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Article

Wastewater-Based Surveillance of Respiratory Syncytial Virus Reveals a Temporal Disconnect in Disease Trajectory across an Active International Land Border

Mackenzie Beach, Ryland Corchis-Scott, Qiudi Geng, Ana M. Podadera Gonzalez, Owen Corchis-Scott, Ethan Harrop, John Norton, Andrea Busch, Russell A. Faust, Bridget Irwin, Mehdi Aloosh, Kenneth K. S. Ng, and R. Michael McKay*

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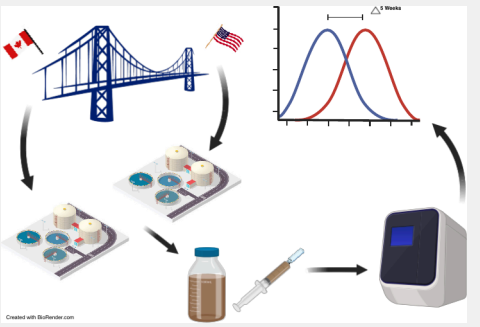
ABSTRACT: Conventional metrics for tracking infectious diseases, including case and outbreak data and syndromic surveillance, can be resource-intensive, misleading, and comparatively slow with prolonged data collection, analysis and authentication. This study examined the 2022−2023 Respiratory Syncytial Virus (RSV) season in a contiguous metropolitan area connected by an active international land border, affording an opportunity for comparison of the respiratory virus season spanning two independent public health jurisdictions. Time-lagged cross correlation and qualitative examination of the wastewater signals showed that the peak of the Detroit (MI, USA) RSV season predated the peak in Windsor (ON, Canada) by approximately 5 weeks. A strong positive relationship was observed between RSV N-gene concentrations in wastewater and hospitalization rates in Windsor-Essex (Kendall's $\tau = 0.539$, $p \leq 0.001$, Spearman's $\rho = 0.713$, $p \leq 0.001$) as well as Detroit (Kendall's $\tau = 0.739$, $p \leq 0.001$, Spearman's $\rho = 0.888$, $p \leq 0.001$). This study demonstrated that wastewater surveillance can reveal regional differences in infection dynamics between communities and can provide an independent measure of the prevalence of RSV, an underreported disease. These findings support the use of wastewater surveillance as a cost-effective tool in monitoring of RSV to enhance existing surveillance systems and to better inform public health disease mitigation strategies.



KEYWORDS: *good health and well-being, pathogen, public health, RT-qPCR, RSV, time-lagged cross-correlation, wastewater surveillance*

INTRODUCTION

Respiratory syncytial virus (RSV) is a highly transmissible respiratory virus that mainly targets the lower respiratory tract. It is an enveloped, single-stranded RNA virus that belongs to the Paramyxoviridae family, and can be classified into two antigenic groups, RSV A and RSV B[1]. Like many viruses, RSV shows seasonal infection dynamics with a season lasting from late fall to early spring in the northern hemisphere.[1,2] Although both RSV subtypes are generally present within RSV seasonal epidemics, RSV A is typically more prevalent and can cause severe disease among infants and children.[3] Clinical presentation ranges from presenting like a mild cold, to causing pneumonia and/or bronchiolitis.[1,4] RSV has an R_0 of 3.0 ± 0.6, meaning each individual infected with RSV, infects an average of three people.[5] For comparison, influenza has an average R_0 of 2−3, whereas the Delta variant of SARS-CoV-2 had an R_0 of 5−8.[6] Those most vulnerable to severe RSV infection are children under the age of five, the elderly, and immunocompromised individuals.[1] Globally, RSV leads to 3.1 million hospitalizations and >100,000 deaths annually.[7] RSV infection increases risk of secondary infections, specifically pneumococcal pneumonia, which poses an even higher health risk as the immune system is already weakened by the viral infection.[8] Many individuals infected with RSV are asymptomatic,[9] thereby increasing the risk of unknowingly spreading RSV throughout the community and rendering wastewater-based surveillance (WBS) an actionable strategy.

WBS can survey entire communities for pathogens by quantifying the occurrence of pathogen-associated biomarkers in the wastewater entering the treatment plant[10] or at locations upstream of the plant.[11] In the U.S., the average sewer residence time (also referred to as sewer transit time), which is the amount of time it takes waste to travel from a residence to a treatment plant, was 3.3 hours.[12] This allows for the collection and analysis of temporally relevant data. Interest in WBS accelerated with an increase in both research and active monitoring networks following the onset of the COVID-19

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pandemic. WBS for RSV was activated early during the pandemic, motivated by parallels in the presentation of COVID-19 and RSV and the need to discriminate between disease agents to facilitate appropriate interventions.[13] A recent systematic review noted that RSV is likely only shed in stool in 14% of infected individuals; however, it was found in sputum, saliva and mucus at a much higher frequency.[14] It is likely that a mixture of these excretions contribute to wastewater concentrations of RSV facilitating use of WBS for RSV.[15] Moreover, recent analysis of respiratory virus decay found that RSV RNA breaks down relatively slowly, making it unlikely that RNA degradation during sewer transit significantly influences WBS.[16] RSV WBS has been used to gauge disease trajectory at the municipal level[17] and at upstream locations.[18,19] Recently, WBS has expanded to include subtype surveillance[20] and genomic surveillance of RSV from wastewater.[21,22] Adopting WBS to monitor community transmission of RSV is important as it is not a reportable disease in many jurisdictions, including Ontario, Canada, yet RSV can place a significant burden on population health and the health system.[23] WBS can fill the gap in clinical data as these data are not comprehensive, showing the extent of disease prevalence. Indeed, WBS can function as an independent measure of disease prevalence allowing for earlier public health intervention than clinical surveillance alone or acting to complement clinical testing.[23] Since WBS relies on measuring the pooled waste of a community, it incorporates RNA/biomarkers shed by asymptomatic or mild cases that may not be captured by clinical testing. WBS has also been shown to predict increases in the incidence of respiratory viruses prior to clinical testing.[24−26] Another benefit of WBS is that it is minimally invasive and circumvents ethical concerns and potential stigma associated with clinical testing of individuals. Finally, WBS can be implemented at flexible geographic scales to monitor both large, underserved regions and individual buildings. Scalability and near real-time data generation make it a valuable tool for public health interventions.[27] Further, WBS can be extended to gain a better understanding of disease trajectory and pathogen spread across jurisdictional boundaries, including international borders.[28] The Ambassador Bridge, which connects Windsor, ON to Detroit, MI, is the busiest border crossing between Canada and the USA[29] with over 40,000 people who cross daily, with many commuting to work.[30] Detroit and Windsor are also connected by a tunnel traversing the Detroit River which accommodates over 4 million vehicles annually.[31] The high daily traffic crossing the border suggest it is likely that pathogens cross freely as well (within human hosts), which has the potential to affect the populations of both cities. As international neighbors, Detroit and Windsor have independent health systems for the management of infectious diseases. We conducted a longitudinal WBS survey of RSV across the 2022−2023 respiratory season through monitoring of wastewater collected from treatment facilities serving the communities on each side of the border. The aims of this investigation were to test WBS against clinical metrics of RSV prevalence and to examine the differences in the severity and trajectory of the RSV seasons.

METHODS

Sample Collection

Wastewater samples were collected 3× weekly from two wastewater treatment plants (WWTPs) in Windsor, ON between September 1, 2022, and March 31, 2023. Combined, the monitored facilities serve over 270,000 people and treat the waste of 54% of the population of Windsor-Essex County. Samples were collected once per week from the Detroit Water Resource Recovery Facility (WRRF) operated by the Great Lakes Water Authority (GLWA). The WRRF has three separate interceptors that transport untreated wastewater to the treatment plant for processing. Collectively, this facility serves approximately 3.1 million people, which represents a third of the population of Michigan. Additionally, samples were collected 3× weekly from the Chatham Water Pollution Control Plant (WPCP) located in Chatham, ON between October 23, 2022, and March 21, 2023. This facility services the urban core of the rural municipality and supports a population of approximately 47,000. Sample collection frequency was coordinated with the operational schedules of both the plants and the lab in which this study was conducted.

Wastewater samples were collected using autosamplers that collect and composite small volumes of sewage from the influent stream at regular intervals over 24 h. One liter samples were transported in coolers on ice to the laboratory for same day processing. Composite samples are more accurate in representing community trends compared to "grab" samples, which are collected at a single point in time.[32]

Sample Processing

Composite wastewater samples were concentrated by filtration using 0.22 μm Sterivex PES cartridge filters (MilliporeSigma, Burlington, MA, USA) attached by luer-lock fitting to a large barrel syringe fitted into a caulking gun. The caulking gun allowed for additional pressure to be applied to increase the volume of liquid passing through the cartridge filter. Volumes ranging from 50 mL-120 mL of wastewater were processed through each filter. Immediately following filtration, filters were sealed, then flash frozen in liquid nitrogen.

After the filtration process, the filters were thawed, and then nucleic acids were extracted to be amplified using RT-qPCR. Samples were extracted using either the AllPrep PowerViral DNA/RNA kit (Qiagen, Germantown, MD, USA) modified by the addition of 5% 2-mercaptoethanol (v/v) or the RNeasy PowerMicrobiome Kit (Qiagen) with the same modification. Samples were not treated with DNase upon extraction with either kit, and RNA was eluted in 50 μL of RNase-free water.

RT-qPCR and Amplicon Sequencing

RT-qPCR, a well-established method for the quantification of RSV genetic material,[33] was used to quantify RSV RNA within samples. Specifically, an assay that targets the N-gene for both RSV A and RSV B was selected for use in this study. The specificity of this assay was previously assessed in silico and in vitro.[13] RT-qPCR assays for Pepper Mild Mottled Virus (PMMoV) were performed alongside RSV and used to normalize results for variability due to flow or other parameters,[34] as it can be used as a human fecal indicator.[35] Detailed information on RSV and PMMoV quantification is found in the Supporting Information (SI). To validate the assay used to measure the concentration of RSV N-gene, RT-qPCR amplicons obtained from RSV-positive wastewater samples were sequenced (Figure S1). Methods for amplicon sequencing are found in the SI.

Clinical Data

The number of weekly positive tests for RSV in the Windsor-Essex County and Chatham-Kent regions for the 2022−23 respiratory season (September 1, 2022, to March 31, 2023) were sourced from the Public Health Ontario (PHO) dashboard, which was accessed on April 19th, 2024.[36] The number of weekly positive tests should not be confused with test positivity rate. We elected to rely on the number of positive tests since relatively few RSV tests were conducted in both Windsor-Essex and Chatham-Kent resulting in a skewed percent positivity data set. This was especially evident at the beginning of the study period where very few tests for RSV were conducted, resulting in high percent positivity data that were likely not reflective of RSV prevalence. Hospitalization rate data for Windsor-Essex for the 2022−23 respiratory season (September 1, 2022, to March 31, 2023) were gathered from the Discharge Abstract Database (DAD) in

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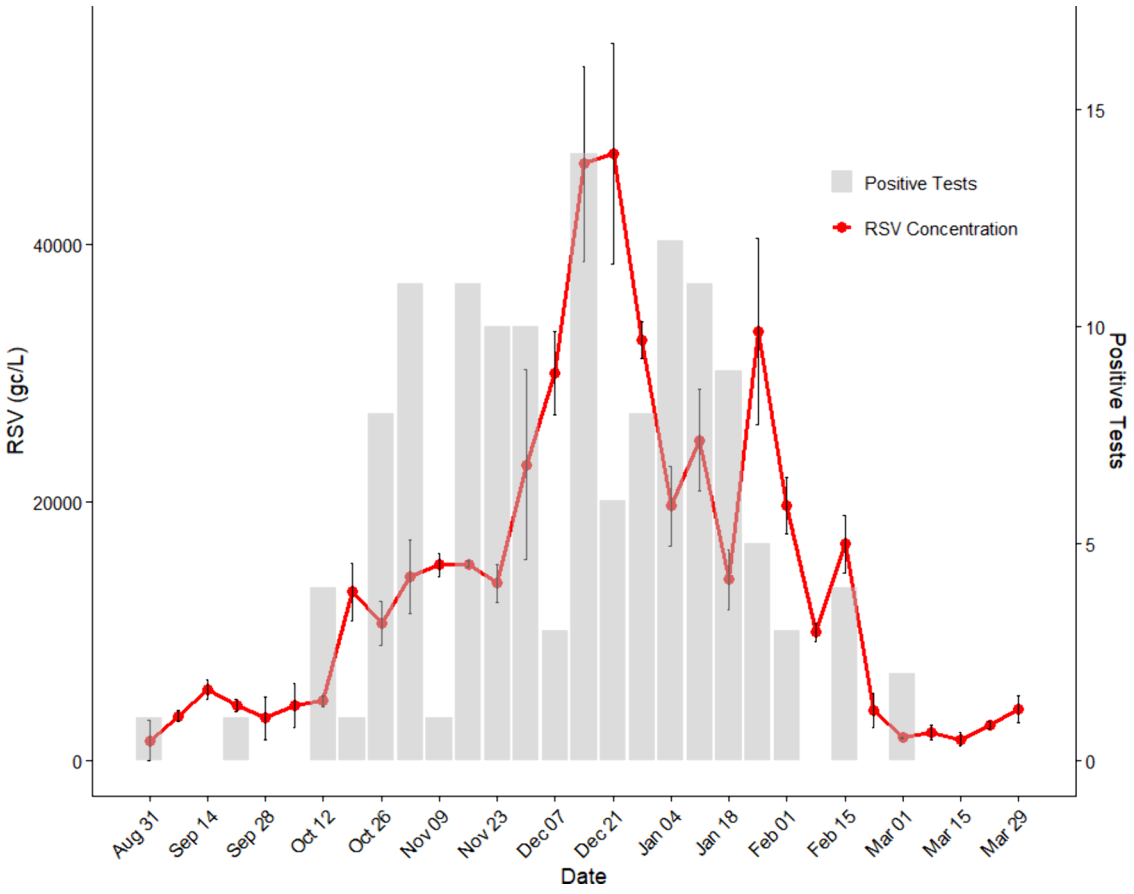

Figure 1. Positive tests for RSV in Windsor-Essex and the aggregate population-weighted wastewater concentrations for RSV in Windsor from September 2022 to March 2023. RSV RNA concentration in wastewater (red line) is displayed alongside the number of positive RSV tests for the Windsor-Essex region (gray bars) by epidemiological week.

collaboration with the Windsor-Essex County Health Unit (WECHU). The hospitalization rate data for Windsor-Essex included hospitalizations of residents of Windsor-Essex with any of the ICD10 codes: 12.1, J20.5, J21.0, and/or B97.4. These data were accessed on April 17th, 2024. Hospitalization data were not available for Chatham-Kent. The number of weekly positive tests for RSV for the 2022−23 respiratory season were available for Windsor-Essex but were not available for Detroit. Alternately, RSV-associated hospitalization data for Michigan were used for analysis. Hospitalization data for Michigan were accessed on April 19th, 2024, from the US Centers for Disease Control and Prevention (CDC) RSV Hospitalization Surveillance Network (RSV-NET) interactive dashboard, with data spanning from September 1, 2022, to March 31, 2023. This dashboard reports laboratory-confirmed RSV-associated hospitalizations.[37] Hospitalization rates for RSV in Michigan were calculated based on surveillance conducted in 5 counties (located in close proximity to Detroit in the southeast of the state), including Clinton, Eaton, Ingham, Washtenaw and Genesee.[37] This represents approximately 12% of the population of Michigan and is considered a representative measure of regional trends in RSV hospitalization.[28,37]

Data Analysis and Visualization

Time Lagged Cross Correlation (TLCC), Kendall's and Spearman's Correlations and data visualization were conducted in R version 4.3.3.[38] Graphs were created using the ggplot2.[39] Block-wise averaging was used to downsample data with higher than weekly resolution, condensing data into a single mean measurement for each week within the study. RSV prevalence metrics, including hospitalization rates, were only available at a weekly resolution. Averaging of the RSV concentration allowed for comparisons to be drawn to clinical measures of RSV prevalence and an evenly spaced data set. For the Windsor-Essex WWTPs, concentrations were combined using a population-weighted mean to account for one plant serving a higher percentage of the population. A total of 31 weekly mean RSV RNA concentrations, as well as PMMoV RNA concentrations, were produced for Windsor. A total of 122 measured samples for RSV and PMMoV were combined through weekly downsampling. Downsampling was not possible for measurements of RSV RNA concentration for samples collected from the WRRF, as those samples were collected weekly. No samples were collected from the GLWA WRRF during epidemiological weeks 39, 45, 48 (2022) and 12 (2023). Missing data were filled using linear interpolation prior to further analysis. Following interpolation, a population-weighted mean was used to combine RSV signals from the three Detroit interceptors into a single measurement representative of Detroit. PMMoV concentration data were treated identically. This produced 31 data points for RSV RNA concentration as well as 31 data points for PMMoV concentration for Detroit. A total of 81 samples were used to produce these weekly averages. A total of 63 wastewater samples were collected from the Chatham WPCP between October 23, 2022, and March 21, 2023. These were also combined into weekly averages to produce 23 data points for RSV RNA concentration and PMMoV concentration. All gene concentrations that are measured within the wastewater are reported as gene copies per L (gc/L).

Time-Lagged Cross Correlation and Peak Synchrony

Examination of quantile-quantile plots showed the data were not normally distributed, and thus nonparametric methods of analysis were used. TLCC determines at what shift (or lag) peak synchrony occurs between two time series. Peak synchrony is the shift that produces the strongest correlation coefficient between series and can be measured by shifting one time series in relation to the other and conducting Spearman's correlations between the two timeseries at each shift. This can reveal if a "leader-follower" relationship exists.

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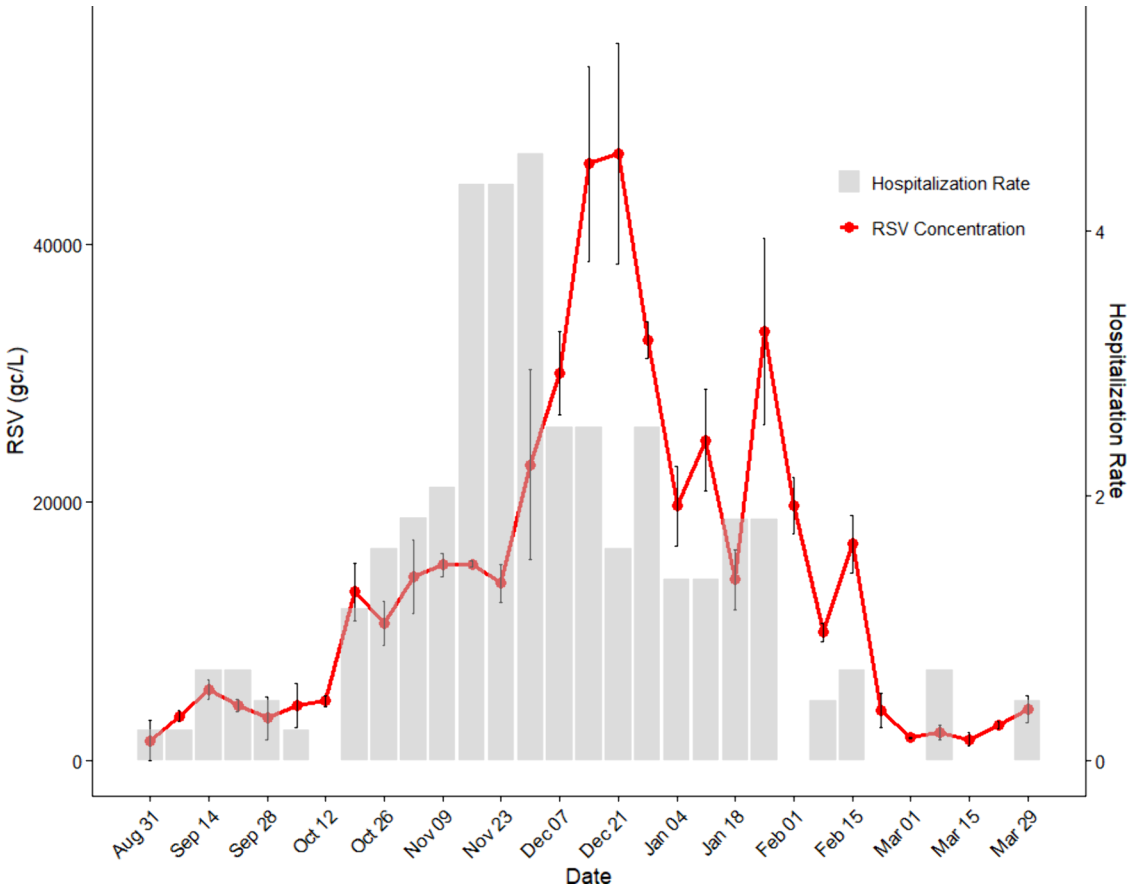

Figure 2. RSV-associated hospitalization rates and the aggregate population-weighted wastewater concentrations for RSV in Windsor from September 2022 to March 2023. RSV RNA concentration in wastewater (red line) is shown with the rate of RSV-related hospitalizations for the Windsor-Essex region (gray bars) by epidemiological week.

TLCC is an established method for determining the relationship between time series and has been widely used in WBS.[40−43] Here, a bootstrapped version of TLCC (the ccf_boot function in the R package "funtimes"[44]) was used to help to account for potential autocorrelation in RSV concentration and clinical metrics. Traditional cross-correlation methods can produce biased results because of inherent autocorrelation. Bootstrapped TLCC can be used to analyze timeseries, while also confirming that the results are reliable even if the data exhibits autocorrelation by using shuffled versions of the data set to ensure that the relationships found are accurate.[45] Bootstrapped TLCC was used to determine if the RSV concentrations within Windsor wastewater was a leading indicator of RSV hospitalization rates and positive tests in Windsor-Essex. Additionally, TLCC was used to determine if RSV concentrations in Detroit wastewater were a leading indicator of RSV hospitalization rates in Michigan. TLCC was used to compare RSV wastewater signals in Windsor-Essex to RSV signals in Detroit to determine if there are differences in the temporal trends of RSV infection between the two halves of this transnational metropolitan region. Finally, TLCC was employed to determine if RSV wastewater signal in Chatham was temporarily shifted in comparison to RSV signal in Detroit.

Correlations between RSV N-Gene and Traditional Measures of RSV Prevalence

Kendall's Tau and Spearman's rank correlation coefficient were used to measure the relationship between the RSV signal within the wastewater and the number of positive tests and hospitalization rates in Windsor-Essex. Similar analyses were conducted for Detroit but only hospitalization rate was used as data describing laboratory testing results were unavailable. These methods were also employed to measure the association between RSV signal in Chatham wastewater and the number of weekly positive tests for RSV.

RESULTS

RT-qPCR targeting the N-gene of RSV was used to quantify RSV RNA concentrations in wastewater samples collected at both the Detroit and Windsor WWTPs over a series of 31 weeks, spanning the 2022−23 respiratory virus season. The N-gene is highly conserved and allows for accurate detection of both RSV A and B subtypes.[40,46] Sequencing RT-qPCR amplicons obtained from RSV positive wastewater samples confirmed the specificity of the assay (Figure S1). No instances of spurious or off-target amplification were observed.

Comparisons between Windsor RSV wastewater signal and clinical data focused on two primary metrics: hospitalization rates and number of positive tests. The study period concluded when the wastewater signal fell below the limit of detection of the assay which largely corresponded to when the number of positive tests in the Windsor-Essex region indicated the cessation of the RSV season. Qualitative inspection of the data supported that the RSV gene concentrations from wastewater closely matched the number of positive tests and hospitalization rates in Windsor-Essex (Figures 1 and 2). Similarly, the RSV concentrations also closely matched hospitalization rate case data from five counties in southeast Michigan used as a proxy for Detroit (Figure 3). Additionally, visual comparison of wastewater signal and positive tests for RSV in Chatham, located in an adjacent county 70km east of Windsor, showed that wastewater monitoring data matched an increase in the number of positive tests for RSV from the end of October 2022 to January 2023 (Figure S2).

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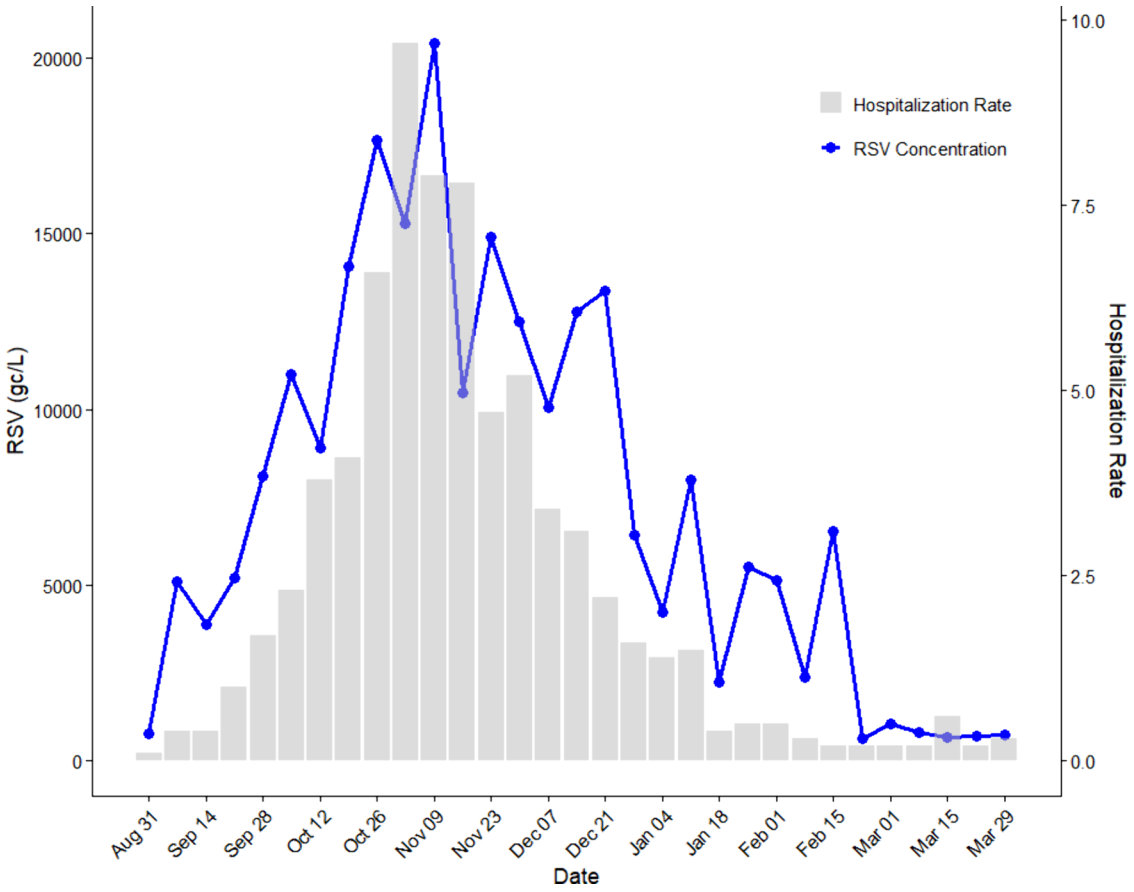

Figure 3. RSV-associated hospitalization rates and the aggregate population-weighted wastewater concentrations for RSV in Detroit, MI from September 2022 to March 2023. RSV RNA concentration in wastewater (blue line) is shown with the rate of RSV-related hospitalizations per 100,000 population for Michigan (gray bars) by epidemiological week.

TLCC was performed between the number of positive tests and hospitalization rates in Windsor-Essex against concentrations of the RSV N-gene within the wastewater (Figure S3). The TLCC for Windsor-Essex showed that the strongest correlation against both metrics was at a lag of 0 days (Peak Synchrony = 0). This indicates that the wastewater signal neither led nor lagged the positive test data or hospitalization rates observed within the municipality on an epidemiological week basis. A strong positive agreement was observed between RSV concentration and positive tests in Windsor-Essex (Kendall's $\tau = 0.536$, $p \leq 0.001$, Spearman's $\rho = 0.731$, $p \leq 0.001$). Normalization of the signal using PMMoV RNA concentrations did not improve the relationship between wastewater signals and RSV positive tests (Kendall's $\tau = 0.476$, $p \leq 0.001$, Spearman's $\rho = 0.671$, $p \leq 0.001$). Another strong positive agreement was seen between RSV concentration and hospitalizations (Kendall's $\tau = 0.539$, $p \leq 0.001$, Spearman's $\rho = 0.713$, $p \leq 0.001$). Again, normalization did not improve the relationship between the two factors (Kendall's $\tau = 0.499$, $p \leq 0.001$, Spearman's $\rho = 0.675$, $p \leq 0.001$) (Figure S4). Correlation between the number of positive RSV tests in Chatham-Kent and the concentration of RSV RNA in Chatham wastewater was moderately high (Kendall's $\tau = 0.438$, $p \leq 0.001$, Spearman's $\rho = 0.597$, $p \leq 0.001$) despite the shorter period over which the association was investigated. Normalization of the data with PMMoV concentrations marginally improved the correlation with the number of positive tests (Kendall's $\tau = 0.517$, $p \leq 0.001$, Spearman's $\rho = 0.674$, $p \leq 0.001$). A strong positive correlation was observed between RSV N-gene concentration and Michigan hospitalization rates (Kendall's $\tau = 0.739$, $p \leq 0.001$, Spearman's $\rho = 0.888$, $p \leq 0.001$). Normalization did not improve the relationship (Kendall's $\tau = 0.634$, $p \leq 0.001$ Spearman's $\rho = 0.811$, $p \leq 0.001$). (Figure S5). When TLCC was performed between the RSV concentrations and the Michigan hospitalization rates, results showed that the strongest correlation had a lag time of 0 days (Peak Synchrony = 0) (Figure S3).

When TLCC was performed between wastewater signals in Windsor-Essex and Detroit, the peak synchrony exhibited a lag time of −5 (Figure S6). This indicates that the peak of the RSV season in Detroit preceded that of Windsor-Essex by approximately 5 weeks during the study period. Visual inspection of both normalized and non-normalized RSV concentration data supports this temporal disconnect between the RSV seasons in Windsor and Detroit (Figure 4, Figure S7). No change was observed in peak synchrony when TLCC was run using data normalized by PMMoV (Figure S6). TLCC carried out between Chatham wastewater signals for RSV and wastewater signals for RSV in Detroit corroborate the 5-week difference between the RSV season in Ontario and in Michigan (Figure S8).

DISCUSSION

This study tracked the RSV prevalence across two cross-border communities forming a large contiguous metro region separated by the busiest land border between Canada and

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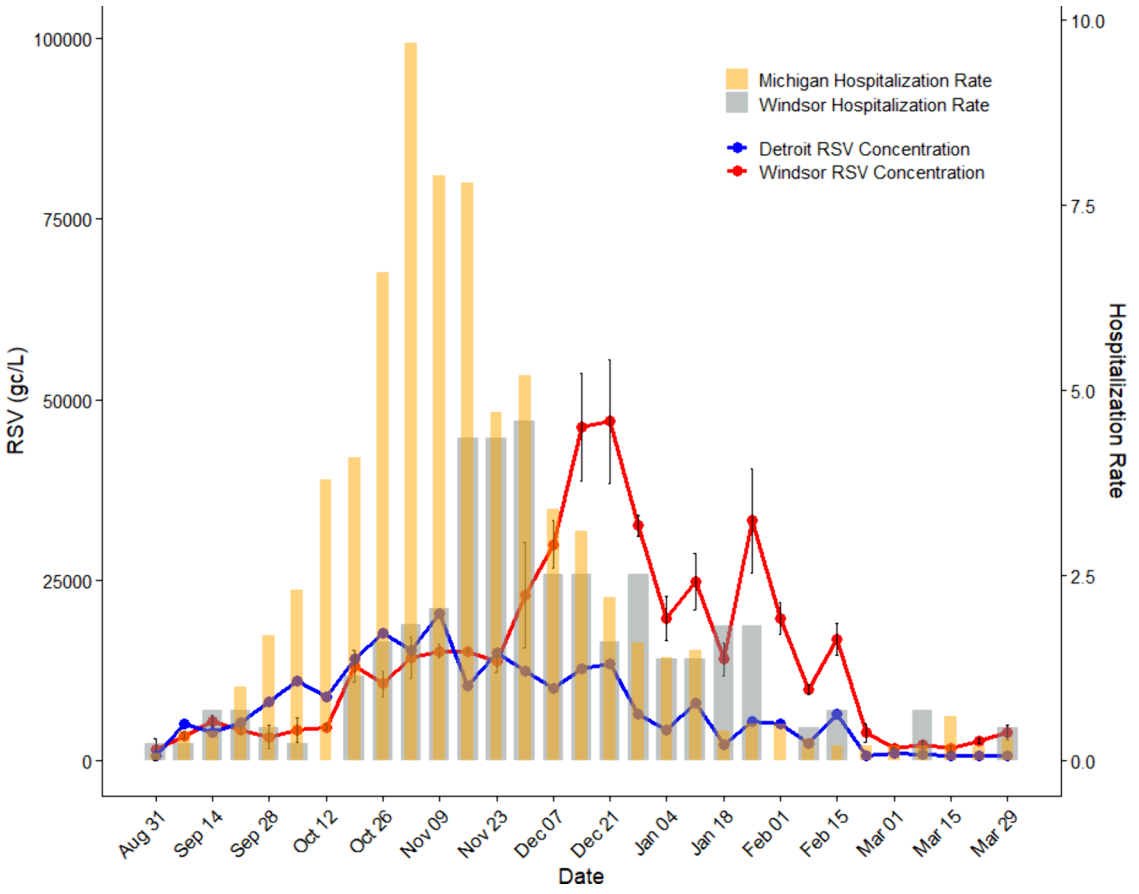

Figure 4. RSV-associated hospitalization rates and the aggregate population-weighted wastewater concentrations for RSV in Windsor and Detroit from September 2022 to March 2023. RSV RNA concentration in wastewater (red line) is shown with the rate of RSV-related hospitalizations per 100,000 population for the Windsor-Essex region (gray bars) by epidemiological week. RSV RNA concentration (blue line) and rate of RSV hospitalizations for Michigan (yellow bars) by epidemiological week are also shown.

the USA.[29] Over the 31-week study period, RSV signals within the wastewater for both Windsor and Detroit were correlated with conventional case and outbreak public health surveillance measures, including the number of positive tests and hospitalizations attributed to RSV infection. The results reinforce a recent nationwide analysis covering 176 sites across the U.S. showing WBS of RSV correlated with RSV positivity and hospitalization rates.[47] Concordance between clinical metrics and wastewater signal in these municipalities was supported by surveillance at a third nearby city in Ontario where moderate correlations existed despite the shorter surveillance window (Figure S2). TLCC indicated that RSV signals within the wastewater neither led nor lagged when compared to clinical metrics, giving a peak synchrony of zero for both Windsor and Detroit (Figure S3). This finding contradicts the results of many SARS-CoV-2 wastewater monitoring programs, where WBS was shown to be a leading indicator of COVID-19 clinical metrics.[24,42,48] Likewise, a lead time of at least 12 days was demonstrated for WBS of RSV when compared to pediatric RSV hospitalizations.[49,50] The synchrony between aggregate wastewater and clinical metrics observed in the present study was recently demonstrated for six other locations in Michigan over the same time period[47] and the lack of lead time could be related to a number of factors. The present study targeted wastewater solids for virus detection, yet previous work on SARS-CoV-2 has demonstrated that methods targeting the free-and suspended viral fraction from wastewater supernatant may afford longer lead times and enhance the early alert potential of WBS.[42] Alternatively, shorter RSV incubation time may have contributed to this observation. Viral shedding for RSV is suggested to last between 2 to 8 days, with peak viral load occurring around 3.5 days.[51] RSV shed during the active disease period can also continue to be present for up to 20 days after infection.[52] This is known as convalescent shedding, when a patient is no longer infectious nor presents symptoms but continues to shed the virus.[53] Convalescent shedding can confound the ability of WBS to predict infection as cases that have resolved may still be contributing to the signal, which could inflate RSV concentration in wastewater without concomitant increases in RSV prevalence. Clinical data were also only available on an epidemiological week basis meaning wastewater data, which were collected multiple times per week for the Ontario sites, had to be compressed and considered on an epidemiological week basis to allow for comparison. This may have hidden lead-time, as downsampling data reduces resolution. This issue is not unique to this study, as others have observed that a lack of lead time can be attributed to weekly resolution of clinical data.[54] There can also be delays with clinical testing. Often, clinical data takes days to weeks to be released whereas wastewater testing can provide same-day results. This offsets the lack of lead time, allowing for the data to be reported to public health officials more rapidly than

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traditional surveillance metrics with the potential for more timely public health interventions.

Like many respiratory viruses, RSV is an underreported disease. As such, clinical data are not complete as many cases are asymptomatic or subclinical; yet WBS is sensitive to all infections where shedding occurs. This difference likely negatively impacts correlations between RSV concentrations in wastewater and clinical metrics. The current study supports this, as correlations between RSV RNA concentrations in wastewater and clinical measures of disease prevalence are lower in less populous sewersheds (Table S2). This was also observed in a recent study which suggests that wastewater data may be more accurate than clinical data in small population centers.[55] This conclusion was drawn using data for wastewater surveillance of SARS-CoV-2 during the pandemic when many resources were being put into clinical testing for COVID-19. Thus, it is even more likely that wastewater surveillance for underreported illnesses, such as RSV, is more accurate than clinical data across a wide variety of sewersheds. The burden of RSV infections within the first five years of life are still poorly defined which limits the comparisons between WBS and traditional metrics.[56] Simply put, clinical metrics for most respiratory illnesses, including RSV, underestimate disease prevalence.[57]

This study also compared Windsor-Essex hospitalization rates to RSV N-gene concentrations in wastewater (Figure 2). A visual inspection of the data show that the peak of hospitalization rates for RSV in Windsor-Essex occurred prior to the peak of the RSV signal in the wastewater. However, TLCC confirmed that the peak synchrony was zero. This observed visual discrepancy between the hospitalization rate and the wastewater signal could be caused by multiple factors. Hospitalization rate does not accurately reflect the prevalence of RSV within a community as hospitalizations represent the most severe cases. Even among hospitalized patients, especially those >65 years of age, true prevalence of RSV infection is likely underestimated.[58] While RSV primarily impacts young children and the elderly, people of all ages can contract the virus.[1] However, it is more likely that these individuals are asymptomatic[9] or present with milder symptoms who do not require hospitalization, yet they still contribute virus to wastewater. Finally, convalescent shedding may contribute to the peak concentration of RSV RNA found in wastewater being delayed in comparison to hospitalization rates. We note that the second clinical measure of RSV prevalence within Windsor-Essex (number of positive tests) was in concordance with wastewater surveillance results (Figure 1). The correlation between RSV concentrations in wastewater and clinical metrics might be increased through an improvement of the quality of both clinical data and the quality of wastewater data. Clinical data for RSV are limited and cases of RSV are likely underreported. Designating RSV a reportable disease and making daily case data publicly available would enhance data reliability as fewer cases would go unreported and temporal resolution of clinical data sets would be improved. Additionally, increasing the frequency of wastewater testing to daily would help to account for variability inherent in wastewater samples which may lead to better prediction of disease trends. Finally, separating the RSV A and RSV B subtypes in both clinical and wastewater surveillance data would enable more detailed analyses, allowing for subtype-specific correlations. This approach could enhance assay accuracy and improve the reliability of trend predictions and the strength of correlations.

With the large volume of traffic including commuters passing daily between Windsor and Detroit, it would be expected that these connected cities have similar RSV seasons; yet this study showed that the RSV season in Windsor was delayed by ∼5 weeks when compared to Detroit. One possible reason for the discrepancy may have been the lingering effect of SARS-CoV-2 border restrictions in reducing the volume of casual trips across the border, potentially providing an additional barrier that may reduce cross-border transmission of disease.[28] The onset of the 2022−23 respiratory season occurred following the removal of pandemic era border restrictions, however, border traffic was approximately 25% lower in the fall of 2022 compared to prepandemic levels.[28]

Another consideration relates to population demographics and socioeconomic factors. The population of Detroit trends younger than that of Windsor. Children ages of 0−5 make up roughly 7% of Detroit's population,[59] whereas this same age group contributes only 4.8% to the population of Windsor.[60] This difference in population structure may influence the rate of transmission of RSV as it helps to determine the number of susceptible individuals. Although the RSV seasons appeared to start simultaneously with a rise in both clinical metrics and in the wastewater signal occurring concurrently (Figure 4), Detroit's RSV trajectory peaked sooner than in Windsor. The difference in timing of peak RSV signal could be attributed to the higher population under five years of age in Detroit. Socioeconomic status is also known to factor into the health outcomes of many diseases including RSV, with higher incidence of hospitalization reported among patients from low socioeconomic status neighborhoods.[58,61] Demonstrable socioeconomic gaps exist between Detroit and Windsor as reflected by a poverty rate of 33.8% for the City of Detroit in 2023[62] compared to 10.8% for the City of Windsor in 2024.[63] Linked to socioeconomic status is equitable access to healthcare. Ontario has a publicly funded health system which improves access to primary care for lower income individuals.[64] Earlier access to primary care could improve health outcomes and reduce hospitalizations in comparison to Detroit, which was nearly twice the rate recorded for Windsor (Figure 4.).

Beyond the temporal discrepancy between the RSV wastewater signal in Windsor and Detroit, a visual inspection of the data reveals that RSV concentrations in Windsor were almost double those in Detroit during the peak of the 2022−23 RSV season (Figure 4). WBS is context dependent and sewer infrastructure and wastewater composition influence signal strength. The concentration of biomarkers is only reflective of disease incidence in the monitored sewershed. Comparison of relative strength of wastewater signal between sewersheds is complicated by obfuscating factors and likely not reflective of differences in disease incidence between sewersheds. However, biomarker normalization can help to account for dilution in wastewater influent caused by precipitation or nonsanitary inputs and improve data comparability. Peak PMMoV-normalized concentrations of RSV are not different between Windsor and Detroit, indicating that dilution can explain some of the difference in RSV concentration between the cities (Figure S7). This is supported by flow data (daily volumes of sewage being treated). The WWTPs in Windsor have much lower combined mean daily flow (∼65.8 megalitres/day) compared with the WRRF in Detroit (∼754.6 megalitres/day). PMMoV normalization data do not fully account for the observed discrepancies as the hospitalization rate in Detroit is

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still higher than in Windsor relative to the concentration of N-gene in wastewater. Finally, RSV prevalence may be similar in Windsor and in Detroit leading to similar normalized wastewater signals but cases severe enough to cause hospitalization may be higher in Detroit.

Beyond RSV, the region experienced surges of COVID-19 and Influenza A over this same period, both of which were also monitored by wastewater surveillance.[28,65,66] Similar to RSV, a disconnect in disease trajectory was also reported for Influenza A between Windsor and Detroit, with wastewater surveillance confirming an earlier onset of disease in Windsor.[28] While some of the same factors presented here may have contributed to the uncoupling of the Influenza A trajectory between these cross-border communities, the timing of vaccine distribution and establishment of herd immunity was identified as particularly important given that flu vaccines are generally distributed in Michigan up to two months earlier than in Ontario.

The ability of WBS to accurately detect onset of the RSV season has important implications to achieve better health outcomes related to RSV infection moving forward.[7] In North America, three vaccines for RSV have recently become available to vulnerable populations. GSK Arexvy and Pfizer Abrysvo were both authorized for use in Canada and the USA in 2023, the former available for adults over the age of 60 while the latter can be administered to women during weeks 32 through 36 of pregnancy.[67,68] In the USA, the Food and Drug Administration approved use of a third vaccine, Moderna's mRESVIA in May, 2024 for adults aged 60 and over.[68] There have also been advances in prophylaxis with the recent approval of nirsevimab, a long-acting monoclonal antibody therapy complementing the long-standing use of palivizumab as preventive options for infants at high risk of severe RSV.[1,68] Here, the value of WBS for RSV was recently demonstrated in a cost-consequence analysis for Ontario showing that WBS-guided prophylaxis could result in a 3-fold reduction in RSV-related hospitalizations and medically attended emergency room/outpatient RSV infections compared to clinical surveillance-guided prophylaxis.[23]

Recognizing inherent limitations of clinical data, wastewater-based surveillance can be used to guide the timely rollout and distribution of RSV prevention strategies that will promote better health outcomes to populations at risk.[23] Additionally, wastewater surveillance can be used to ascertain the effectiveness of novel RSV treatments at a community scale and potentially warn of the evolution of RSV variants with resistance to current vaccines and therapies.

This study has several limitations. The temporal resolution of wastewater data for Detroit were limited, with weekly testing potentially missing some variability in RSV signal. Similarly, hospitalization data were only available weekly, leading to lower data resolution. The Detroit wastewater treatment facility monitored does not serve the Michigan counties from which the hospitalization data were derived and instead covers five surrounding counties. The spatial disconnect between the catchment of the GLWA WRRF and the area from which RSV clinical data were drawn may have influenced results by reducing the strength of the correlation between the wastewater signal and clinical data. However, the CDC state on the RSV-NET site that these data are considered a proxy for statewide incidence[69] and the GLWA WRRF serves a substantial portion of the population of Michigan (~30%) making it likely that each is representative of RSV incidence for the entire state. The strong correlation between hospitalization rate and wastewater signals support that they are each proxies for statewide RSV incidence. This study was also limited to a single respiratory season, which is insufficient to draw conclusions about trends in RSV trajectory. The study was conducted one year post COVID-19 pandemic, and one year prior to the introduction of RSV vaccines. Both the pandemic and increase in vaccines could influence future respiratory seasons.[70] Lastly, the WWTPs in both Windsor and Detroit do not serve all residents, meaning that individuals infected by the virus outside of this jurisdiction would not be accounted for in wastewater surveillance data yet they may have contributed to clinical data. Future studies should span multiple seasons in order to capture temporal patterns and gather a better understanding of the potential impact that could come from the introduction of RSV vaccines. It would also be beneficial to integrate subtype specific analysis, separately categorizing RSV A and RSV B, as this would further enhance the accuracy of RSV surveillance in wastewater.

CONCLUSIONS

Wastewater surveillance for RSV closely matched clinical metrics used to measure disease prevalence in both Windsor and Detroit. However, the data were not found to be predictive of clinical measures of RSV prevalence. Despite this, WBS can be an effective and rapid measure of RSV transmission within the community, especially considering delays in release of clinical data. This study successfully showcased the ability of WBS for RSV to measure the regional difference in infection dynamics, as it showed a delay of roughly 5 weeks in the Windsor RSV season compared to the RSV season in Detroit. This study was conducted to gain a better understanding of the trajectory of RSV seasons in both Windsor and Detroit. It also demonstrated that it is possible to accurately monitor RSV within a community using wastewater surveillance, allowing for an additional way in which viral transmission patterns can be studied. We demonstrated that the cross-border neighbors Windsor and Detroit have temporally staggered RSV seasons despite their spatial proximity. Wastewater surveillance can act as a nonbiased measure of the prevalence of a multitude of diseases and allows for easier comparisons across jurisdictional boundaries.

ASSOCIATED CONTENT

Data Availability Statement

Sequence data were uploaded to the NCBI Sequence Read Archive (SRA) with the following BioSample accessions: SAMN43299432, SAMN43299433, SAMN43299434. RT-qPCR data encompassing gene quantifications presented are available for download in CSV format from a GitHub repository (https://github.com/OntarioWastewaterSurveillanceConsortium/sars-cov-2-data).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00168.

Additional methods and results including RT-qPCR methods, visualizations of TLCC results, visualizations of correlations between wastewater and clinical data (PDF)

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AUTHOR INFORMATION

Corresponding Author

R. Michael McKay − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada; Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, United States; Great Lakes Center for Fresh Waters and Human Health, University of Michigan, Ann Arbor, Michigan 48109, United States;* orcid.org/0000-0003-2723-5371; Email: Robert.Mckay@uwindsor.ca

Authors

Mackenzie Beach − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada;* orcid.org/0009-0009-1397-3681

Ryland Corchis-Scott − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada;* orcid.org/0000-0003-2525-1730

Qiudi Geng − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada;* orcid.org/0000-0003-0150-4975

Ana M. Podadera Gonzalez − *Department of Chemistry and Biochemistry, University of Windsor, Windsor, ON N9B 3P4, Canada;* orcid.org/0000-0001-5278-0449

Owen Corchis-Scott − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada*

Ethan Harrop − *Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON N9B 3P4, Canada*

John Norton − *Great Lakes Water Authority, Detroit, Michigan 48226, United States*

Andrea Busch − *Great Lakes Water Authority, Detroit, Michigan 48226, United States*

Russell A. Faust − *Oakland County Health Division, Oakland County, Michigan 48341, United States;* Present Address: Public Health Informatics, University of Illinois Chicago, Chicago, IL 60607 USA

Bridget Irwin − *Windsor-Essex County Health Unit, Windsor, ON N9A 4J8, Canada;* Present Address: BC Centre for Disease Control, 655 West 12th Avenue, Vancouver, BC V5Z 4R4 Canada

Mehdi Aloosh − *Windsor-Essex County Health Unit, Windsor, ON N9A 4J8, Canada; Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, ON L8S 4L8, Canada;* orcid.org/0000-0001-6763-1067

Kenneth K. S. Ng − *Department of Chemistry and Biochemistry, University of Windsor, Windsor, ON N9B 3P4, Canada*

Complete contact information is available at: https://pubs.acs.org/10.1021/envhealth.4c00168

Author Contributions

MB: Conceptualization, Formal analysis, Investigation, Writing−original draft, Writing−review and editing, Visualization. RC-S: Formal analysis, Investigation, Writing−review and editing. QG: Methodology, Validation, Investigation, Writing−review and editing. AP: Methodology, Validation, Investigation, Writing−review and editing. OC-S, EH: Investigation, Writing−review and editing. JN, AB: Resources, Writing−review and editing. AB: Resources, Writing−review and editing. RF, BI, MA: Formal analysis, Data curation Writing−review and editing. KN: Writing−review and editing, Supervision, Funding acquisition. RM: Conceptualization, Writing−review and editing, Supervision, Project administration, Funding acquisition.

Notes

The authors declare no competing financial interest.

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