Using wastewater for population-level colorectal cancer surveillance: a future research agenda

Elizabeth Wurtzler,[1] Erica K Barnell,[1,2] Catherine Morrison,[1] Clayton Grass ,[1]
Natalie DuPré,[3] Donald J Biddle,[4] Allie Jin,[5] Sandy Kavalukas,[6] Rochelle H Holm ,[7]
Ted R Smith [7]

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[1]Geneoscopy Inc, St. Louis, Missouri, USA
[2]Department of Medicine, School of Medicine, Washington University, St. Louis, Missouri, USA
[3]Department of Epidemiology and Population Health, School of Public Health and Information Sciences, University of Louisville, Louisville, Kentucky, USA
[4]Department of Geographic and Environmental Sciences, Center for Geographic Information Sciences, University of Louisville, Louisville, Kentucky, USA
[5]School of Medicine, University of Louisville, Louisville, Kentucky, USA
[6]Hiram C. Polk Jr., M.D., Department of Surgery, School of Medicine, University of Louisville, Louisville, Kentucky, USA
[7]Center for Healthy Air Water and Soil, Christina Lee Brown Envirome Institute, School of Medicine, University of Louisville, Louisville, Kentucky, USA

Correspondence to
Dr Rochelle H Holm;
rochelle.holm@louisville.edu
and Dr Ted R Smith;
ted.smith@louisville.edu

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ABSTRACT

Background Individual screening for colorectal cancer (CRC) is typically performed through either direct clinical examination, such as a colonoscopy, or an approved non-invasive test that measures CRC markers shed from the tumour into stool. There are known barriers to current screening recommendations.

Methods We present feasibility data demonstrating the detection of CRC-associated RNA biomarkers in community wastewater. RNA expression values for GAPDH (housekeeping marker) and CDH1 (colorectal neoplasia-associated marker) were quantified via droplet digital PCR. The CDH1 region evaluated in this study represents overall CDH1 concentration in wastewater samples, with elevated expression shown to be associated with colorectal neoplasia. Four neighbourhood clusters were assessed in this study: three CRC clusters (CRC Clusters #1–#3) and one control region (Control Cluster #1).

Results Average normalised colorectal neoplasia-associated RNA markers (CDH1/GAPDH) for CRC clusters were 20.0 (CRC Cluster #1), 2.2 (CRC Cluster #2) and 4.0 (CRC Cluster #3); average normalised colorectal neoplasia-associated RNA markers (CDH1/GAPDH) for Control Cluster #1 were 2.6.

Conclusion Our data provide proof of principle for a novel application of wastewater surveillance to track potential cancer burden. We demonstrate that CDH1 is detectable in wastewater, potentially accelerating the development of this approach for epidemiological studies. Further investigation with additional samples and closer alignment with documented case activity will be necessary in future population-level CRC surveillance research.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The recent trend of increasing colorectal cancer (CRC) incidence in younger populations underscores the need for improved public health strategies to complement existing screening methods, such as colonoscopy and stool-based tests. Wastewater can be used to surveil a range of urinary or faecal shed health targets, but its feasibility for CRC community surveillance remains unclear.

WHAT THIS STUDY ADDS

⇒ This proof-of-concept study on wastewater surveillance shows that CDH1, which is associated with CRC, is detectable in wastewater. These findings suggest the need for an expanded research agenda.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Detecting elevated CRC markers across broader community zones, before high incidence is recorded by tertiary care centres or state cancer registries, could help target areas for practical, cost-effective, community screening. This approach may enable early diagnosis while also identifying regions with a lower likelihood of case discovery, enabling timely clinician alerts.

INTRODUCTION

Prevention and early detection of colorectal cancer (CRC) are critical, as colon and rectal cancers have an estimated 154 270 new cases annually in the USA, making them the third most common cancer and the second leading cause of cancer-related deaths.[1] CRC screening can be completed using an invasive procedure, such as a colonoscopy, or an approved non-invasive test that measures CRC markers shed from the tumour into the stool, such as the multitarget stool DNA test[2,3] or the multitarget stool RNA test (mt-sRNA, ColoSense).[4] Faecal immunochemical tests, which detect haemoglobin in stool, are less sensitive and more specific than multitarget stool tests.[5] Shed markers enter the community wastewater system when a patient flushes the toilet, likely several times per day. A research agenda is needed to assess the feasibility of population-based testing for human RNA biomarkers in wastewater, which could eventually enable the detection of the CRC burden at the neighbourhood level.

In part due to the increasing incidence of early onset CRC (individuals <50 years of age),[1] a large portion of new CRC cases in the USA present as late diagnoses. Recently, screening guidelines have recommended lowering the age for initiation of screening from 50 to 45.[6] However, non-compliance with recommended guidelines, coupled with rising incidence in younger individuals under 45 years of age, has led to CRC becoming a high-morbidity disease in younger patients.[1,6,7] Further, disparities in CRC screening persist, most notably among Hispanics, Black individuals and American Indian and Alaskan Native populations.[8] Structural and social determinants of health, including access to health insurance coverage, also matter in CRC treatment for minoritised communities.[9]

Wastewater has been used for infectious disease surveillance, but also has the potential to monitor other population-level diseases.[10 11] Evidence from the COVID-19 pandemic suggests that community-level wastewater data can inform public health actions, particularly when paired with geotargeted digital advertising that promotes protective behaviours.[12] Considering existing cancer prevention and care gaps, leveraging wastewater to detect elevated CRC markers on a broader scale within community zones prior to tertiary care or state cancer registry high-incidence determination could help target areas for practical and cost-effective community screening, enabling early diagnosis while also identifying regions with relatively lower likelihood of case discovery for timely clinician alerts.

In this study, we present proof-of-principle data demonstrating the detection of colorectal neoplasia-associated RNA biomarkers in wastewater. These wastewater biomarkers adapt elements of the mt-sRNA test,[4] which has shown high sensitivity and specificity for CRC and advanced adenomas in average-risk individuals aged 45 or older. Using conventional wastewater-based epidemiology methods, this study aimed to evaluate whether RNA biomarkers used in the mt-sRNA test could be feasibly adapted to detect CRC biomarkers in neighbourhood-level wastewater samples.

MATERIALS AND METHODS

A retrospective study at a tertiary care centre analysed patients with CRC from 2021 to 2023. Based on a preliminary study of a notable trend among a clinician's patients with cancer, the residential addresses of these patients[13] were mapped to identify geographical clusters of high CRC incidence rates in the county. Clusters were defined as areas with >4 CRC cases within a half-mile radius, as this number represents nearly a fourfold increase in cases (ie, Clusters #1 and #2). Next, CRC sex-standardised and age-standardised rates were calculated from state cancer registry data[14] for census tracts across Kentucky using diagnoses captured between 1995 and 2018 from the Kentucky Cancer Registry database, and the Getis Ord Gi* spatial statistic was applied to identify census tracts of high CRC incidence in Jefferson County, Kentucky; one additional cluster (ie, Cluster #3) was added based on similar demographics to Clusters #1 and #2. In total, we selected three geographical areas (ie, CRC Clusters #1, #2 and #3) with high CRC incidence, common to the tertiary care centre-derived regions and/or the statewide cluster analysis. CRC Clusters #1, #2 and #3 have a high distribution of Black or African American residents, a known factor associated with disparities in CRC screening uptake.[8]

An additional sewershed (Control #1), where none of the tertiary care or state cancer registry patients lived, was selected as a population control, like CRC Cluster #2, based on population size, race and ethnicity distribution and median household income (table 1).

All sewersheds were residential, neighbourhood-level sites. Population demographics were drawn from the ESRI Business Analyst (Esri, Redlands, CA) data via the Enrich tool. No industrial wastewater flow was reported upstream of any of the four sewershed locations.

Wastewater samples were collected three times over the course of the day via the grab method from one manhole in each of four sewersheds on 26 July 2023 (N=12). The sewersheds include both combined storm and sanitary sewers and separate sanitary sewers (online supplemental table 1); 175 mL of influent wastewater was collected at each site at approximately 7:00, 10:00 and 13:00. At these site locations during the study period, the sewer utility determined that 24-hour composite samplers were not feasible due to manhole locations. We selected sampling at various times throughout the day to thus obtain a more representative sample of household toilet usage. Since sewage travel time is less than 24 hours in the study area and patients may flush the toilet often throughout the day, this approach improves coverage. Patients who are away from home during the day may not be represented in the 13:00 sample but could be included in either the 7:00 or 10:00 sample. Raw wastewater samples were stored at −20°C until analysis; 49 mL of each sample was analysed. The mean of the triplicate samples over the course of the day was normalised.

Samples were blinded during quantification assay runs. The CDH1 region evaluated in this study represents overall CDH1 concentration in those samples; this is the same CDH1 region targeted by the ColoSense assay,[4] whose elevated expression is associated with colorectal neoplasia. Before RNA extraction, the cellular material from each sample was pelleted using differential centrifugation.[15] Samples were lysed, and RNA was extracted using an EMAG (bioMérieux, Marcy l'Étoile, France) according to previously described methods.[15] Extracted RNA concentrations were quantified using a Qubit. RNA expression values for GAPDH (housekeeping marker) and CDH1 (colorectal neoplasia-associated marker) were quantified via droplet digital PCR using previously described methods.[4]

Table 1 Characteristics for the four clusters evaluated as part of this study, which included three colorectal cancer clusters (CRC Clusters #1–#3) and one region to serve as a control area (Control Cluster #1)

Sewershed	Region status	Total population	% Black	% white	% Hispanic	Median household income	Rate of tertiary care centre patients with CRC per 100 people	Rate of recent KY cancer registry patients with CRC per 100 people
CRC Cluster #1	Cancer cluster	610	83.67	11.78	2.95	US$19 202	0.66	0.33
CRC Cluster #2	Cancer cluster	342	91.64	4.48	0	US$50 000	1.2	0.58
CRC Cluster #3	Cancer cluster	797	90.24	5.13	2.19	US$37 204	0.13	0
Control Cluster #1	Control area	343	66.98	27.1	0	US$67 919	0	0

The total population associated with each sewershed area is provided. The distributions of race and ethnicity, median household income, the rate of patients with CRC treated by colorectal surgeons at a tertiary care centre per 100 people and the rate of CRC among all patients with CRC captured by the state cancer registry per 100 people for each sewershed area are also provided.

CRC, colorectal cancer; KY, Kentucky.

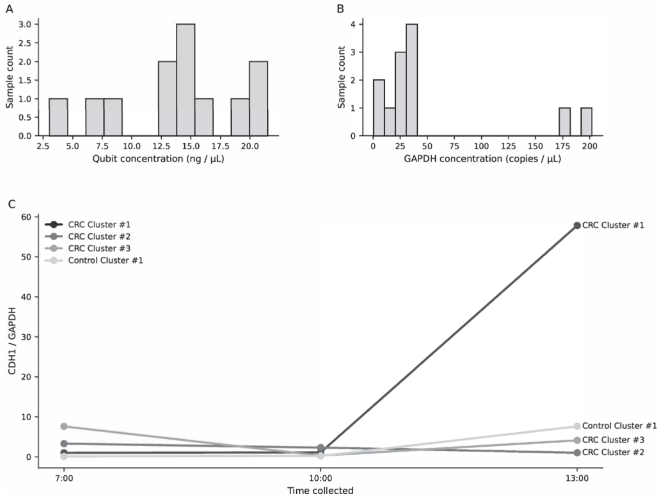

Figure 1 Assessment of RNA concentration values for all replicates across the four sewershed areas (CRC Clusters #1–#3 and Control Cluster #1). (A) Histogram of Qubit concentrations (ng/µL) for all samples assessed across the four sewershed areas. (B) Histogram of GAPDH concentrations (copies/µL) for all samples assessed across the four sewershed areas. (C) Line plot of normalised colorectal neoplasia-associated RNA markers (CDH1/GAPDH) for all samples assessed across the four sewershed areas parsed by various time points (7:00, 10:00 and 13:00). CRC, colorectal cancer.

RESULTS

Total eukaryotic RNA was successfully extracted from all wastewater samples analysed. All replicates from all four clusters showed detectable levels of human RNA, including CDH1, which is a colorectal neoplasia-associated biomarker.

Average Qubit concentration across all samples was 13.8 ng/µL (range: 3.03–21.5 ng/µL), and average GAPDH concentration was 52.1 copies/µL (range: 0.45–202 copies/µL) (figure 1A,B, online supplemental table 1). Colorectal neoplasia-associated RNA biomarkers (CDH1), when normalised to the housekeeping transcript (GAPDH), showed low concentration values for all clusters at 7:00 and 10:00 sampling time points, but showed a spike in value at 13:00 for CRC Cluster #1, which affected the mean value of sampling detections (figure 1C). Average normalised colorectal neoplasia-associated RNA markers (CDH1/GAPDH) for CRC clusters were 20.0 (CRC Cluster #1), 2.2 (CRC Cluster #2) and 4.0 (CRC Cluster #3); average normalised colorectal neoplasia-associated RNA markers (CDH1/GAPDH) for Control Cluster #1 were 2.6 (online supplemental table 1). Although statistical testing could not be performed due to the small sample size in this study, CRC Cluster #1 had known tertiary care centre patients (table 1) and high average normalised CDH1/GAPDH. While the Control Cluster did not have any known tertiary care clinical cases at the time of this study, two of the three CDH1/GAPDH measurements were less than 1.0 (online supplemental table 1).

DISCUSSION

The findings of this study underscore the potential feasibility of expanding wastewater-based epidemiology as a community-level and population-level surveillance tool for CRC. By integrating environmental surveillance with biomolecular detection of CRC-associated markers, this study provides encouraging, though not definitive, preliminary data that help address the current limitations of CRC screening methodologies. We show that measurement is feasible, but individual measurements (both between and within sites) exhibit substantial variation.

Previous research in this field has primarily focused on using wastewater to detect cancer treatment medications or environmental contaminants associated with cancer-related health outcomes.[16 17] While some studies have explored the detection of mitochondrial DNA mutations in wastewater, these mutations were not specific to a particular cancer type.[18 19] In contrast, the data presented here represent the first use of specific human RNA biomarkers to detect CRC in wastewater.

The recent trend of increasing CRC incidence in younger populations underscores the need for improved public health approaches. While existing screening methods like colonoscopy and stool-based tests remain effective, their reliance on individual compliance presents challenges, particularly in communities facing structural and social determinants of health barriers. While mail-based self-sampling has reduced the burden of the conventional visit-based CRC screening approach,[20] wastewater surveillance could further improve access to screening. While infectious disease wastewater surveillance is often conducted once or two times a week for baseline monitoring, from a public health perspective, wastewater CRC surveillance could be implemented with a single yearly sample to guide geographic boundaries for community-based intervention.[14]

Although our preliminary data justify further study, this study has several limitations. Detection of colorectal neoplasia-associated biomarkers in the control area, where there are no known tertiary care centres or Kentucky cancer registry patients, needs further survey. Some residents in this area may be receiving cancer treatment elsewhere, though it would likely be limited. Given the retrospective nature of the study, the timing of wastewater sampling, and the number of known and active CRC cases, it remains unknown how the CDH1/GAPDH ratio in wastewater specifically relates to CRC incidence. For example, it is not known whether diagnosed or undiagnosed CRC cases are contributing to the higher CDH1/GAPDH ratio. The small sample size and geographical scope limit the generalisability of findings. Additionally, the sensitivity and specificity of wastewater-based epidemiology for detecting CRC biomarkers require validation in larger populations.

This proof-of-principle work calls for an expanded research agenda to determine the feasibility of population-based testing for human RNA biomarkers in wastewater, which could eventually enable the detection of CRC burden at the neighbourhood level. Key areas for study include: (1) Exploring additional sampling time points and sites may allow a more quantitative trend evaluation of biomarker levels; (2) Developing a better understanding of signal integrity, perhaps by tracing the wastewater of consenting patients with CRC from the household plumbing source, neighbourhood manhole and downstream treatment plant to determine sewer transport conditions and decay from patient source and understand the individual versus neighbourhood level influences (ie, how easily can one individual skew measured wastewater quantities); (3) Using cancer registry data associated with county-wide wastewater surveillance to determine if CRC biomarkers detected in wastewater follow incidence gradients spatially; (4) Widening the geographical distance between cluster and control groups to better determine the limitations of detecting clusters (based on clinical boundaries), the negative predictive value of wastewater surveillance, and biomarker stability under wastewater system conditions, including how sewage transport, infrastructure type or other built environment factors might affect sampling results; (5) Further development of intervention strategies that exploit

CRC risk information from wastewater surveillance to increase the effectiveness of home-test or other targeted clinical screening programmes; and (6) Ongoing biomedical ethics and medical privacy discussions where only a single diagnosed cancer case may be included within a sewershed.

CONCLUSION

This basic proof-of-concept for a novel wastewater surveillance application to track potential cancer burden demonstrates that CDH1, which is associated with CRC, is detectable in wastewater and may accelerate the field's development for epidemiological studies. Wastewater surveillance could complement traditional screening by enabling a broader, community-level assessment of CRC risk without requiring individual participation. This proof-of-principle work demonstrates the earliest steps of feasibility testing toward extending wastewater analysis to detect CRC-associated RNA biomarkers. The finding that CDH1 is detected is promising and needs an expanded research agenda and larger sample size for statistical power to enable more definitive findings. By combining environmental surveillance with targeted public health interventions, wastewater-based epidemiology holds promise for contributing to CRC prevention and early detection strategies, ultimately improving outcomes for high-risk communities.

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Competing interests EW, EKB, CM and CG are employees of Geneoscopy Inc. EW and EKB are inventors of intellectual property owned by Geneoscopy Inc. ND, DJB, AJ, SK, RHH and TS declare no competing financial interests.

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ORCID iDs
Clayton Grass https://orcid.org/0009-0007-3683-8437
Rochelle H Holm https://orcid.org/0000-0001-8849-1390
Ted R Smith https://orcid.org/0000-0003-2804-4294

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