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Presence of antibiotic resistance genes in the receiving environment of Iqaluit's wastewater treatment plant in water, sediment, and clams sampled from Frobisher Bay, Nunavut: a preliminary study in the Canadian Arctic

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Abstract

Antibiotic resistance (AR) is a growing health concern worldwide and the Arctic represents an understudied region in terms of AR. This study aimed to quantify AR genes (ARGs) from effluent released from a wastewater treatment plant (WWTP) in Iqaluit, Nunavut, Canada, thus creating a baseline reference for future evaluations. Water, sediment, and truncate softshell clam (*Mya truncata*) tissue samples were compared from the wastewater, the receiving environment of Frobisher Bay, and nearby undisturbed freshwaters. The pharmaceuticals and personal care products (PPCPs) atenolol, carbamazepine, metoprolol, naproxen, sulfapyridine, and trimethoprim were found in the wastewater, but the PPCPs were undetectable in the receiving environment. However, the relative abundances of ARGs were significantly higher in wastewater than in the receiving environment or reference sites. Abundances did not significantly differ in Frobisher Bay compared to undisturbed reference sites. ARGs in clams near the WWTP had similar relative abundances as those from pristine areas. The lack of ARG detection is likely due to Frobisher Bay tides flushing inputs to levels below detection. These data suggest that the WWTP infrastructure does not influence the receiving environment based on the measured parameters; more importantly, further research must elucidate the impact and fate of AR and PPCPs in Arctic communities.

Key words: wastewater, contaminants, antimicrobial resistance, food security

Introduction

Antibiotic resistance (AR) genes (ARGs) allow bacteria to become resistant to antibiotics and naturally exist in soils; however, human activity has been contributing to their development and release (Dcosta et al. 2011), including wastewater (Graham et al. 2019*a*, 2019*b*). Few studies have characterized the impacts of wastewater effluents on receiving environments in the Canadian Arctic, and fewer are concerned about the spread of antibiotic-resistant (AR) genes (ARGs). The wastewater treatment plant (WWTP) in Iqaluit (Nunavut, Canada), located at the head of the Koojesse Inlet of Frobisher Bay, releases wastewater effluent into Frobisher Bay annually (Neudorf et al. 2017). Neudorf et al. (2017) found that Iqaluit's primary treatment reduced ARGs but ineffectively controlled its entire release into the environment.

The impacts in the receiving environment remain a concern, and information is limited. For example, Krumhansl et al. (2015) found sediments at >500 m distance from the Iqaluit wastewater discharge point to be anoxic and devoid of benthic invertebrates. These impacts concern the local population, which harvests truncate softshell clams (*Mya truncata*); while harvesters avoid the inlet nearest the WWTP, the clams may be within distances impacted by wastewater effluent (Manore et al. 2020). In addition, the clams closest to the Iqaluit WWTP are impacted by wastewater effluent (Schaefer et al. 2022), but the presence of AR bacteria has not been determined.

Further, the AR results depend on the conditions of receiving environment. Hayward et al. (2018) focused on tundra wetland systems into which settlement lagoons discharge;

they concluded that wastewater discharges increase ARG abundances, but their fate is influenced by wetland hydrology. On the other hand, Chaves-Barquero et al. (2016) in Cambridge Bay, NU, did not consider the concentration of pharmaceuticals and ARGs to be a concern at the time of their study. As such, the impacts of wastewater discharges and the factors that affect their fate remain largely unelucidated in Arctic communities.

This study investigated the variation of ARGs and pharmaceutical and personal care products (PPCP) within Iqaluit's WWTP and its associated discharge into Frobisher Bay, Nunavut. By examining ARGs and pharmaceuticals and personal care products (PPCPs), we determined whether the discharges were detrimental to Iqaluit's water quality and the potential for human exposure to AR bacteria.

Methods

Study location

Iqaluit is the capital city of the Nunavut territory and is situated on Baffin Island, Canada (2016 population, 7740). The city is located within Koojesse Inlet, near the top of Frobisher Bay, which is macrotidal (12 m) with 17 km^3 of seawater flushing the Bay during a single tide (Hsiao 1992). At the time of sampling, the wastewater system in Iqaluit was comprised of mechanical screening (Salsnes filter) prior to release, which was discharged continuously year-round into an open channel, i.e., directly into the marine environment (see Fig. 1). The associated lagoon was for overflow or when the plant was not functional and was released monthly (Neudorf et al. 2017). Approximately 7.2×10^8 L of wastewater is released annually (Neudorf et al. 2017).

Analysis of PPCPs

A total of 28 pharmaceuticals were sampled in this work using the organic diffusive gradients in thin film (o-DGT) passive sampler as reported previously (Stroski et al. 2020), including 17-estradiol, 17-ethynylestradiol, atenolol, atrazine, carbamazepine, clarithromycin, clofibric acid, diclofenac, enrofloxacin, erythromycin, estrone, fenoprofen, fluoxetine, gemfibrozil, ibuprofen, ketoprofen, metoprolol, naproxen, paroxetine, propranolol, roxithromycin, sulfamethazine, sulfamethoxazole, sulfapyridine, sulfisoxazole, sulfachloropyridazine, sulfadimethoxine, and trimethoprim. These PPCPs were monitored as they are commonly found in wastewater (Ying et al. 2009; Gagnon and Lajeunesse 2012; Challis et al. 2016), including in arctic regions (Stroski et al. 2020), and existing laboratory methods and capacity were in place. The assembly, extraction, and calculation of time-weighted average (TWA) water concentrations o-DGT are detailed elsewhere (Challis et al. 2016) and reported for Frobisher Bay, NU (Stroski et al. 2020). Laboratory and field blanks were extracted with each set of samples and had negligible levels of all analytes measured. Analyte concentrations were determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS) using an Agilent 1200 Series LC pump and Agilent 6410B MS/MS (Agilent Technologies, Mississauga, ON) in electrospray ionization positive and negative mode. Limits of detections (LOD) and quantifications (LOQ) are found in the Supplementary material, while chromatographic and MS/MS method details are found elsewhere (Challis et al. 2016).

Sample collection for ARGs

Samples were collected 8–10 August 2019 (Table 1 and Fig. 1). From the WWTP, we sampled the wastewater influent, the retention lagoon, and discharged effluent and sediment as it flowed along the tidal flats to Frobisher Bay. At varying distances, we sampled the water in Frobisher Bay; additionally, the clam tissues, sampled for another project during the same time period, were graciously provided to us (Schaefer et al. 2022; see Table 1). Additional water samples from Lake Geraldine and the Sylvia Grinnell River, representing pristine water sources, were also examined; however, their shoreline conditions were too rocky to collect any sediment. Finally, a soil sample near the WTTP, but not exposed to wastewater, was also collected as an additional control. All sampling was done with the support of the Amaruq Hunters and Trappers Association (Nunavut Scientific Research Licence No. 01 031 18 N-M) and under a license approved by Fisheries and Oceans Canada (Licence No. S-19/20–1040-NU).

Waters were aseptically collected in 1L-polypropylene bottles after a triple on-site rinse; sediments were obtained in 50 mL polypropylene centrifuge tubes; both were collected by hand. Cells from wastewaters (50–150 mL) and natural waters (900 mL) were filtered (Whatman 0.2 μm membrane filters). All samples were kept cool (<10 °C) during transport and frozen (−15 °C) during storage. DNA from sediment and soil were extracted directly.

DNA extractions

Sediment, soil (0.5 g), and water filters (cells harvested from water) were homogenized by a FastPrep24 cell disruptor (MP Biomedicals; 6.0 speed 2 × 20 s) and extracted using DNeasy PowerSoil kit (Qiagen; Venlo, The Netherlands). The clam tissues (10–20 g) in 50 mL solution of 10 mM PBS (pH 7.4) and protease K were digested in a Seward Stomacher (Seward, Worthing, UK); the DNA was extracted by DNeasy Blood and Tissue kit (Qiagen). Both extraction methods followed the manufacturer's recommended protocols. The purity and quantity of extracted DNA were 1.7–2.0 (A260/A280) and 10–150 ng/μL, respectively, as determined by the micro-UV-spectrophotometer. The extraction kits were selected based on these purities and yields for each sample type.

Sediment and wastewater samples were diluted 1:20 with molecular-grade water to minimize co-eluted polymerase chain reaction (PCR) inhibitors; the DNA from the clams were diluted 1:200. This was determined by serially diluting the extractions (i.e., from soils and clams) pre-spiked with 10^9 genes of *E. coli* 16S rRNA. The resultant PCR efficiencies and expected threshold abundances were compared against a "neat" standard curve.

Pre-screening ARGs

To distinguish between ARGs from the wastewater and those that may naturally occur, extracted DNA (1 μg) from sediment exposed to wastewater effluent and environmental

Fig. 1. Map of sample locations in August 2019. Key: ▲, wastewater samples; △, Frobisher Bay samples; ○, clam samples; ■, wastewater effluent-contaminated sediment (and water); and □, "clean" soil sample. Credit: contains information licenced under the Open Government Licence-Canada (https://open.canada.ca/en/open-government-licence-canada). Map projection: NAD_1983_UTM_ZONE_19 N; it was created in ESRI.

soil samples (nearby, not in contact with wastewater) were analyzed using the Open Array platform (Chinese Academy of Sciences—Xiamen; e.g., Zhu et al. (2013)). We selected 11 gene targets from the results (Table S1): *sul*1, *erm*B, *acr*A, *qac*H, *dfr*A, *tet*39, *qnr*A, *cph*A, *amp*C, bla_{TEM}, and *aph*A for qPCR (see below). Table S2 summarizes gene information. In addition, a 16S-rRNA gene target was used as a surrogate measure of "total bacteria," to which all relative abundances were calculated.

Quantitative polymerase chain reactions (qPCR)

Quantitative PCR was used to target 11 ARGs in the samples. Each 10 μL reaction consisted of 2 μL of diluted DNA template, 5 μL of ssoFAST-EvaGreen qPCR reagent (BioRad; Hercules, CA, USA), 0.2 μm primers and was made to volume with molecular-grade water. Reaction conditions included initial denaturation (94 °C, 3 min), 30–50 cycles of primer annealing (5 sec, 60 °C), and denaturation (94°C, 3 sec); all

Table 1. Sample locations and brief descriptions.

Sample	Location	Description
Wastewater	Influent* ($n = 3$)	Raw sewage as it enters the treatment plant, 50 mL each.
	Discharge No. 1 N63°44.6817/W68°32.3427	Sediment ($n = 3$, 10 g each, composited) and water ($n = 1$; 100 mL) at an outlet 50 m from the treatment plant; the water begins to flow on the tidal flats.
	Discharge No. 2 N63°44.5483/W68°32.2508	Same as above, ~300 m from the outlet as the discharged water flows on the tidal flats
	Discharge No. 3 N63°44.5171/W68°32.2117	Same as above, ~300 m from the outlet as the discharged water flows on the tidal flats
	Retention lagoon ($n = 2$) N63°44.6829/W68°32.1864	Raw sewage from the retention lagoon next to the treatment plant.
Frobisher Bay	Bay No. 1 N63°44.6829/W68°31.9472	Water sample (1000 mL) from Koojesse inlet, closest to the WWTP
	Bay No. 2 N63°43.6829/W68°31.1491	Water sample (1000 mL) from Koojesse inlet, 2.1 km from the outlet.
	Bay No. 3 N63°43.1289/68°26.9805	Water sample (1000 mL) from Koojesse inlet, 5 km from the outlet, near Apex community.
	Bay No. 4 N63°39.5901/W68°46.4771	Water sample (1000 mL) from the western end of Frobisher Bay, distant (SW-15 km) from discharge, near Kituriaqannigituq. This represents a "control" sample, unlikely impacted by discharges.
Other	Geraldine Lake N63°45.3667/W68°30.1360	Freshwater samples from the lake's southwestern shoreline, close to the drinking water intake.
	Sylvia Grinnell river N63°46.8088/W68°37.1494	Freshwater sample from upstream of Frobisher Bay.
	Environmental soil sample * N63°44.6829/W68°32.1864	This was a soil sample (10 g) collected near the lagoon, but it was not in direct contact with the wastewater.
Clams (*Mya truncata*) **	Clams No. 1 ($n = 7$)	Direct wastewater exposure; tidal flats near the outlet.
	Clams No. 2 ($n = 8$)	Potential wastewater exposure (SE-3 km), near "Tundra Ridge"
	Clams No. 3 ($n = 5$)	Potential wastewater exposure (S-5 km); popular clam harvesting location "Apex"
	Clams No. 4 ($n = 6$)	Potential wastewater exposure (S-4.9 km); Monument Island is an uninhabited island at the mouth of the Koojesse inlet. Also a popular harvest location
	Clams No. 5 ($n = 6$)	Collected near an uninhabited island, Aupalajat, which is distant (W-5.2 km) from discharge with a geographical barrier (Peterhead inlet).
	Clams No. 6 ($n = 6$)	At the western end of Frobisher Bay, distant (SW-15 km) from discharge, near Kituriaqannigituq. It represents a "control" sample, unlikely impacted by discharges.

*Samples analyzed by quantitative microarray analysis.
**Locations were adapted from Schaefer et al. (2022)

were conducted on a BioRad iCycler5 (BioRad instrument) in triplicate. Blanks and standards were routinely run with samples. In addition, a post-analytical melt curve (Δ0.2 C/s) was run to verify amplification quality and specificity. Detections were valid when at least two replicates were within one cycle of each other without aberrant fluorescent signals.

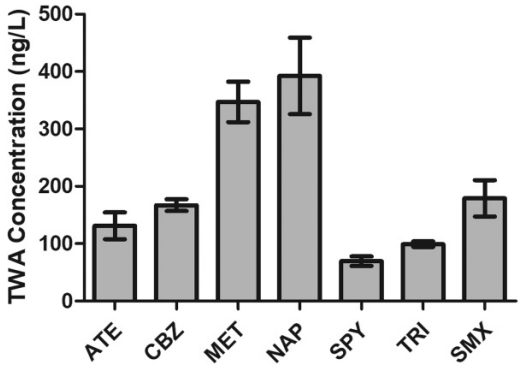
Standards comprised of spiked DNA (10^2–10^8 copies/μL) in previously UV-irradiated sample matrix (i.e., extracts from sediment, marine water, or clam tissue); the 10 min exposure under UV sterilizing conditions prevents existing DNA from becoming PCR-able. Therefore, DNA standards were prepared from PCR amplicons, purified with a Qiagen PCR Purification Kit, and quantified by UV-micro-spectrophotometry; sequences were verified by Sanger Sequencing (GATC-Eurofins Genomics).

Data analysis

The abundances of genes were log-transformed prior to statistical analysis to improve sample distribution (normality). Absolute abundances were presented for the "total bacteria" (as measured by 16S-rRNA gene targets) and represented genes detected per mL (filtered water) or gram (clam tissue, sediment or soil). In contrast, relative abundances have been normalized to the 16S-rRNA gene counts and provide a sense of whether the selection of ARGs in the system has been enriched. Due to data distributions, non-parametric tests (e.g, Mann Whitney and Kruskal Wallis) were used for statistical comparisons.

Repeat sample events were cancelled due to the 2019 SARS-CoV-2 pandemic. However, for data comparisons, we grouped like samples: wastewater effluent ($n = 3$), the sediment on

Fig. 2. Concentrations of compounds detected in Iqaluit Lagoon August 2019. Mean (±SD $n = 3$) time-weighted averages of pharmaceuticals in Iqaluit municipal wastewater lagoon: atenolol (ATE), carbamazepine (CBZ), metoprolol (MET), naproxen (NAP), sulfapyridine (SPY), trimethoprim (TRI), and sulfamethoxazole (SMX).



which the wastewater effluent flowed ($n = 3$), freshwater samples (Lake Geraldine and Sylvia Grinnell River), Frobisher Bay water ($n = 4$), and clams (5–8 clams per location; see Table 1).

Results and discussion

Pharmaceutical and personal-care products

Compounds detected were atenolol, carbamazepine, metoprolol, naproxen, sulfapyridine, and trimethoprim, but only in the sewage lagoon (Fig. 2). The compounds were found at levels similar (in the ng/L range) to other wastewater systems in Nunavut (Chaves Barquero et al. 2016; Stroski et al. 2020), including work in Iqaluit previously (Stroski et al. 2020). They were also not detected outside of the lagoon itself, which is consistent with previous work at this location (Stroski et al. 2020). The lack of detection suggests that compounds are being (a) degraded through photolytic or biological means within the lagoon or before discharge or (b) the large body of water the compounds enter (i.e., Frobisher Bay) act to dilute so much as to make the concentrations negligible. There was no evidence of these compounds in the drinking water source (Lake Geraldine) and the upstream river reference (all were non-detects).

Wastewater composition

The pre-screening assay provided relative abundances of 308 ARGs, 53 genetic elements, and 11 critical taxonomies associated with resistance genes (Tables S1 and S3). Among the taxonomy results, differences in gene frequency helped select ARG targets most relevant to the wastewater. For example, the influent comprised 33% Bacteroidetes, 26% Firmicutes, 9.7% *Acinetobacter* sp., and 8.2% *Pseudomonas*, representing human-gut microbiota (Thomas et al. 2011). In contrast, the soil had half the percentages of Bacteroidetes and Firmicutes and <1% of the latter two genera.

Based on the ARG pre-screen, the wastewater had a higher richness of resistance genes (positive gene detections) of each antibiotic "type" (see Table S4 for the complete list). The wastewater and soil had the following %positive detections of resistance genes (respectively; selected gene targets for the qPCR are also mentioned) per antibiotic class: aminoglycosides (61%, 22%; *aphA3*), beta-lactamases (54%, 19%; *ampC*, *cphA*, bla_{TEM}), fluoroquinolones (80%, 50%; *qnrA*), multidrug resistance genes (83%, 55%; *qacH*, *acrA*), macrolide–lincosamide–streptogramin B (59%, 39%; *ermB*), phenicols (40%, 20%), sulfonamides (71%, 57%; *sul1*, *dfrA*), tetracyclines (50%, 36%; *tet39*), trimethoprim (29%, 18%; *dfrA*) vancomycin (38% 13%), other resistance genes (53%, 24%), and mobile genetic elements (79%, 55%).

Wastewater received only primary treatment at the time of sampling (August 2019). The WWTP was able to reduce the bacterial gene concentrations from $10^{8.6\ (\pm 0.1)}$ in the influent to $10^{7.6\ (\pm 0.5)}$ genes/mL (90% reduction). When examining ARG distribution between wastewater influent ($10^{-0.7}$ genes/16S-rRNA) and effluent ($10^{-1.2}$), there appears to be a slight shift in their relative abundances. Neudorf et al. (2017) found similar removal rates. However, here, for most genes, there were no significant differences (*t* test, $p > 0.05$; see Supplementary Table S5); exceptions included lower effluent concentrations for *qac*H and *dfr*A and higher concentrations of *sul*1. Primary treatment often remains ineffective in removing ARGs (Graham et al. 2019*a*); any removal would be attributed to bacteria physically removed with the solids during primary treatment.

Following primary treatment, the discharged wastewater flowed >200 m along the upper-tidal flat to Frobisher Bay. Most ARG values representing wastewater discharges and sediment were not statistically different (Table 2). However, lower relative abundances of *qnr*A, *dfr*A, and bla_{TEM} genes were found in the sediment than in the flowing waters; the differences could be influenced by indigenous bacteria on the tidal flat surface on which the discharged effluent flowed. We do not anticipate selective pressures from discharged pharmaceuticals on the bacteria (PPCP concentrations are low); instead, we were detecting the presence and fate of faecal bacteria and the ARG they contained.

ARGs in the water

Relative concentrations of ARGs became diluted once they entered Frobisher Bay but remained detectable (Table 2). To determine whether they impacted the Bay, we compared the concentrations in Frobisher Bay with those of inland freshwater sources (i.e., Sylvia Grinnell River and Lake Geraldine). The two inland sites represent "pristine" (minimally impacted) sites.

Although bacteria and fungus levels were orders of magnitude lower in Frobisher Bay and freshwater samples than in wastewater (Table 2), the relative abundances helped discern whether selective pressures remained. From the results, the marine and freshwater samples had comparable concentrations for all but three genes: *qnr*A, *cph*A, and *amp*C, which were similar in all locations. However, non-wastewater samples had lower relative abundances for the other genes. The decreased total bacteria and decline in relative abundances further reduce ARG risks to Frobisher Bay.

Table 2. Relative abundances of ARGs (log ARG/16S-rRNA), bacteria, and fungus (either genes/mL or genes/g) at four grouped locations.

Gene	Wastewater outflow (3 locations)	Wastewater sediment* (3 locations)	Freshwater (nearby river and lake)	Frobisher Bay (4 locations)	K-Wallis statistic* (H_2 and p-values)
sul1	–2.1 (0.5)	–1.5 (0.6)	–3.6 (0.0)	–4.0 (0.2)	6.7/0.04
ermB	–1.8 (0.2)	–2.8 (1.1)	–5.0 (0.2)	–4.6 (0.8)	6.6/0.04
acrA	–3.0 (0.2)	–2.5 (0.4)	–3.9 (0.3)	–3.9 (0.2)	6.6/0.04
qacH	–2.3 (0.6)	–2.5 (1.5)	–4.8 (1.2)	–5.0 (0.7)	6.6/0.04
dfrA	–2.8 (0.3)	–4.2 (0.9)	–4.0 (0.7)	–3.9 (0.7)	5.5/0.06
tet39	–1.4 (0.5)	–2.0 (0.9)	–5.2 (0.4)	–3.4 (1.3)	7.9/0.02
qnrA	–2.9 (1.5)	–4.0 (0.6)	–5.2 (1.5)	–4.0 (0.9)	3.5/0.17
cphA	–2.9 (0.5)	–2.0 (0.4)	–2.9 (0.4)	–2.5 (0.6)	0.2/0.91
ampC	–3.7 (1.1)	–3.1 (0.6)	–3.0 (0.2)	–3.9 (0.4)	3.3/0.19
bla_{TEM}	–2.5 (0.4)	–3.1 (0.6)	–3.0 (0.4)	–3.2 (0.3)	5.5/0.07
aphA3	–3.2 (0.3)	–3.5 (0.5)	–4.3 (0.1)	–4.5 (0.8)	6.9/0.03
Bacteria	7.9 (0.3)	9.9 (0.4)	5.3 (0.4)	6.4 (0.3)	7.8/0.02
Fungus	6.9 (1.0)	9.1 (0.6)	4.9 (0.3)	5.6 (0.7)	4.8/0.09

Note: Values represent sample means (log-transformed) with standard deviations in parentheses. ∗Statistical comparisons did not include sediment values.

Table 3. The relative abundance of each ARG selected, with increasing distance from the WWTP effluent outlet.

Gene	**Wastewater discharge**	**Bay No. 1,** near wastewater	**Bay No. 2**	**Bay No. 3**, Apex	**Bay No. 4**, Kituriaqannigituq
sul 1	–2.05	–3.75	–4.12	–3.93	–4.12
ermB	–1.80	–3.65	–4.27	–5.25	–5.25
acrA	–3.02	–3.90	–3.80	–3.71	–4.21
qacH	–2.33	–4.46	–4.36	–5.54	–5.70
dfrA	–2.83	–3.10	–4.06	–3.65	–4.71
tet39	–1.43	–2.24	–2.39	–4.13	–4.81
qnrA	–2.92	–3.03	–3.64	–3.98	–5.20
cphA	–2.88	–1.65	–2.67	–2.74	–3.00
ampC	–3.70	–3.54	–3.76	–3.89	–4.37
bla_{TEM}	–2.54	–2.94	–3.01	–3.40	–3.63
aphA3	–3.21	–3.53	–4.93	–5.23	–4.33

Note: The colour gradient shows the highest (red) to lowest (green) relative abundance.

However, some concerns become highlighted when one examines the ARG more closely in Frobisher Bay by comparing relative gene abundances from the discharge point. A clear inverse trend was observed between gene abundances and distance (Table 3), which suggests that wastewater discharges may impact water conditions in Frobisher Bay. This analysis remains rudimentary as actual travel distance would not be direct but would be influenced by the complex hydrological dynamics of circulation and tidal fluctuations. However, sample collection began at high tide, and the influx of marine waters could have influenced the results. Kituriaqannigituq (Bay No. 4) is located at a different inlet and unlikely to be influenced by Iqaluit's wastewater; as a "control" site, it provides a context of expected gene concentrations in the Bay.

ARGs detected in clams

Similarly, we detected resistance genes in the tissues of truncate softshell clams sampled at multiple sites (Table 4). The Kruskal–Wallis test showed no significant differences among the ARG at the six sampling locations and no clear trends or patterns (Table S6). However, higher bacteria levels were found in Koojesse Inlet (near the point of wastewater discharge (Clam No. 1), Apex (Clam No. 3), and Monument Island (Clam No. 4); $H_5 = 12.8$, $p = 0.03$; Table 4).

It is hypothesized that the reason for no significant differences in ARG levels in *M. truncata* was related to their storage following harvest. As previously mentioned, clams were harvested by Schaefer et al. (2022) for their biometrics; as part of their study, clams were held in artificial seawater (2–4 °C) before dissection. *M. truncata* filters 2.5 L/h (Petersen et al. 2003; Bernard and Noakes 1990) and can rapidly digest the ARG-containing wastewater bacteria. As such, it was likely that the bacteria would have been flushed from the clams. This "depuration" method has been utilized to reduce potential health hazards of bacteria (Metcalf et al. 1979) and viruses (Polo et

Table 4. Relative abundances of ARGs (log ARG/16S-rRNA), bacteria, and fungus (log genes/g of tissues) in clam tissues.

Gene	Clam No. 1 Wastewater ($n = 7$)	Clam No. 2 Tundra Ridge ($n = 8$)	Clam No. 3 Apex ($n = 5$)	Clam No. 4 Monument Island ($n = 6$)	Clam No. 5 Aupalajat ($n = 6$)	Clam No. 6 Kiturialqannigituq ($n = 6$)	Kruskal Wallis p-value
sul1	−4.3 (0.3)	−4.4 (0.3)	−3.9 (0.2)	−4.1 (0.1)	−3.7 (0.2)	−3.9 (0.1)	0.20
	100%	88%	100%	100%	83%	100%	
ermB		−4.5 (0.5)	−5.7		−4.5		0.74
	0%	50%	20%	0%	17%	0%	
acrA	−3.7 (0.3)	−3.1 (0.3)	−3.1 (0.2)	−3.6 (0.3)	−2.9 (0.4)	−3.2 (0.4)	0.72
	100%	100%	100%	100%	67%	100%	
qacH	−3.2 (0.4)	−3.1 (0.3)	−3.3 (0.2)	−5.8 (0.3)	−3.6 (0.7)		0.15
	43%	50%	80%	50%	67%	0%	
dfrA	−3.7 (0.5)	−3.0 (0.5)	−3.8 (0.7)	−3.8 (0.4)	−3.5 (0.8)	−3.8 (0.6)	0.72
	43%	50%	80%	83%	50%	83%	
tet39	−5.4	−3.6 (0.1)	−4.4 (0.6)	−4.7 (0.8)	−3.6 (1.2)	−4.5 (0.3)	0.56
	14%	38%	60%	67%	50%	50%	
qnrA	−4.7 (0.6)	−4.9 (0.7)	−4.3 (0.6)	−4.0 (0.7)	−5.2 (0.3)	−4.2 (0.3)	0.51
	86%	63%	80%	100%	100%	83%	
cphaA	−4.2 (0.3)	−4.0 (0.4)	−4.0 (0.4)	−3.6 (0.2)	−3.5 (0.2)	−4.2 (0.2)	0.34
	86%	63%	100%	100%	100%	83%	
ampC	−4.0 (0.2)	−4.1 (0.1)	−3.7 (0.3)	−4.0 (0.2)	−3.5 (0.4)	−3.3 (0.4)	0.42
	86%	25%	100%	100%	83%	67%	
bla_{TEM}	−4.8 (0.2)	−4.2 (0.1)	−4.2 (0.3)	−4.4 (0.4)	−4.4 (0.2)	−4.3 (0.1)	0.36
	86%	75%	60%	83%	83%	83%	
aphA3	−3.9 (0.8)	−4.4 (0.3)	−3.7 (0.7)	−4.7 (0.4)	−4.1	−3.6 (0.4)	0.63
	43%	50%	60%	67%	17%	33%	
Total bacteria (genes/g)	4.5 (0.2)	4.2 (0.1)	4.5 (0.1)	4.7 (0.1)	4.2 (0.1)	4.2 (0.1)	0.03
Total fungus (genes/g)	5.7 (0.3)	5.6 (0.3)	5.1 (0.5)	5.3 (0.2)	5.2 (0.2)	5.3 (0.6)	0.88

Note: Values represent sample means (log-transformed) with standard deviations in parentheses (in brackets); frequency (%) of detection in samples are also mentioned.

al. 2014), and the same process could have happened here. Further investigation is required to scrutinize depuration impacts. Nevertheless, an environmental risk would remain for harvesters in contact with potentially contaminated seawater. Therefore, improving wastewater treatment would confer the greatest anthropogenic and environmental benefits.

Conclusions

It does not appear that the concentrations of ARG have been significantly elevated in Frobisher Bay due to wastewater discharge from Iqaluit's municipal treatment plant. Relative abundances were highest in the wastewater effluent, which potentiates the possible impact, and diminishing relative abundances could be seen in Koojesse Inlet from the discharge point. However, the relative abundances were equivalent to those from upstream (albeit freshwater) and distant marine undisturbed (reference site) samples, suggesting no significant enrichment could be found. Additionally, total bacteria (per 16S rRNA gene counts) similarly declined, and with the reduction of relative abundance, the absolute amounts of genes being detected would be much lower.

Similar patterns were seen with the detectable PPCP. A contributing factor for both PCPP and ARG fate is likely due to the seawater dilutions (e.g., Zhao et al. 2019) and efficient flushing of Frobisher Bay during tidal fluctuations. Similar flushing could possibly help reduce associated risks with the clams, but this requires further investigation.

The genes selected for analysis in this study do not represent the full range of genes that could confer antibiotic resistance. A disadvantage of this investigation is that ARGs not selected may be polluting the waters of Frobisher Bay undetected. This study, however, provides a reference point for any future quantification risk, building on the earlier work of Neudorf et al. (2017). Following planned upgrades to the sewage treatment works, further studies may investigate how effective the upgrade has been with ARG and pathogen risks.

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Data availability

Data generated or analyzed during this study are available in the University of Strathclyde repository (https://pureportal.strath.ac.uk/en/data sets/data-for-presence-of-antibiotic-resistance-genes-in-the-receiving). DOI: 10.15129/5aed02b6-abf2-479d-98b2-c3b154834d7b.

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Competing interests

The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/as-2023-0011.

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