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Authors

Diaz-Decaro, JD
Launer, B
Mckinnell, JA
et al.

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Author for correspondence:

J.D. Diaz-Decaro, E-mail: jdiazdecaro@ph.lacounty.gov and jdiazdecaro@gmail.com

J.D. Diaz-Decaro^{1,2}, B. Launer³, J.A. Mckinnell³, R. Singh⁴, T.D. Dutciuc⁴, N.M. Green¹, M. Bolaris³, S.S. Huang⁴ and L.G. Miller³

¹Los Angeles County Public Health Laboratories, Downey, CA, USA; ²UCLA Fielding School of Public Health, Los Angeles, CA, USA; ³LA BioMed at Harbor-UCLA Medical Center, Torrance, CA, USA and ⁴University of California, Irvine School of Medicine, Irvine, CA, USA

Abstract

Skilled nursing home facilities (SNFs) house a vulnerable population frequently exposed to respiratory pathogens. Our study aims to gain a better understanding of the transmission of nursing home-acquired viral respiratory infections in non-epidemic settings. Symptomatic surveillance was performed in three SNFs for residents exhibiting acute respiratory symptoms. Environmental surveillance of five high-touch areas was performed to assess possible transmission. All resident and environmental samples were screened using a commercial multiplex polymerase chain reaction platform. Bayesian methods were used to evaluate environmental contamination. Among nursing home residents with respiratory symptoms, 19% had a detectable viral pathogen (parainfluenza-3, rhinovirus/enterovirus, RSV, or influenza B). Environmental contamination was found in 20% of total room surface swabs of symptomatic residents. Environmental and resident results were all concordant. Target period prevalence among symptomatic residents ranged from 5.5 to 13.3% depending on target. Bayesian analysis quantifies the probability of environmental shedding due to parainfluenza-3 as 92.4% (95% CI: 86.8–95.8%) and due to rhinovirus/enterovirus as 65.6% (95% CI: 57.9–72.5%). Our findings confirm that non-epidemic viral infections are common among SNF residents exhibiting acute respiratory symptoms and that environmental contamination may facilitate further spread with considerable epidemiological implications. Findings further emphasise the importance of environmental infection control for viral respiratory pathogens in long-term care facilities.

Introduction

Long-term care facilities present a unique public health problem: a highly susceptible population in a crowded institutional setting constantly exposed to respiratory pathogens from the flow of visitors, personnel and other residents. Nursing home-acquired infections cost the US healthcare system roughly \$673 million to \$2 billion annually and are a significant concern in long-term care populations where the prevalence of the co-morbid disease is high [1]. Nearly half (49%) of long-term care populations are arthritic and over a quarter are suffering from other chronic ailments [2]. Among acute morbidities, influenza, upper respiratory tract infections and nursing home-acquired pneumonia have presented a challenging and prevalent public health concern [3–8]. Vaccines are available, but even for the most seasonal respiratory tract infection (i.e., influenza) vaccine efficacy is <70% [9]. And even in highly vaccinated nursing home populations, influenza outbreaks still occur leading to substantial morbidity and mortality [10]. Outbreaks in long-term care facilities have been caused by a variety of respiratory pathogens including influenza B, coronavirus, parainfluenza and *Bordetella pertussis* [11–13].

Previous data have suggested that the physical environment plays a prominent role in respiratory disease transmission. Influenza A H1N1 has been shown to survive on common surfaces for up to 17 days, remaining infectious for at least a week [14, 15]. Other respiratory pathogens such as coronavirus 229E remain infectious for at least 5 days on a variety of materials including ceramics, rubber and glass [16]. Despite appropriate hygiene and prevention control, residual pathogenic microbial contamination persists in healthcare environments [17]. Data on fomite contamination of respiratory pathogens in endemic (i.e., non-outbreak) settings is limited. Respiratory viral contamination is a particular concern given that the environmental burden of respiratory pathogens may facilitate transmission, exacerbate existing health conditions and be a potential source of outbreaks. This study is part of a larger collaboration known as PROTECT Project, a pilot investigation to study decolonization of nursing

home residents against healthcare-associated infection. The intent of this sub-study is to assess baseline epidemiology and report surveillance results of respiratory pathogens from residents and the environments of three skilled nursing home-facilities (SNFs) in Southern California. The probability of viral shedding in SNFs due to symptomatic residents is estimated by applying Bayes' Theorem providing evidence on the importance of infection control in long-term care facilities.

Methods

Specimen collection

Between May 2015 and July 2015 infection control nurses at each participating site identified patient residents with clinical symptoms suggestive of influenza-like illness (ILI), i.e., fever, congestion, rhinorrhea, cough (with or without sputum production), shortness of breath, or other pulmonary complaints (pleurisy, wheezing). Symptomatic patients underwent nasal and environmental swabbing using a viral collection system involving flocked tipped swabs (one per nostril or two per surface) placed in M4 viral transport media. For residents with suspected ILI, nursing staff returned 3 days later to swab five common high-risk exposure objects (bed side table/bed rail, call button/remote/phones, door knobs (room and bathroom combined), light switch, bathroom handles (toilet flush handle and sink handles combined) using the above described viral collection system. Two-hundred sixty environmental samples were collected. All samples were transported on cold pack, immediately frozen and stored frozen at -70°C prior to testing. All specimens were processed and tested at the Los Angeles County Public Health Laboratories.

Respiratory multiplex testing

All resident and environmental swabs were processed using the FilmArray RP v.1 (BioFire Diagnostics, Salt Lake City, Utah) an FDA-approved multiplex nested polymerase chain reaction (PCR)-based respiratory assay capable of detecting 17 viral targets (adenovirus, coronavirus (HKU1, NL63, 229E and OC43), human metapneumovirus, rhinovirus/enterovirus, influenza A, influenza A subtypes H1, H3 and (H1N1)pdm09, influenza B, parainfluenza types 1, 2, 3 and 4 and respiratory syncytial virus) and three bacterial targets (*Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*). Prospective and retrospective studies have shown the reliable diagnostic performance of the FilmArray RP in detecting a variety of respiratory pathogens in vulnerable populations [18–20]. A complete overview of our surveillance and multiplex testing algorithm is found in Figure 1.

Application of Bayes' Theorem in environmental surveillance

Fagan nomograms are clinical graphical Bayesian tools that determine disease probability conditional on the probabilities of input parameters [21]. The input parameters include (1) a prior (pre-test) probability defined as the disease prevalence in the population and (2) the diagnostic performance measures of the test being used to determine the presence of disease. Input parameters determine the posterior (post-test) probability of disease providing a much more confident result interpretation. While the use of Fagan nomograms has been limited to clinical practice the underlying probabilistic mechanics of Bayes' Theorem have had a wide range of applications [22–24]. Because we are using a

clinical diagnostic test for environmental surveillance purposes, the pre-test probability cannot be defined to just disease prevalence within SNF populations. From an infection control perspective, the potential for transmission of respiratory pathogens in the environment is due to symptomatic individuals, but also fomites in the general environment [25, 26]. Therefore, the total prevalence of transmissible pathogens within SNFs includes disease prevalence in residents and also the unknown disease prevalence in the general environment. Thus, application of the Fagan nomogram is only applicable by including Bayes' pre-test probability as minimum disease prevalence, which we obtained from symptomatic surveillance efforts. Logically, the disease prevalence in our SNF environments is either equal to or greater than the minimum disease prevalence found among residents.

The second Bayesian parameter included in our Fagan nomogram is the positive and negative likelihood ratio calculated from the diagnostic measures (sensitivity and specificity) reported in the FDA-approved summary report of the BioFire FilmArray RP. Both Positive (LR+) and Negative Likelihood Ratios (LR–) were calculated directly from sensitivity and specificity: $\text{LR}+ = \text{Sensitivity}/1-\text{Specificity}$, $\text{LR}- = 1-\text{Sensitivity}/\text{Specificity}$. By standard methods, sensitivity is also called the true positive rate and $1-\text{specificity}$ is called the false positive rate [27].

Using the above Bayesian parameters of minimum disease prevalence and the diagnostic performance measures of the FilmArray RP, the post-test probability can then be interpreted as the minimum probability of environmental contamination of high contact surfaces due to either shedding from an environmental source or a symptomatic resident. Likelihood confidence intervals are calculated to provide uncertainty estimates of our post-test probabilities using established methods [28].

All statistical analyses and data visualizations were done using R Studio (Version 1.0.143).

Institutional Review Board approval was obtained from the Los Angeles County Public Health Department, University of California, Los Angeles and the University of California, Irvine.

Results

Demographics

The demographics including comorbidities of all patients at risk are listed in Table 1. The source population for this study included facilities whose mean annual admission was 762 patients and 47 033 resident days. The mean length of stay among patients at all SNFs was approximately 2 months. A majority of residents were white females between the ages of 65 and 85, and a third of the total population was >85 years of age. Nearly all residents were admitted from local hospitals, and many had existing comorbidities at the time of the study including diabetes, fecal incontinence and a wound and/or rash (Table 1).

Symptomatic surveillance

Fifty-two residents were identified as symptomatic for acute respiratory illness. Ten of 52 residents (19%) had a detectable viral pathogen: parainfluenza type 3 ($n=4$), rhinovirus/enterovirus ($n=4$), RSV ($n=1$) and influenza B ($n=1$). (Fig. 2) All positive results were from two SNFs with no FilmArray RP targets detected from a third SNF. Parainfluenza-3 (13.3%) and rhinovirus/enterovirus (10.0%) were the most common targets detected. Additional targets identified include influenza B, RSV and

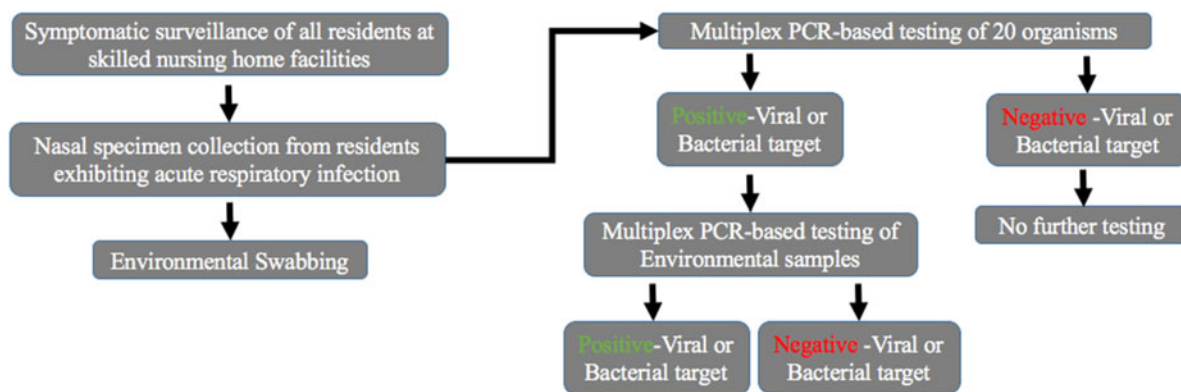


Fig. 1. Surveillance and testing algorithm for symptomatic resident and environmental multiplex testing. Once multiplex PCR confirmed either a viral or bacterial target in a symptomatic resident sample, environmental samples were then screened for the same targets.

rhinovirus/enterovirus (5.5% each). Total period prevalence is stratified per facility and per target in [Table 2](#).

Environmental Surveillance and Bayes' theorem

A total of 260 environmental surfaces were swabbed during environmental surveillance. Among residents with detectable viral

infection, environmental contamination of the same pathogen was found in 20% (2/10) of high-contact surfaces tested (i.e., bedrail, doorknobs). The FilmArray RP confirmed the presence of parainfluenza type 3 and rhinovirus/enterovirus in the environment. All positive viral environmental specimens were concordant with confirmed resident results. No bacterial respiratory pathogens were detected among any resident or environmental samples.

Using disease prevalence from symptomatic surveillance as the minimum pre-test probability and the LRs calculated from the reported BioFire FilmArray RP target-specific sensitivity and specificity, true environmental contamination probability due to shedding is 92.4% (95% CI: 86.8–95.8%) for parainfluenza 3 and 65.6% (95% CI: 57.9–72.5%) for rhinovirus/enterovirus. A Fagan nomogram for parainfluenza 3 using the appropriate

Table 1. Skilled nursing home facility-level characteristics

Facility-level variable	SNF1	SNF2	SNF3
Annual volume	N		
Admissions	562	832	892
Resident days	32 638	49 928	58 532
Mean length of stay (days)	58	60	66
Demographics and insurance	%		
Age			
<65	18	38	12
65–85	46	42	50
85+	36	20	38
Male	34	46	41
Race			
White	92	91	79
Black	2	3	10
Asian/Pacific Islander	6	6	11
Hispanic ethnicity	25	27	9
<High school education	32	19	11
Medicare insured	38	20	18
Admitted from hospital	95	94	92
Illness and comorbidities			
Mechanical ventilation	0	0	0.2
End stage renal disease	7	8	9
Diabetes	40	42	34
Wounds or rash	83	68	90
Fecal Incontinence	35	35	34

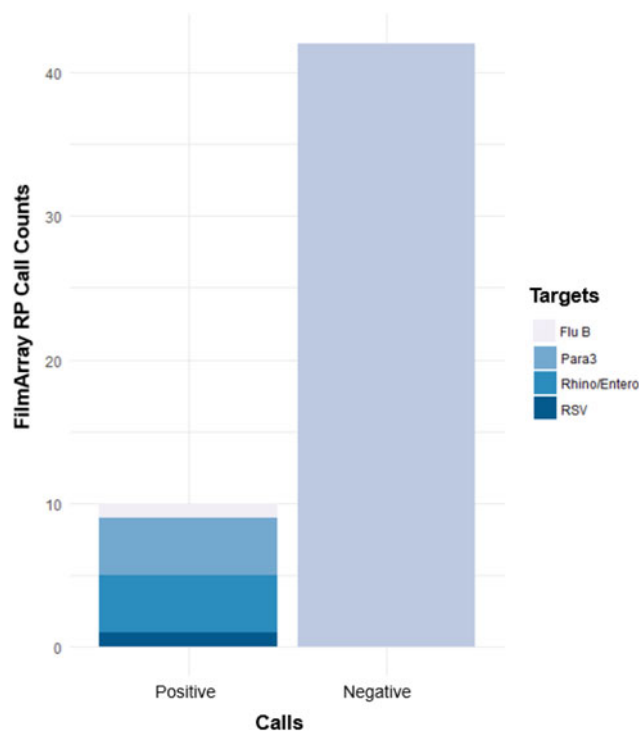


Fig. 2. Summary of symptomatic surveillance based on BioFire FilmArray RP positive and negative call counts separated by target. 10 out of 52 (19.2%) symptomatic residents had a detectable viral infection.

Table 2. Epidemiological summary of symptomatic surveillance showing prevalence and overall burden from confirmed targets found in SNF 1 and SNF 2

	Positive target detected	Prevalence (%)	Estimated number of annual cases ^a
SNF1	7	23.3	28
Para 3	4	13.3	16
Rhino/Enterovirus	3	10.0	12
SNF2	3	16.6	12
Flu B	1	5.5	4
Rhino/Enterovirus	1	5.5	4
RSV	1	5.5	4

^aAssuming period prevalence is sustained during a 12-month period.

parameters is found in Figure 3. Of note, each parameter is used as a linear scale bisected to yield the minimum post-test probability. Prevalence and LR- can also be used to give the minimum post-test probability of a negative result, meaning <1% probability of parainfluenza 3 environmental shedding upon a positive diagnostic result. Results from the complete Bayesian analysis is found in Table 3.

For our Bayesian analysis, minimum pre-test probability is limited to only the disease prevalence as determined from our symptomatic surveillance. However, if known, including environmental disease prevalence would allow greater approximation of the post-test probability of environmental contamination due to shedding within the SNF environment. To assess how increased

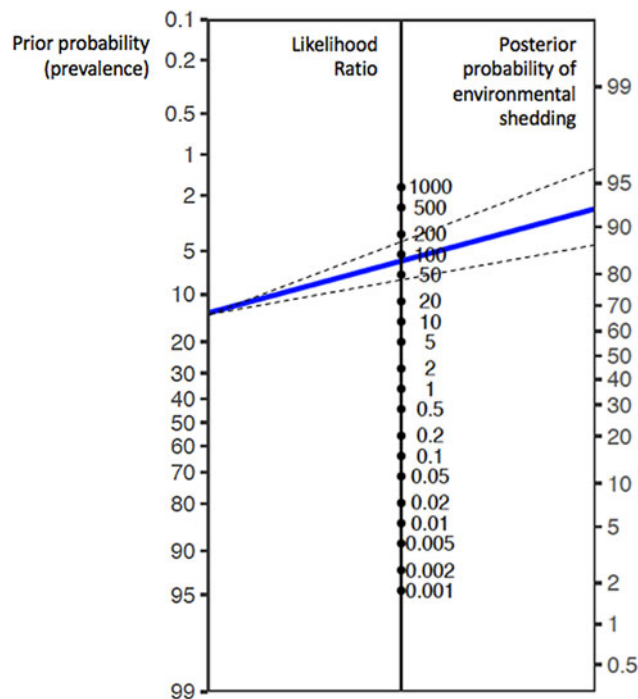


Fig. 3. Fagan nomogram of parainfluenza 3 virus incorporating disease prevalence from symptomatic surveillance in SNF1 and positive LR calculated from reported diagnostic measures of the FilmArray Respiratory Panel. Collectively, disease prevalence and diagnostic measures yield the post-test probability (blue line) of environmental shedding on a high contact surface. Dotted lines bracket 95% CI area.

disease prevalence would affect environmental contamination due to shedding, we extend our Bayesian analysis by simulating how post-test probability would be affected by altering parainfluenza 3 and rhinovirus/enterovirus pre-test probabilities (i.e., disease prevalence). As illustrated in Figure 4, viral detection is saturated, marked by an exponential decay relationship between environmental contamination due to shedding and increased true disease prevalence, which occurs at higher prevalence for either viral target. Figure 4 suggests that evidence of environmental transmission is more likely to occur when disease prevalence is high but also limited by the detection method.

Discussion

Over the next few decades, older populations will continue to grow at an accelerated rate increasing the demand for long-term care facilities and creating new public health challenges in managing respiratory health. As of 2012, the long-term care facility workforce functioned in hospices, adult day service centers, home health agencies, assisted living communities and nursing homes totaling 58 600 workers for the 4 million Americans in long-term care facilities (one long-care service worker for every ~67 patient residents) [29, 30]. While nursing homes comprise nearly a third of all long-term care facilities and are expected to increase, appropriate surveillance of respiratory tract infections among the elderly should be an important public health priority as most of this population is highly susceptible to respiratory outbreaks [31].

The viral pathogens found among residents and in the environment in this study have all been previously implicated in previous outbreaks affecting vulnerable populations in healthcare settings [10, 32]. From symptomatic surveillance, we confirmed the presence of parainfluenza 3, rhinovirus/enterovirus, RSV and influenza B infection in non-epidemic, i.e., endemic settings. Parainfluenza type 3 was implicated as the source of an outbreak in an adult hematology unit that occurred over a 5-month period [33]. The source of the outbreak was a chronically infected resident that had been placed in isolation, suggesting an environmental component of transmission in the outbreak. In the present study, all four parainfluenza type 3 specimens found during surveillance were detected in the same SNF population (SNF1) within a 1-week period, plausibly suggesting intra-facility transmission. However, confirmation of intra-facility transmission could be resolved only by whole genome sequencing to compare viral genetic profiles and phylogeny. Evidence of parainfluenza type 3 environmental contamination due to shedding was observed as a symptomatic resident's call button/TV remote positively detected the virus. What role, if any, this shedding had on parainfluenza type 3 transmission to other residents is unclear; however, application of Bayes' Theorem reveals that environmental shedding of parainfluenza 3 due to this resident is highly probable.

The significance of the environmental shedding reported in our study increases upon considering that sustained transmission is plausible in a semi-closed population such as a nursing home environment [34]. This strengthens the use of prevalence of infection as a pre-test probability performed during our Bayesian analysis. We additionally found evidence of rhinovirus/enterovirus shedding in the environment of the same SNF population (SNF1) on resident door knobs. Once again, using a Fagan nomogram helps interpretation: given a 10% prevalence of rhinovirus/enterovirus in this subpopulation and given 92.7% true positive

Table 3. Application Bayes' Theorem to calculate minimum Post-test probability of environmental contamination from confirmed targets detected during environmental surveillance

Confirmed target from environmental surveillance	Diagnostic performance of the BioFire FilmArray RP				Prior Probability: Minimum prevalence in the environment ^a	Post-test probability: Minimum probability of environmental contamination ^b (95% CI)
	Sen (95% CI)	Spe (95% CI)	LR+ (95% CI)	LR- (95% CI)		
Parainfluenza 3	0.958 (0.789–0.999)	0.988 (0.978–0.994)	79.8 (42.9–100)	0.01 (0.006–0.289)	13.3%	92.4% (86.8–95.8)
Rhinovirus/Enterovirus	0.927 (0.882–0.958)	0.946 (0.926–0.962)	17.1 (12.4–23.7)	0.05 (0.05–0.12)	10.0%	65.6% (57.9–72.5)

Sen, sensitivity; Spe, specificity; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

^aTotal prevalence would have included prevalence of respiratory pathogens on surfaces and inanimate objects within SNFs, however, this is unknown.

^bMinimum probability of environmental contamination given a positive test result on the BioFire FilmArray RP and a minimum prevalence as observed from our symptomatic surveillance.

rate and 5.4% false positive rate, the probability of shedding in the environment by rhinovirus/enterovirus from a symptomatic individual is 65.6% suggesting the possible presence of either a different pathogen as the aetiological cause of infection or minimal viral shedding in the environment. RSV, influenza B and rhino/enterovirus were all detected from resident samples collected on the same day from one SNF, however, no co-infection was detected. The co-circulation and burden from multiple respiratory viruses among vulnerable populations is unknown but evidence

suggests respiratory tract infections may be compounded by the synergistic effect of multiple pathogens [35].

Based on our clinical and environmental surveillance, clear epidemiological facility differences were present. While SNF 1 had the most viral targets confirmed by multiplex testing, SNF 2 had greater viral diversity. Additionally, SNF 3 had no targets detected at all. Our observations may reflect differences in sanitation and hygiene control, but also may be due to unequal foot traffic flow at each facility. High traffic flow would allow greater

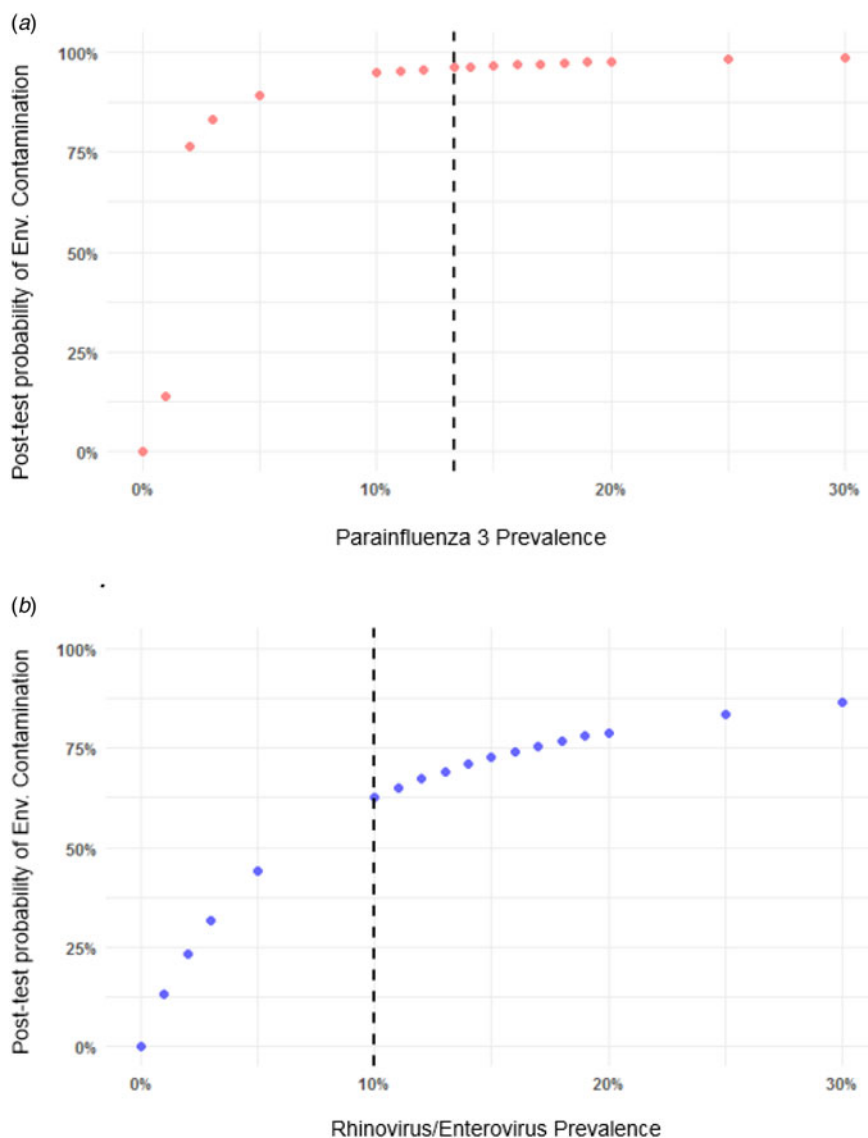


Fig. 4. Post-test probability of environmental contamination with increased prevalence of respiratory disease. Dashed line indicates viral prevalence as found during symptomatic surveillance (parainfluenza 3:13.3%, rhinovirus/enterovirus:10.0%).

opportunity for resident exposure, colonization and infection; and conversely, low traffic flow would minimise the opportunity of the same. Overall, our study did not coincide with the wintertime seasonality of most viral respiratory infections, which explains why no targets were confirmed in SNF 3, but additionally provides evidence that our reported disease prevalence for each detected viral pathogen is likely an underestimate.

The confirmed presence and absence of respiratory pathogens in our study supports some national trends. Based on Western United States Census Region RSV 2015 data gathered from the National Respiratory and Enteric Virus Surveillance System (NREVSS), the RSV clinical sample identified in this study coincided with a period of low viral isolation among the general US population [36]. NREVSS data suggest a high percent positive rate for antigen detection of parainfluenza 3 in the USA compared with RSV at the same time [37]. While influenza A was not found in any clinical and environmental sample, influenza B infection was confirmed, supporting national and regional-level outpatient surveillance from the 2014 to 2015 flu season, where influenza B was the prevalent circulating influenza strain [38]. Additionally, influenza B strains are often most prevalent at the end of most influenza seasons, which coincides with when our study was conducted (i.e., late spring, early summer). Seasonal trends of other respiratory pathogens may help explain the low detection rates reported in our study.

Our results provide evidence supporting a recent report revealing major gaps in the knowledge, practice and policy in infection control of environmental surfaces in healthcare settings [39]. In our study, environmental contamination was highly likely shed from symptomatic residents. Fagan nomograms are methods of Bayesian analysis incorporating conditional probabilities for evaluating environmental contamination and person-to-person transmission, but only if a baseline prevalence is known. Our data suggest environmental contamination is site specific with possible viral shedding only found in SNF 1. This evidence suggests minor differences in the adherence to environmental hygiene practices within and between facilities. Recommendations for basic and environmental infection control practice in health-care facilities have been created by the CDC and the Healthcare Infection Control Practices Advisory Committee [40]. Per recommendations, high-level disinfectants on noncritical areas or environmental surfaces are not required in long-term care facilities, however, a more frequent cleaning schedule of high-contact surfaces is suggested.

The main limitation of our study is related to respiratory viral detection. While some residents were confirmed, a majority of residents had no detectable respiratory infection. Results do not exclude the presence of other respiratory pathogens not included in the FilmArray RP. Despite the high sensitivity of the FilmArray RP, detection may also have been hindered by low microbial load or equally likely, routine facility-specific cleaning procedures preventing the capture of pathogens altogether. Another significant issue is that the FilmArray RP is only FDA approved for processing nasopharyngeal swab (NPS) specimens; however, due to resident co-morbidities, a non-invasive sample collection method (i.e., nares swabs) was preferred. However, nares swab specimen processing on the FilmArray RP is effective for detecting respiratory pathogen [41]. Additionally, the time of collection may not have coincided with high viral titres. For instance, sampling outside of the general incubation of parainfluenza infections (2–4 days) or environmental shedding (3–10 days) may have affected detection [42]. Additionally, the FilmArray RP is FDA-cleared

only for *in vitro* diagnostic use with no clear application for environmental testing. However, environmental swab testing and processing have been recommended as a method to prevent environmental contamination during routine clinical testing [43]. Another limitation of our study is our sampling and testing method of screening environmental samples only when the FilmArray RP confirmed a positive result among symptomatic residents. While our surveillance and testing algorithm would have benefited from screening all environmental samples, we could not do these further testing due to funding limitations. We encourage future studies to thoroughly screen all environmental samples to provide a comparable baseline between positive and negative results screened by the FilmArray RP.

Our results suggest that heightened surveillance among vulnerable populations in a crowded institutional setting may help identify residents with transmissible respiratory infections, thereby enhance prevention efforts. Ideally, year-round surveillance activities, especially during influenza season would provide a clearer picture on the role of the environment on respiratory pathogen transmission. Additionally, evidence for inter-facility circulation of respiratory viruses may be under reported and may be amenable to intervention. For long-term care facility staff, heightened awareness about the potential for viral respiratory pathogen spread is necessary as well as reinforcement of standard infection control practices of ILI patients. To our knowledge this is the first report to use the BioFire FilmArray RP for environmental monitoring for respiratory pathogens and also the first report to use this technology for testing samples on a strictly older population in a LTC setting.

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Declaration of Interest. No conflicts of interest.

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