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 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.

Keywords: wastewater, contaminants, antimicrobial resistance, food security

Introduction

Antibiotic resistance (AR) genes 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., 2019a; Graham et al., 2019b). 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. 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 a*l., 2017). Neudorf et al. (2017) found that Iqaluit's primary treatment reduced antibiotic-resistance genes (ARG) 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 > 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 the 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 wastewater treatment plant and its associated discharge into Frobisher Bay, Nunavut. By examining ARGs and PPCPs (pharmaceuticals and personal care products), 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 (12m) with 17km^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 Figure 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 X10^8 L of wastewater is released annually (Neudorf et al., 2017).

Analysis of pharmaceutical and personal care products

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 SI, while chromatographic and MS/MS method details are found elsewhere (Challis *et al*., 2016).

Sample collection for antibiotic resistance genes

Samples were collected 8-10th August, 2019 (Table 1 and Fig.1). From the wastewater treatment plant, 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 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 50mL polypropylene centrifuge tubes; both were collected by hand. Cells from wastewaters (50-150mL) and natural waters (900mL) were filtered

(Whatman 0.2µm 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 2x20s) and extracted using DNeasy PowerSoil kit (Qiagen; Venlo, The Netherlands). The clam tissues (10-20g) in 50 mL solution of 10mM 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 PCR (polymerase chain reaction) 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 (1µg) from sediment exposed to wastewater effluent and environmental 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 eleven 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 summarises 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 eleven 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, 3min) and 30-50 cycles of primer annealing (5sec, 60˚C) and denaturation (94˚C, 3sec); all 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 (Δ-0.2C/second) 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-minute 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 AR genes 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

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 (Figure 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 antibiotic resistance genes, 53 genetic elements, and 11 critical taxonomies associated with resistance genes (Table 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 wastewater treatment plant 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 Supplemental 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.*, 2019a); any removal would be attributed to bacteria physically removed with the solids during primary treatment.

Following primary treatment, the discharged wastewater flowed >200m 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.

Antibiotic resistance genes 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.

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 #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.

Antibiotic-resistant genes 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 #1), Apex (Clam #3) and Monument Island (Clam #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 litres per hour (Peterson 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 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. 2017) 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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Author contributions: M.S. did the statistical analysis and drafted the manuscript; C.M.S. collected and provided the clams; K.M.J., D.D. and M.H. conceptualized and managed the project and acquired the funding; K.H.L and C.S.W. analyzed PPCPs; C.W.K. sampled and analyzed the antibiotic resistance genes, and coordinated that aspect of the project; all authors reviewed and edited the manuscript.

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299 **Data availability**: Data generated or analyzed during this study are available in the
300 University of Strathclyde repository (https://pureportal.strath.ac.uk/en/datasets/data-for-
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374

375 **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 #1 N63°44.6817/ W68°32.3427	Sediment (n=3, 10 g each, composited) and water (n=1; 100 mL) at an outlet 50m from the treatment plant; the water begins to flow on the tidal flats.
	Discharge #2 N63°44.5483/ W68°32.2508	Same as above, ~300m from the outlet as the discharged water flows the on tidal flats
	Discharge #3 N63°44.5171/ W68°32.2117	Same as above, ~300m from the outlet as the discharged water flows the on 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 #1 N63°44.6829/W68°31.9472	Water sample (1000 mL) from Koojesse inlet, closest to the wastewater treatment plant
	Bay #2 N63°43.6829/W68°31.1491	Water sample (1000 mL) from Koojesse inlet, 2.1km from the outlet.
	Bay #3 N63°43.1289/68°26.9805	Water sample (1000 mL) from Koojesse inlet, 5km from the outlet, near Apex community.
	Bay #4 N63°39.5901/W68°46.4771	Water sample (1000 mL) from the western end of Frobisher Bay, distant (SW-15km) 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 #1 (n=7)	Direct wastewater exposure; tidal flats near the outlet.
	Clams #2 (n=8)	Potential wastewater exposure (SE-3km), near “Tundra Ridge”
	Clams #3 (n=5)	Potential wastewater exposure (S-5km); popular clam harvesting location “Apex”
	Clams #4 (n=6)	Potential wastewater exposure (S-4.9km); Monument Island is an uninhabited island at the mouth of the Koojesse inlet. Also a popular harvest location
	Clams #5 (n=6)	Collected near an uninhabited island, Aupalajat, which is distant (W-5.2km) from discharge with a geographical barrier (Peterhead inlet).
	Clams #6 (n=6)	At the western end of Frobisher Bay, distant (SW-15km) from discharge, near Kituriaqannigituq. It represents a “control” sample, unlikely impacted by discharges.

376
377 * Samples analysed by quantitative microarray analysis.
378 ** Locations were adapted from Schaefer et al. (2021)
379

380 **Table 2**. Relative abundances of ARGs (log ARG/16S-rRNA), bacteria and fungus (either
381 genes/mL or genes/g) at four grouped locations. Values represent sample means (log-
382 transformed) with standard deviations in parentheses. *Statistical comparisons did not
383 include sediment values.

384

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

385
386
387

388 **Table 3**. The relative abundance of each ARG selected, with increasing distance from the
389 WWTP effluent outlet. The colour gradient shows the highest (red) to lowest (green) relative
390 abundance.

Gene	**Wastewater discharge**	**Bay #1**, near wastewater	**Bay #2**	**Bay #3**, Apex	**Bay #4** Kituriaqannigituq
sul1	-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

391

Table 4. Relative abundances of ARGs (log ARG/16S-rRNA), bacteria and fungus (log genes/g of tissues) in clam tissues. Values represent sample means (log-transformed) with standard deviations in parentheses (in brackets); frequency (%) of detection in samples are also mentioned.

Gene	**Clam #1** Wastewater (n=7)	**Clam #2** Tundra Ridge (n=8)	**Clam #3** Apex (n=5)	**Clam #4** Monument Island (n=6)	**Clam #5** Aupalajat (n=6)	**Clam # 6** Kituriaqannigituq (n=6)	Kruskal Wallis *p*-value
sul1	-4.3 (0.3) 100%	-4.4 (0.3) 88%	-3.9 (0.2) 100%	-4.1 (0.1) 100%	-3.7 (0.2) 83%	-3.9 (0.1) 100%	0.20
ermB	 0%	-4.5 (0.5) 50%	-5.7 20%	 0%	-4.5 17%	 0%	0.74
acrA	-3.7 (0.3) 100%	-3.1 (0.3) 100%	-3.1 (0.2) 100%	-3.6 (0.3) 100%	-2.9 (0.4) 67%	-3.2 (0.4) 100%	0.72
qacH	-3.2 (0.4) 43%	-3.1 (0.3) 50%	-3.3 (0.2) 80%	-5.8 (0.3) 50%	-3.6 (0.7) 67%	 0%	0.15
dfrA	-3.7 (0.5) 43%	-3.0 (0.5) 50%	-3.8 (0.7) 80%	-3.8 (0.4) 83%	-3.5 (0.8) 50%	-3.8 (0.6) 83%	0.72
tet39	-5.4 14%	-3.6 (0.1) 38%	-4.4 (0.6) 60%	-4.7 (0.8) 67%	-3.6 (1.2) 50%	-4.5 (0.3) 50%	0.56
qnrA	-4.7 (0.6) 86%	-4.9 (0.7) 63%	-4.3 (0.6) 80%	-4.0 (0.7) 100%	-5.2 (0.3) 100%	-4.2 (0.3) 83%	0.51
cphaA	-4.2 (0.3) 86%	-4.0 (0.4) 63%	-4.0 (0.4) 100%	-3.6 (0.2) 100%	-3.5 (0.2) 100%	-4.2 (0.2) 83%	0.34
ampC	-4.0 (0.2) 86%	-4.1 (0.1) 25%	-3.7 (0.3) 100%	-4.0 (0.2) 100%	-3.5 (0.4) 83%	-3.3 (0.4) 67%	0.42
bla_{TEM}	-4.8 (0.2) 86%	-4.2 (0.1) 75%	-4.2 (0.3) 60%	-4.4 (0.4) 83%	-4.4 (0.2) 83%	-4.3 (0.1) 83%	0.36
aphA3	-3.9 (0.8) 43%	-4.4 (0.3) 50%	-3.7 (0.7) 60%	-4.7 (0.4) 67%	-4.1 17%	-3.6 (0.4) 33%	0.63
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

Figure 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.

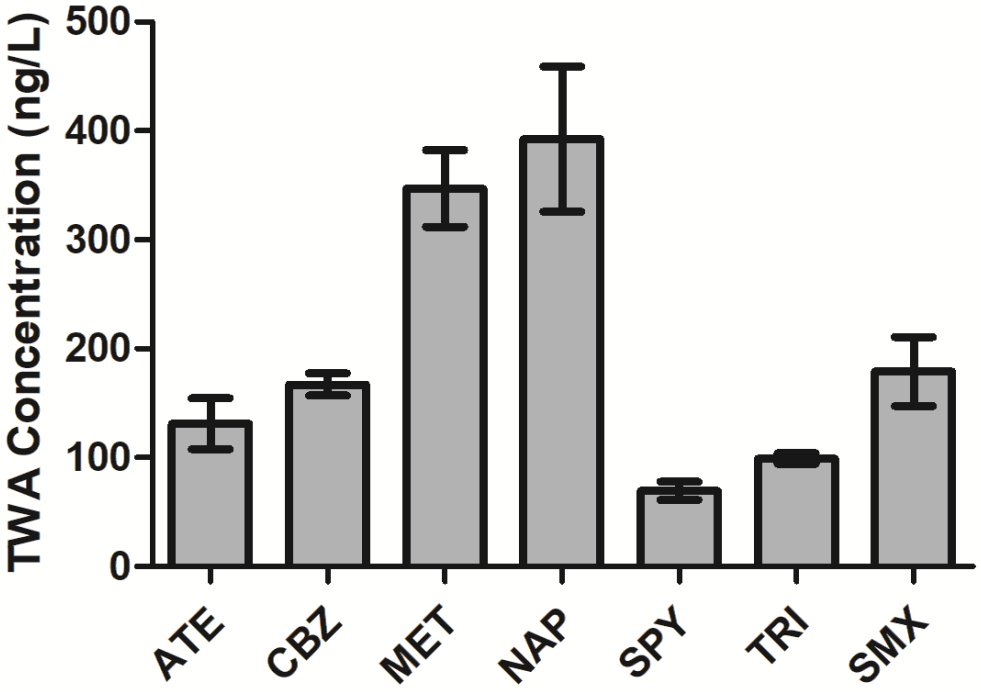

Figure 2: Concentrations of compounds detected in Iqaluit Lagoon August 2019. Mean (±SD n=3) time-weighted averages of pharmaceuticals in Iqaluit municipal wastewater lagoon: atenolol (ATE), carbamazepine (CBZ), metoprolol (MET), naproxen (NAP), sulfapyridine (SPY), trimethoprim (TRI) and sulfamethoxazole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

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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Supplemental Table S1. Initial screening results of relative abundances (per 16S rRNA) and rank of antibiotic resistance genes in wastewater and nearby soils.

		Relative abundances		Overall abundance ranking	
		Plant influent	Nearby soils	Plant influent	Nearby soils
Aminoglycoside	aac3-Via	-1.85	-1.34	21	3
Aminoglycoside	aadA_99	-1.86	-3.63	23	27
Aminoglycoside	aadA17	-1.95	-3.82	25	36
Aminoglycoside	aadA2-1	-2.36	-4.02	34	47
Aminoglycoside	aadA16	-2.67	-3.63	46	28
Aminoglycoside	aac3ia	-2.80		55	
Aminoglycoside	aadA10	-2.93	-3.90	62	42
Aminoglycoside	aadA6	-2.98	-4.05	68	49
Aminoglycoside	aadA5	-3.13	-4.08	76	51
Aminoglycoside	aph3-III	-3.29		81	
Aminoglycoside	aphA3	-3.33		85	
Aminoglycoside	aadB	-3.35	-5.09	89	104
Aminoglycoside	sat4	-3.36	-5.86	91	138
Aminoglycoside	aph(3'')-ia	-3.40	-5.65	93	132
Aminoglycoside	ant6-ia	-3.57		107	
Aminoglycoside	aadA7	-3.59	-3.29	108	19
Aminoglycoside	aac(3)-iid_iii_iif_iia_iie	-3.71	-4.16	116	58
Aminoglycoside	ant6-ib	-3.75		120	
Aminoglycoside	aac(6')-Ib	-3.82		126	
Aminoglycoside	aph3via	-3.95		130	
Aminoglycoside	Aac6-Aph2	-4.01		135	
Aminoglycoside	spcN	-4.12	-4.26	139	61
Aminoglycoside	aph6ic	-4.21	-4.13	142	57
Aminoglycoside	aph4ib	-4.27	-3.91	143	45
Aminoglycoside	aph6ia	-4.28	-4.77	144	88
Aminoglycoside	aacC2	-4.41		154	
Aminoglycoside	acc3-iva	-4.52	-4.90	158	91
Aminoglycoside	aac(6)-im	-5.11		191	
Aminoglycoside	aac(6)-ir	-5.13	-5.24	193	114
Aminoglycoside	aac(6)-ig	-5.14	-5.56	194	129
Aminoglycoside	aac(6')-II	-5.14		195	
Aminoglycoside	aph3-ib	-5.29		203	
Aminoglycoside	aph3-viia	-5.45		211	
Aminoglycoside	aacA_aphD	-5.47		213	
Aminoglycoside	aac(6)-iz	-5.52	-4.95	217	96
Aminoglycoside	aac(6)-iw	-5.78		226	
Aminoglycoside	aacA43	-5.83		229	
Aminoglycoside	apmA		-5.12		106
Aminoglycoside	aph(2')-Id				
Aminoglycoside	strB				
Aminoglycoside	aph_viii				
Aminoglycoside	ArmA				
Aminoglycoside	ant4-ib				
Aminoglycoside	aadA2-2				
Aminoglycoside	aac(6)-iic				
Aminoglycoside	aadD				
Aminoglycoside	aadA9				

Aminoglycoside	spec_aph				
Aminoglycoside	aac(6)-ij				
Aminoglycoside	aphA1				
Aminoglycoside	aac(3)-ib				
Aminoglycoside	aac(6')I1				
Aminoglycoside	aadE				
Aminoglycoside	aac(3)-id_ie				
Aminoglycoside	aph4-ia				
Aminoglycoside	aac(6')-Iy				
Aminoglycoside	str				
Aminoglycoside	aac(6)-is_iu_ix				
Aminoglycoside	strA				
Aminoglycoside	aac(3)-xa				
Aminoglycoside	aac(6)-iv_ih				
Beta lactamase	ampC	-2.53	-4.71	41	83
Beta lactamase	cphA	-2.68	-4.70	47	81
Beta lactamase	blaTEM	-2.74		51	
Beta lactamase	bl3_cpha	-3.00		72	
Beta lactamase	blaOXY-1	-3.26	-3.89	80	39
Beta lactamase	bla-ACT	-3.32	-4.01	84	46
Beta lactamase	blaMIR	-3.49	-4.94	100	95
Beta lactamase	blaOXY-2	-3.63		109	
Beta lactamase	blaVEB	-3.71	-5.34	117	117
Beta lactamase	blaCTX-M-1_3_15	-3.78	-5.15	122	108
Beta lactamase	blaMOX_blaCMY	-3.84		128	
Beta lactamase	blaSHV-11	-3.98		133	
Beta lactamase	blaFOXnew	-4.17		140	
Beta lactamase	blaLEN	-4.38		150	
Beta lactamase	beta_ccra	-4.57		160	
Beta lactamase	blaPSE	-4.59	-4.28	161	63
Beta lactamase	blaOXA10	-4.65		168	
Beta lactamase	blaCARB	-4.69	-4.66	170	80
Beta lactamase	blaPAO_PDC	-4.70		171	
Beta lactamase	cfiA	-4.77		175	
Beta lactamase	bla-L1	-4.96		181	
Beta lactamase	blaACC-1	-5.10		190	
Beta lactamase	nonmobile_blaADC	-5.27		202	
Beta lactamase	cefa_ampc	-5.29		204	
Beta lactamase	Pbp5	-5.45		212	
Beta lactamase	blaCTX-M	-5.47		214	
Beta lactamase	bl1acc	-5.53		218	
Beta lactamase	blaVIM	-5.64		221	
Beta lactamase	blaIMI	-6.04		235	
Beta lactamase	blaGES		-5.27		115
Beta lactamase	imp-marko				
Beta lactamase	KPC				
Beta lactamase	blaIMIR				
Beta lactamase	bla1				
Beta lactamase	NDM new				
Beta lactamase	bla-SME				
Beta lactamase	pbp				
Beta lactamase	blaHERA				
Beta lactamase	blaIND				

Beta lactamase	nonmobile blaBEL				
Beta lactamase	blaGOB				
Beta lactamase	blaZ				
Beta lactamase	blaOCH				
Beta lactamase	cfxA				
Beta lactamase	penA				
Beta lactamase	cepA				
Beta lactamase	blaCMY				
Beta lactamase	ampC_blaDHA				
Beta lactamase	blaPER				
Beta lactamase	blaROB				
Beta lactamase	blaSFO				
Beta lactamase	blaTLA				
Beta lactamase	mecA				
Beta lactamase	blaB-11_13_14				
fluoroquinolone	qnrS2	-1.17	-4.34	7	66
fluoroquinolone	qepA_1_2	-3.34	-3.65	87	29
fluoroquinolone	qnrB46_47_48	-3.56	-4.60	105	78
fluoroquinolone	oqxA	-3.64	-5.69	112	134
fluoroquinolone	QnrS1_S3_S5	-3.72		119	
fluoroquinolone	qnrD	-4.12		138	
fluoroquinolone	QnrB4	-4.36	-4.36	148	68
fluoroquinolone	QnrVC4_VC5_VC7	-5.51		216	
fluoroquinolone	QnrVC1_VC3_VC6				
fluoroquinolone	norA				
MDR	qacH_351	-1.56	-2.97	15	13
MDR	merA-marko	-1.63	-2.77	17	11
MDR	pcoA	-2.05	-4.25	28	60
MDR	arsA	-2.49	-2.89	40	12
MDR	cefa_qacelta	-2.64	-4.11	44	55
MDR	tcrB	-2.73	-5.48	49	124
MDR	mdth	-2.81	-5.37	56	120
MDR	copA	-2.83	-3.88	59	38
MDR	tolC	-2.94	-5.17	63	109
MDR	czcA	-2.97	-3.15	66	17
MDR	acrB	-2.98	-5.34	67	116
MDR	acrA	-3.18		78	
MDR	terW	-3.37		92	
MDR	acrR	-3.56	-5.50	104	127
MDR	mdtg	-3.67	-3.69	115	33
MDR	sugE	-3.80	-5.37	123	119
MDR	bexA_norM	-3.81		125	
MDR	marR	-4.06		137	
MDR	pbrT	-4.40	-4.56	152	76
MDR	mexB	-5.80		228	
MDR	mtrD	-5.96		231	
MDR	adeI				
MDR	cmr				
MDR	mtrE				
MDR	silE				
MDR	cadC				
MGE	IS26	-0.98	-1.83	4	5
MGE	int1	-1.23	-2.00	8	8

MGE	IS21-ISAs29	-1.24	-4.31	9	65
MGE	tnpA-7	-1.24	-3.87	10	37
MGE	IS6100	-1.29	-1.70	12	4
MGE	IS613	-1.62	-5.10	16	105
MGE	TN5403	-1.65	-4.07	18	50
MGE	Tp614	-1.74	-5.03	19	100
MGE	Tn3	-1.81	-4.46	20	72
MGE	IS200-2	-1.98	-4.11	26	54
MGE	tnpA-6	-2.14	-3.89	30	40
MGE	pBS228-IncP-1a	-2.35	-3.91	33	43
MGE	ISEcp1	-2.37	-3.50	35	25
MGE	tnpA-5	-2.46	-2.98	39	16
MGE	IS200-1	-2.75	-4.92	52	93
MGE	IncN_rep	-2.75	-4.94	53	94
MGE	trb-C	-2.88	-3.65	60	30
MGE	IS3	-2.99		69	
MGE	intI3	-3.03	-5.18	74	110
MGE	IS1247	-3.07	-3.31	75	20
MGE	IS91	-3.35		88	
MGE	IS1133	-3.44		95	
MGE	IS256	-3.44	-5.06	96	102
MGE	IncN_korA	-3.49		101	
MGE	TN5	-3.52		103	
MGE	pAKD1-IncP-1ß	-3.56	-4.04	106	48
MGE	IncP_oriT	-3.64	-3.71	113	34
MGE	IncQ_oriT	-3.75	-5.49	121	125
MGE	orf39-IS26	-4.19		141	
MGE	intI2	-4.28		145	
MGE	PAMBL-1-F_377old	-4.41		153	
MGE	IS630	-4.44	-4.77	156	87
MGE	trfa	-4.46	-2.98	157	15
MGE	orf37-IS26	-4.55		159	
MGE	IS6_257	-4.63		166	
MGE	traN	-4.72	-3.49	172	24
MGE	mobA	-4.73	-4.82	173	89
MGE	IncF_FIC	-4.92		180	
MGE	EAE_05855	-4.98		182	
MGE	IS1111	-5.43		209	
MGE	cro	-5.66	-5.95	222	141
MGE	ISCR1	-6.02		234	
MGE	IncW_trwAB		-5.93		140
MGE	IS5_IS1182				
MGE	IncI1_repI1				
MGE	IncHI2-smr0018				
MGE	IS15DI				
MGE	tra-A				
MGE	IncN_oriT				
MGE	tnpA-1				
MGE	tnpA-2				
MGE	tnpA-3				
MGE	tnpA-4				
MLSB	msr(E)	-0.82	-2.23	3	10
MLSB	erm(F)	-1.51	-1.93	14	6

MLSB	erm(Q)	-1.86	-4.27	22	62
MLSB	erm(B)	-1.92	-4.55	24	75
MLSB	mphA	-2.04	-4.12	27	56
MLSB	ere(A)	-2.40	-4.22	37	59
MLSB	mefA	-2.71	-4.09	48	52
MLSB	mdtA	-2.81		57	
MLSB	lnuC	-2.90		61	
MLSB	lnuB	-3.00	-4.91	70	92
MLSB	mef(B)	-3.00	-3.40	71	21
MLSB	erm(E)	-3.33	-4.39	86	69
MLSB	erm(O)	-3.50	-4.75	102	86
MLSB	ermX	-3.63		110	
MLSB	lmrA	-4.30	-4.52	146	73
MLSB	vatB	-4.31		147	
MLSB	erm(35)	-4.43	-5.36	155	118
MLSB	pikR2	-4.91	-5.69	179	133
MLSB	pica	-5.05	-4.41	187	70
MLSB	lnu(F)	-5.07		188	
MLSB	ermA_ermTR	-5.15		196	
MLSB	vgaA	-5.29		205	
MLSB	erm(A)	-5.58		219	
MLSB	lsa(C)	-5.70		223	
MLSB	lnuA	-5.77	-5.41	225	121
MLSB	erm(36)	-5.98		232	
MLSB	erm(S)	-5.98	-5.07	233	103
MLSB	oleC		-5.73		136
MLSB	emrB_qacA				
MLSB	ermK				
MLSB	mphB				
MLSB	carB				
MLSB	msr(D)				
MLSB	msr(A)				
MLSB	vat(E)				
MLSB	erm(D)				
MLSB	vat(A)				
MLSB	ermY				
MLSB	erm(34)				
MLSB	cfr				
MLSB	erm(G)				
MLSB	vga(A)LC				
MLSB	vgaB				
MLSB	erm(42)				
MLSB	ermT				
MLSB	msr(C)				
Multidrug	acrF	-2.96	-5.44	65	122
Multidrug	mdtE_yhiU	-3.00		73	
Multidrug	ttgB	-3.31	-3.43	83	23
Multidrug	floR	-3.36	-3.89	90	41
Multidrug	emrD	-3.45	-5.80	99	137
Multidrug	mexE	-3.63	-3.79	111	35
Multidrug	adeA	-4.38		151	
Multidrug	ttgA	-4.59		162	
Multidrug	oprD	-4.61	-3.67	164	32

Multidrug	mepA	-4.79	-4.99	177	97
Multidrug	mexA	-5.08		189	
Multidrug	multidrug resistance	-5.22		198	
Multidrug	pmrA				
Multidrug	ceoA				
other	ere(B)	-2.81	-4.58	58	77
other	catB3	-3.71	-4.70	118	82
other	Arr2	-3.97		131	
other	ARR-3	-3.98		134	
other	catB8	-4.05		136	
other	fabK	-4.64	-4.73	167	84
other	qacA_B	-5.02		185	
other	mcr-1	-5.25	-5.20	200	112
other	bacA	-5.43		207	
other	fosb				
other	mcr-2				
other	fosX				
other	catA1				
other	nimE				
other	nisB				
other	qnrA				
other	qacF_H				
Phenicols	cmlA5	-2.56	-5.12	43	107
Phenicols	qnrB-bob_resign	-3.17	-4.30	77	64
Phenicols	catQ	-3.81	-4.35	124	67
Phenicols	catA2	-3.92		129	
Phenicols	cat	-3.97		132	
Phenicols	catP	-5.04		186	
Phenicols	cmlA1				
Phenicols	cmx(A)				
Phenicols	catA3				
Phenicols	catB2				
Phenicols	catB9				
Phenicols	cat(pC221)				
Phenicols	cmlV				
Phenicols	fexA				
Phenicols	optrA				
Sulfonamide	sul1 NEW	-1.32	-1.98	13	7
Sulfonamide	dfrA1	-2.39	-5.05	36	101
Sulfonamide	dfrA12	-2.67	-5.71	45	135
Sulfonamide	folA	-4.59	-5.64	163	131
Sulfonamide	sulA_folP	-4.65		169	
Sulfonamide	sul2				
Sulfonamide	sulIII				
tetracycline	tet39	-1.27	-3.67	11	31
tetracycline	tetR	-2.13	-4.10	29	53
tetracycline	tetM	-2.44	-3.42	38	22
tetracycline	tetE	-2.74	-4.63	50	79
tetracycline	tet(32)	-2.78	-4.99	54	98
tetracycline	tetG_F	-2.95	-3.54	64	26
tetracycline	tetPA	-3.43	-3.91	94	44
tetracycline	tet44	-3.45	-5.46	98	123
tetracycline	tetD	-3.67	-4.74	114	85

tetracycline	tetT	-4.62	-5.20	165	111
tetracycline	tetbP	-4.73		174	
tetracycline	tetH	-5.73		224	
tetracycline	tetB	-5.78		227	
tetracycline	tet(36)	-5.90		230	
tetracycline	tetL				
tetracycline	tetA				
tetracycline	tetX				
tetracycline	tetC				
tetracycline	tet40				
tetracycline	tetK				
tetracycline	tetS				
tetracycline	tetJ				
tetracycline	tetU				
tetracycline	tetQ				
tetracycline	tetPB				
tetracycline	tet(38)				
tetracycline	tetW				
tetracycline	tetO				
trimethoprim	dfra14	-2.29	-5.62	31	130
trimethoprim	dfra21	-2.34	-4.85	32	90
trimethoprim	dfrA15	-3.82		127	
trimethoprim	dfrA22	-4.77		176	
trimethoprim	dfrA10	-4.89	-5.91	178	139
trimethoprim	dfrA18				
trimethoprim	dfrBmulti				
trimethoprim	dfra17				
trimethoprim	dfra7				
trimethoprim	dfrA25				
trimethoprim	dfrA27				
trimethoprim	dfra5				
trimethoprim	dfrAB4				
trimethoprim	dfrA8				
trimethoprim	dfrC				
trimethoprim	dfrG				
trimethoprim	dfrK				
Vancomycin	vanTG	-4.38		149	
Vancomycin	vanA	-4.99	-5.21	183	113
Vancomycin	vanHB	-5.19		197	
Vancomycin	vanHD	-5.23		199	
Vancomycin	vanRD	-5.42		206	
Vancomycin	vanRC4	-5.43		208	
Vancomycin	vanD	-5.45		210	
Vancomycin	vanRB	-5.50		215	
Vancomycin	VanB	-5.63	-5.00	220	99
Vancomycin	vanYD		-5.52		128
Vancomycin	vanSB				
Vancomycin	vanRA				
Vancomycin	vanG				
Vancomycin	vanSA				
Vancomycin	vanSC				
Vancomycin	vanC2_vanC3				
Vancomycin	vanXA				

Vancomycin	vanWB
Vancomycin	vanTE
Vancomycin	vanXB
Vancomycin	vanTC
Vancomycin	vanC
Vancomycin	vanYB
Vancomycin	vanRC

Supplemental Table S2. Target genes and PCR primers.

Resistance type [a]	Gene	Forward (F)/ Reverse (R) primers	Associated antibiotics (examples) [b]	Resistance mechanism	Host bacteria (examples) [b]
Aminoglycoside	*aphA*3	F: AAAAGCCCGAAGAGGAACTTG R: CATCTTTCACAAAGATGTTGCTGTCT	Kanamycin	Antibiotic inactivation	*Escherichia coli, Klebsiella pneumoniae, Moraxella, Pseudomonas, P. aeruginosa*
Beta lactamase	*ampC*	F: TGGCGTATCGGGTCAATGT R: CTCCACGGGCCAGTTGAG	Cephalosporinases and penicillins e.g. amoxicillin, piperacillin, cefoxitin, cephalexin, cefazolin and ceftriaxone	Antibiotic inactivation	*Escherichia coli, Shigella flexneri, Shigella sonnei*
	cphaA	F: GCGAGCTGCACAAGCTGAT R: CGGCCCAGTCGCTCTTC	Cephalosporinases and penicillins e.g. amoxicillin, piperacillin, cefoxitin, cephalexin, cefazolin and ceftriaxone	Antibiotic inactivation	*Aeromonas hydrophilia*
	bla_{TEM}	F: AGCATCTTACGGATGGCATGA R: TCCTCCGATCGTTGTCAGAAGT	Cephalosporinases and penicillins e.g. amoxicillin, piperacillin, cefoxitin, cephalexin, cefazolin and ceftriaxone	TEM beta-lactamase	Frequently found in *E. coli* and *Klebsiella pneumoniae.*
Quinolone	*qnr*A	F: AGGATTTCTCACGCCAGGATT R: CCGCTTTCAATGAAACTGCAA	Low-level resistance to quinolone	Antibiotic target protection	Many Gram-negative bacteria
Multidrug	*qacH*	F: CATCGTGCTTGTGGCAGCTA R: TGAACGCCCAGAAGTCTAGTTTT	Multiple antibiotics	Antibiotic efflux pump	*Pseudomonas aeruginosa*
	acrA	F: CAACGATCGGACGGGTTTC R: TGGCGATGCCACCGTACT	Multiple antibiotics	Antibiotic efflux pump	-
Mobile genetic element [c]	*int1*	F: GGCTTCGTGATGCCTGCTT R: CATTCCTGGCCGTGGTTCT	Class 1 integron gene	n/a	Particularly in Gram-negative bacteria
MLSB	*ermB*	F: TAAAGGGCATTTAACGACGAAACT R: TTTATACCTCTGTTTGTTAGGGAATTGAA	Macrolides e.g. erythromycin and Azithromycin	Antibiotic target alteration	*Enterococcus faecium*
Sulfonamide [d]	*sul1*	F: CGCACCGGAAACATCGCTGCAC R: TGAAGTTCCGCCGCAAGGCTCG	Sulfonamides	Antibiotic target replacement	Gram-negative pathogenic bacteria, e.g. *E. coli* and *Salmonella*
	dfrA1	F: GGAATGGCCCTGATATTCCA R: AGTCTTGCGTCCAACCAACAG	Trimethoprim	Antibiotic target replacement	Gram-negative pathogenic bacteria, e.g. *E. coli* and *Salmonella enterica*
Tetracycline	*tet39*	F: CTCCTTCTCTATTGTGGCTA R: CACTAATACCTCTGGACATCA	Tetracycline, doxycycline and minocycline.	Antibiotic efflux pump	*Acinetobacter baumannii, Ac. junii, Ac. nosocomialis,*

					Klebsiella oxytoca
Total bacteria [e]	*16S-rRNA*	F: AYTGGGYDTAAAGNG R: TACNVGGGTATCTAATCC, TACCRGGGTHTCTAATCC, TACCAGAGTATCTAATTC, CTACDSRGGTMTCTAATC			
Total fungus [f]	*18S-rRNA*	F: AAGTCTGGTGCCAGCAGCCG R: CCCGTGTTGAGTCAAATTAAGC			

a) Zhu, et al. (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences-USA* 110(9) 3435-3440. https://doi.org/10.1073/pnas.1222743110
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c) Luo, et al. (2010) Trends in antibiotic resistance genes occurrence in the Haihe river, China. *Environmental Science & Technology* 44(19): 72220-5. https://doi.org/10.1021/es100233w
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Supplemental Table S3. Key taxonomy of microorganisms commonly associated with ARGs in wastewater influent and nearby soil, not exposed to wastewater.

Guilds	Influent	Nearby soil
Bacteroidetes	32.88%	15.50%
Enterococcus	0.28%	0.00%
A. baumannii	0.05%	0.00%
K. pneumoniae	0.04%	0.00%
Staphylococcus aureus (MRSA)	0.00%	0.00%
P. aeruginosa	0.00%	0.00%
Firmicutes	25.98%	16.32%
Acinetobacter	9.71%	0.06%
Pseudomonas	8.15%	0.85%
Xanthobacter	0.06%	0.11%
Enterobacter	0.00%	0.00%
Total:	77.15%	32.84%

Supplemental Table S4. The number of genes, their relative richness (%), and representative genes (used in this study) from each class of ARGs.

Gene class	Plant influent Detects		Nearby soil Detects		Representative gene(s)
Aminoglycoside	14	100%	11	79	*aph*A3
Beta lactam	29	54%	10	19%	*amp*C, *cph*A, bla_{TEM}
Fluoroquinolone	8	80%	5	50%	*qnr*A
Multidrug resistance	33	83%	22	55%	*qac*H, *acr*A
Mobile genetic element	42	79%	29	55%	
MLSB	27	59%	18	39%	*erm*(B)
Other	9	53%	4	24%	
Phenicol	6	40%	3	20%	
Sulfonamide	5	71%	4	57%	*sul*1, *dfr*A1
Tetracyclines	14	50%	10	36%	*tet*39
Trimethoprim	5	29%	3	18%	*dfrA*
Vancomycin	9	38%	3	13%	

Supplemental Table S5. Comparison of influent and effluent gene concentrations in the wastewater treatment plant.

	Influent *Relative abundance (log(gene/16SrRNA)*	**Effluent** *Mean relative abundance (log(gene/16SrRNA), n=3, standard deviation in brackets.*	**t-test Statistics**	
			t_2	*p*
*acr*A	-3.18	-2.96 (0.20)	1.86	0.20
*amp*C	-2.53	-4.09 (0.94)	-2.87	0.10
aphA	-3.33	-3.17 (0.36)	0.76	0.53
bla_{TEM}	-2.74	-2.47 (0.47)	0.97	0.44
*cph*A	-2.68	-2.94 (0.55)	-0.82	0.59
*dfr*A	-2.39	-2.98 (0.16)	-6.23	0.03
erm(B)	-1.92	-1.76 (0.27)	1.06	0.40
*qac*H	-1.56	-2.58 (0.32)	-5.46	0.03
*qnr*A	-1.17	-3.79 (0.37)	-10.06	0.06
*sul*1	-1.32	-2.29 (0.23)	-7.21	0.02
tet(39)	-1.27	-1.48 (0.64)	-0.56	0.63

Supplemental Table S6. A Kruskal-Wallis test was applied to determine whether there was a statistically significant difference in ARGs present in the *M. truncata* clams at the six sampling locations. A significant level of $p<0.05$ was set; *d.f.* = 5.

ARGs	***H* value**	***p* value**
Σ ARGs	4.2	0.51
acrA	2.2	0.82
ampC	3.4	0.63
aph	4.9	0.42
bla_{TEM}	4.5	0.48
cph	6.7	0.24
dfrA	2.6	0.76
ermB	3.5	0.48
intI1	3.7	0.59
qacH	6.7	0.24
qnr	6.5	0.26
sul1	10.6	0.06
sul2	10.3	0.07
sul3	4.0	0.55
tet39	3.7	0.60