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Surveillance of *Clostridioides difficile* Burden in Hospitals Through Wastewater Analysis

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Background

- *Clostridioides difficile* infection (CDI) is the leading cause of infectious diarrhea in hospitals and is a nationally notifiable disease under surveillance in Canada.[1]
- The 'gold standard' for CDI diagnosis and monitoring relies on confirming a patient's clinical symptoms and medical history with laboratory testing.[1]
- Despite Infection, Prevention & Control measures, hospital-acquired CDI and outbreaks still occur, increasing patient morbidity, mortality, and health care costs.[2]
- Wastewater (WW)-based surveillance is an emerging surveillance tool that enables comprehensive, unbiased, and inclusive assessments of different populations – spatially and temporally.[3]
- **We sought to detect, track, and quantify *C. difficile* across a range of scales using WW-based surveillance.**

Methods

- Samples were collected from the Peter Lougheed Centre (PLC; 517 inpatient beds), Rockyview General Hospital (RGH; 615 beds), and a municipal WW Treatment Plant (WWTP; services a population of 290,069) in Calgary, Alberta, Canada.
- Quantitative PCR (qPCR) targets included *C. difficile* 16S rRNA and toxin A *tcdA* genes (multiplexed).
  - *C. difficile* gene abundances were assessed as raw or normalized relative to the abundance of different fecal biomarkers (e.g., total bacterial 16S rRNA genes, human 18S rRNA genes and *Bacteroides* HF183 16S rRNA genes).
- Kruskal-Wallis and Mann-Whitney statistical tests were used to compare gene abundances between WW sites (P<0.05 is significant).

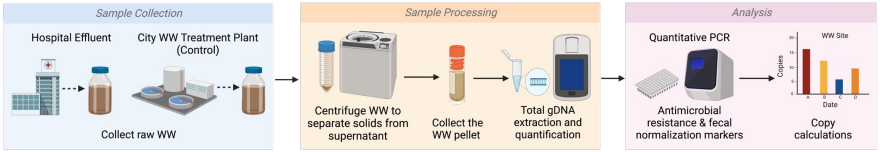
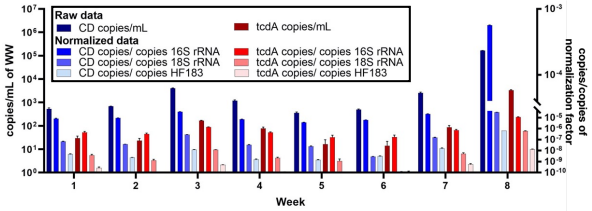


Figure 1. Workflow for the sample collection, processing, and analysis of WW.

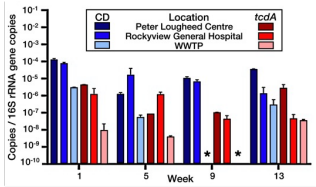
Results

Figure 2. *C. difficile* genes in Rockyview General Hospital WW as measured by qPCR.
A comparison over time of *C. difficile* 16S rRNA (CD) and *tcdA* gene abundance as raw values versus normalized using different fecal biomarkers.



- Samples collected over 8 weeks from the RGH showed significant changes in the levels of total *C. difficile* 16S rRNA and *tcdA* genes over time (P=0.0004 and P=0.0005, respectively, Kruskal-Wallis).
- Similar trends were seen in total *C. difficile* and *tcdA* burden over time when these gene copies were normalized against three fecal biomarker genes.

Figure 3. C. difficile genes in WW as measured by qPCR.
A comparison over time of *C. difficile* 16S rRNA (CD) and *tcdA* gene abundances in WW from two hospitals and a WWTP, as normalized by total bacterial 16S rRNA gene abundance.



- *C. difficile* 16S rRNA and *tcdA* gene abundance were greater in hospital WW (RGH and PLC) than from the WWTP samples (P=0.048 and P=0.012, respectively, Mann-Whitney).
- There was no significant difference in *C. difficile* 16S rRNA and *tcdA* gene abundances between RGH and PLC (P=0.896 and P=0.343, respectively, Mann-Whitney).

Conclusions

Wastewater surveillance is a powerful tool that can monitor the burden the *C. difficile* across a range of scales in real-time.

This tool could augment infection prevention and control and antimicrobial stewardship programs to better understand factors that contribute to colonization and infection.

Thereby, WW-based surveillance could potentially reduce *C. difficile* incidence.

References

1. Public Health Agency of Canada. Canadian Nosocomial Infection Surveillance Program (CNISP) Surveillance for *Clostridium difficile* infection (CDI) CDI Surveillance Protocol. 2022.
2. Alberta Health Services. Hospital-Acquired *Clostridium difficile* Infections Q3 2016-17 Performance Report. 2017.
3. Choi PM, Tscharke BJ, Donner E, O'Brien JW, Grant SC, Kaserzon SL, et al. Wastewater-based epidemiology biomarkers: Past, present and future. TrAC Trends in Analytical Chemistry. 2018;105:453-69.