 21(17.8) | 17(14.4) | 7(5.9) | 13(11.0) | 4(3.4) | 38(32.2) | 27(22.9) | 20(16.9) | 28(23.7) |
| Never | 77(19.7) | 51(13.1) | 28(7.2) | 66(16.9) | 17(4.4) | 109(27.9) | 85(21.8) | 54(13.8) | 78(20.0) |

(Blue blocks indicate p-value<0.05)

- There was a higher frequency of detection of rhinovirus, but not other viral and bacterial pathogens among adults who were active smokers. (Table 1)
- Detection of *S. aureus* was more common among those with influenza (OR: 1.68; 95% CI:1.12, 2.5) and CVEV (OR:1.66; 95% CI:1.01, 2.72) overall. These associations were significantly higher among children (OR: 2.33, and OR: 2.48, respectively). Increased detection of *S. pneumoniae* was observed only for children with RSV (OR: 2.06; 95% CI:1.13, 3.73). Increased detection of *H. influenzae* was not observed for any viral pathogen nor either age group. (Table 2)

Table 2. Odds ratios of codetecting bacterial pathogens among ILI patients with viral detection

| | <i>S. pneumoniae</i> OR 95% CI | <i>H. influenzae</i> OR 95% CI | <i>M. catarrhalis</i> OR 95% CI | <i>S. aureus</i> OR 95% CI |
|-----------------------------|--------------------------------|--------------------------------|---------------------------------|----------------------------|
| Influenza | | | | |
| Overall | 1.13 (0.79-1.62) | 0.70 (0.45-1.08) | 0.43 (0.26-0.71) | 1.68 (1.12-2.50) |
| Adults | 1.25 (0.81-1.93) | 0.73 (0.42-1.25) | 0.38 (0.17-0.86) | 1.47 (0.92-2.37) |
| Children | 0.94 (0.48-1.81) | 0.66 (0.31-1.38) | 0.41 (0.20-0.85) | 2.33 (1.10-4.02) |
| Rhinovirus | | | | |
| Overall | 0.99 (0.68-1.45) | 1.20 (0.80-1.81) | 1.10 (0.72-1.68) | 0.99 (0.63-1.56) |
| Adults | 0.79 (0.49-1.27) | 1.09 (0.65-1.82) | 0.74 (0.38-1.45) | 1.06 (0.64-1.78) |
| Children | 1.73 (0.86-3.48) | 1.54 (0.76-3.10) | 2.00 (0.99-4.03) | 0.80 (0.31-2.03) |
| Coronavirus | | | | |
| Overall | 0.59 (0.38-0.92) | 0.61 (0.36-1.02) | 0.57 (0.33-0.98) | 0.74 (0.43-1.26) |
| Adults | 0.73 (0.44-1.21) | 0.73 (0.40-1.32) | 0.66 (0.32-1.36) | 0.39 (0.22-0.71) |
| Children | 0.42 (0.16-1.12) | 0.47 (0.15-1.45) | 0.77 (0.33-1.95) | 1.11 (0.35-3.48) |
| Coxsackie/Echo virus | | | | |
| Overall | 0.86 (0.54-1.37) | 1.19 (0.72-1.95) | 1.75 (1.09-2.81) | 1.66 (1.01-2.72) |
| Adults | 0.52 (0.26-1.07) | 1.35 (0.70-2.62) | 1.19 (0.54-2.63) | 1.33 (0.69-2.57) |
| Children | 1.11 (0.56-2.24) | 0.81 (0.38-1.73) | 1.66 (0.82-3.35) | 2.48 (1.14-5.39) |
| RSV | | | | |
| Overall | 2.38 (1.53-3.70) | 1.40 (0.87-2.27) | 3.89 (2.48-6.10) | 0.65 (0.35-1.19) |
| Adults | 1.53 (0.72-3.24) | 0.76 (0.29-2.03) | 2.00 (0.83-4.82) | 0.94 (0.38-2.36) |
| Children | 2.06 (1.13-3.73) | 1.24 (0.67-2.26) | 2.42 (1.33-4.40) | 0.52 (0.22-1.23) |

(Green block indicates positive association with p-value<0.05; Blue block indicates negative association with p-value<0.05)

Conclusions

- Young age and day care attendance was associated with more frequent detection of viral and bacterial respiratory pathogens among children with ILI
- Smoking was not associated with more frequent detection of bacterial pathogens among adult ILI cases
- Increased detection of *Staphylococcus aureus* was found for several viral pathogens, whereas increases in *S. pneumoniae* were only observed in pediatric RSV. Further evaluations of viral/bacterial co-detections are warranted, particularly as related to etiology and symptom severity.

Acknowledgements and Disclaimer

- Support for this work was provided by the Department of Defense Global Emerging Infections Surveillance (GEIS) program and Military Infectious Diseases Research Program (MIDRP). This project has been funded in part, with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), under Inter-Agency Agreement [Y1-AI-5072] to Uniformed Services University of the Health Sciences.
- Disclaimer. The views expressed are those of the authors and do not necessarily reflect the official policy or position of Uniformed Services University of the Health Sciences, the Department of the Navy, Army, Department of Defense, the U.S. Government nor the Henry M. Jackson Foundation