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Antibiotic resistance genes, antibiotic residues, and microplastics in influent and effluent wastewater from treatment plants in Norway, Iceland, and Finland

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ABSTRACT

Monitoring antimicrobial resistance genes (ARGs) in wastewater influents (pre-treatment) and effluents (post-treatment) provides insights into community-level circulation, potential amplification during treatment, and risks associated with gene release into surface waters. Pollutants such as antibiotic residues and microplastics (MPs) may influence ARG dynamics, highlighting the need to assess their dynamics across wastewater environments. In this study, we analyzed ARGs and bacterial communities using Oxford Nanopore (ONP) metagenomics and qPCR in wastewater samples from Mekjarvik (Norway), Reykjavik (Iceland), and Mariehamn (Åland, Finland). Antibiotic residues were quantified via high-performance liquid chromatography (HPLC), and MPs were characterized using micro-fourier transform infrared spectroscopy (μ-FTIR) in Mekjarvik and Reykjavik. Metagenomic analysis identified 193 unique ARGs, with the highest average (±SD) in Reykjavik (66.3 ± 4.1), followed by Mekjarvik (61.3 ± 14.1) and Mariehamn (18.0 ± 2.2). ONP sequencing revealed that many ARGs were plasmid-associated, co-occurring with metal stress genes. Common plasmids were *Col440I*, *IncQ2*, and *ColRNAI*. Mercury-related genes dominated metal stress genes (64.9 %), followed by multimetal (23.7 %) and copper (6.4 %). Of 45 antibiotics screened, only sulfamethoxazole and sulfapyridine were consistently detected. Polyethylene (~60 %) was the dominant MP type; Reykjavik influent had the highest MP load (8200 MP/m^3). While treatment reduced ARGs, antibiotic residues, and larger MPs, it was less effective against fine particles and key ARGs, including carbapenemase- and ESBL-associated genes. Clinically relevant ARGs and potential pathogens (e.g., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*) persisted in effluents, highlighting risks to downstream ecosystems. These findings underscore the need for regular monitoring of both influents and effluents to assess treatment performance and safeguard environmental health.

1. Introduction

Antimicrobial resistance (AMR) is a global public health threat, causing millions of infections and deaths annually (Murray et al., 2022; Prestinaci et al., 2015). Wastewater surveillance (WS), i.e. monitoring wastewater influents, is an emerging tool for nearly real-time assessment of AMR at the population level (Hendriksen et al., 2019; Karkman et al., 2020; Tiwari et al., 2022a). Municipal wastewater captures pathogens from diverse sources (households, healthcare), reflecting population-wide excretion through feces, urine, saliva, nasal secretions,

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and skin, regardless of clinical symptoms (Karkman et al., 2020; Tiwari et al., 2023a). Unlike clinical surveillance, which focuses on symptomatic cases, WS also captures asymptomatic carriers and ARGs from commensal gut bacteria (Karkman et al., 2016; Trosvik et al., 2024). Thus, WS can serve as a valuable tool for assessing the circulation of AMR in communities and may also provide indirect insights into antibiotic usage patterns.

The recast of EU's Urban Wastewater Treatment Directive (UWWTD) urges respective countries to regularly montor AMR in both influent and effluent to track WS and improve effluent quality (EC, 2024). Conventional qPCR is a commonly used method for monitoring ARGs, but it is limited to detecting only a few targets per run, making large-scale monitoring time-consuming (Sarekoski et al., 2024; Tiwari et al., 2024). High-throughput qPCR (HT-qPCR) overcomes this by analyzing hundreds of targets simultaneously making it suitable for WS (Karkman et al., 2016; Sarekoski et al., 2024). However, these conventional qPCR methods and also HT-qPCR methods require fixed labs and stable power, delaying responses due to sample transport and preprocessing. Biomeme's portable, battery-powered qPCR device enables on-site testing at WWTPs or remote locations, delivering results within hours and integrating with cloud platforms for real-time data sharing (Frimpong et al., 2023; Zowawi et al., 2021). Metagenomics, requiring no prior knowledge of primers or probes, is valuable for tracking emerging ARGs (Hendriksen et al., 2019; Trosvik et al., 2024). Oxford Nanopore (ONP) sequencing has gained traction for its portability, long-read capabilities, rapid turnaround, and low infrastructure needs, making it suitable for real-time pathogen detection and pandemic response (Taxt et al., 2020). The use of real-time or onsite monitoring tools helps avoid the challenge of degradation of sample quality due to transportation to a centralized laboratory and saves expenses resulting from the use of a conventional costly microbiology laboratory.

Although wastewater treatment plants (WWTPs) are primarily designed to remove nutrients and pathogens (EC, 2024), they also play a role in limiting the environmental spread of AMR (Karkman et al., 2016). However, biological processes—typically activated sludge—foster microbial interactions and create ideal conditions for horizontal gene transfer (HGT) of mobile genetic elements (MGEs) such as plasmids and transposons, making WWTPs hotspots of proliferation of ARGs (Gao et al., 2022; Karkman et al., 2018). The presence of antibiotics, heavy metals, and detergents in wastewater adds selective pressure, further driving HGT and resistance development (Karkman et al., 2018).

DNA damage caused by antibiotics, heavy metals, and other stress factors can trigger the bacterial SOS response, involving specific stress-response genes, which are commonly studied using the SOS *E. coli* assay. The SOS *E. coli* assay is a bioanalytical tool used to analyze genotoxic pressure in environmental bacteria such as those in wastewater (Crane et al., 2021; Serment-Guerrero et al., 2020). It employes an *E. coli* strain carrying a reporter gene (typically *lacZ*) fused to an SOS response gene (e.g., *sulA*). The resulting β-galactosidase activity serves as a proxy for genotoxic stress and helps assess ecological risks associated with sub-lethal DNA damage, including enhanced HGT, mutagenesis, and antibiotic resistance (Crane et al., 2021; Serment-Guerrero et al., 2020).

Wastewater also carries other emerging pollutants such as microplastics (MPs), from various antropogenics sources, possibly affecting ARG dynamics (Feng et al., 2023; Sivalingam et al., 2025). MPs can host bacterial communities, including resistant pathogens, in biofilms within the so-called "plastisphere" (Feng et al., 2023; Mazumder et al., 2023).

Water is a central component of the One Health concept, as it links humans, animals, and the environment (Prata et al., 2022). The release of clinically relevant AMR and ARGs together with various emerging pollutants into the environment may pose significant health risks to humans and animals, underscoring the need for effective wastewater treatment and reducing pollutant loads into recipient waters (Karkman et al., 2018, 2020; Uluseker et al., 2021). The recast of the EU's UWWTD encourages advanced treatment technologies to reduce emerging pollutants such as MPs and microbial contaminants, including ARGs, improving effluent quality (EC, 2024).

This study monitored ARGs via ONP and qPCR in the influent and effluent of three WWTPs: Mekjarvik (Norway), Reykjavik (Iceland), and Mariehamn (Finland). Analysis of antibiotic residues and MPs was conducted in Mekjarvik and Reykjavik. The study had the following objectives: (a) to compare the composition of the influent resistome in three Nordic cities; (b) to assess ARG removal efficiencies across resistance classes and WWTPs in the three cities; (c) to compare resistome profiles obtained through metagenomic analysis with results from qPCR-based monitoring; (d) to examine the association between the prevalence of antibiotic residues and the presence of corresponding ARGs in wastewater; and (e) to evaluate how different treatment processes influence the removal efficiency of MPs. By addressing these objectives, the study advances our understanding of AMR in wastewater systems, supports the implementation of Article 17 of the recast EU UWWTD (EC, 2024), and contributes to the development of a harmonized ARG WS framework across the Nordic region.

2. Methodology

2.1. Site description, and sample collection

Wastewater samples were collected from three WWTPs — Mekjarvik (Stavanger, Norway), Klettagarðar Reykjavik (Iceland), and Mariehamn (Åland, Finland) — representing diverse Nordic regions. Mekjarvik WWTP (Nord-Jæren), among Norway's largest, serves ~300,000 people across Randaberg, Stavanger, Sola, Sandnes, and Gjesdal (Basiry et al., 2024). Operating since 1991, it features primary and secondary treatment, effectively removing phosphorus via chemical precipitation (Basiry et al., 2024). The Klettagarður WWTP in Reykjavik, Iceland's largest, has operated since 2002, serving ~160,000 residents and key industrial areas (Magnusson et al., 2016). It uses only primary treatment with a 3 mm filter before discharging effluent 5500 m offshore at a 30-m depth (Magnusson et al., 2016). Mariehamn WWTP in Åland, serves ~21,000 people across Mariehamn, Jomala, Lemland, Hammarland, Finström, Saltvik, and Sund (Tiwari et al., 2022b). It features primary and secondary treatment, meeting EU UWWTD 1991 standards (EEC, 1991).

For monitoring various parameters, wastewater influent and effluent samples were collected (April 2023–April 2024) (Table S1), following standardized sample collection, preservation, and shipment protocols (Sarekoski et al., 2024). Reykjavik samples were stored at below −75 °C and shipped to the Norwegian Research Centre's (NORCE) chemical-microbiology lab in Stavanger, Norway for analysis. Mekjarvik WWTP, ~200 m from NORCE, facilitated rapid processing. Mariehamn samples were sent in cool boxes to the water microbiology laboratory of the Finnish Institute for Health and Welfare (THL) in Kuopio, Finland, and analyzed within 24 h (Tiwari et al., 2022b).

2.2. Bacterial community analysis and ARG monitoring

2.2.1. Wastewater concentration and DNA extraction

Triplicate aliquots of each sample of influent (35 mL) and effluent (240 mL) were filtered using a Sterivex-GP Pressure Filter Unit (0.22 μm pore size, polyethersulfone membrane, Millipore) for bacterial biomass concentration, as described earlier (Krolicka and Cook, 2025). Aseptically, filters were torn into ~1 cm^2 pieces with sterile forceps and placed in extraction tubes with 1.5 mL of 3M guanidine thiocyanate lysis solution (pH 8.9) and incubated at 85 °C for 10 min in a ThermoMixer C at 500 rpm. The lysate was filtered through a 0.2 μm syringe filter (25 mm cellulose acetate, VWR cat no. 514–1273) into a Falcon tube to minimize foam (Krolicka and Cook, 2025). The lysate was transferred to the M1 Sample Prep Cartridge Kit (Biomeme #3000536R). DNA purification was performed according to the cartridge manual with minor modifications. To increase the total concentration and purity of DNA, (1) 650

μL of elution buffer from the last section of the cartridge was removed, DNA was eluted using approximately 150 μL of elution buffer (2) Prior to the air-drying procedure after the wash step, the syringe and column were shaken vigorously to remove any remaining alcohol used during the washing step (Krolicka and Cook, 2025).

For HT-qPCR analysis, wastewater samples were concentrated using previously described methods, with minor adjustments (Hokajärvi et al., 2021). Influent (70 mL) and effluent (240 mL) samples were pre-centrifuged at 4654 x g for 30 min without a break. The resulting pellets were re-suspended in approximately 900 μL (influent) and 1100 μL (effluent) of supernatant, while the remaining supernatant was discarded. The pellets were stored at −80 °C and then extracted with the Chemagic-360D instrument (Hokajärvi et al., 2021).

2.2.2. Oxford Nanopore metagenomics

Samples collected over three sampling events from Mekjarvik (May–July), Reykjavik (May–July), and Mariehamn (May, August, September) were used for ONP metagenomic sequencing. Composite samples were prepared by pooling triplicates with an equal DNA mass. Multiple displacement amplification (MDA) to enrich the whole metagenome to ensure a sufficient amount of total DNA, was performed using high fidelity EquiPhi29 DNA Polymerase and 5′-phosphorylated random hexamers (ThermoFisher# A65393). The reaction was performed according to the manufacture's instructions. The DNA library was constructed using the Rapid Barcoding Kit (SQK-RBK114-96) and loaded onto an R10.4.1 flow cell for sequencing on a MinION device. Raw sequencing data were processed into nucleotide sequences and filtered for low-quality reads using MinKNOW software in high-accuracy mode. The NanoFilt tool was used for preprocessing, including the removal of reads shorter than 1000 base pairs, reads with an average quality score lower than 9, and the removal of the first 50 bases from each read, to ensure data quality. Human reads were identified and discarded using a combination of the Minimap2 and Kraken2 suite of tools (H. Li, 2018; Wood et al., 2019). Reads mapped to the human genome reference GRCh38 by Minimap2 were discarded, followed by running the extract_kraken_reads.py script from KrakenTools on the initial Kraken2 results to filter out the remaining reads belonging to taxid 9443 (primates) (H. Li, 2018; Wood et al., 2019). Taxonomic classification of the metagenome was performed using Kraken2 with the PlusPF-8 database (Wood et al., 2019; Wood and Salzberg, 2014). Microbial abundances were standardized as a percentage of total genera per sample.

The ABRicate tool, integrated into the Epi2me pipeline, along with the Resfinder database, was employed to identify ARGs (Zankari et al., 2012). Results were presented with and without normalization using gigabase pair sequences. As the "read count" provided by the Epi2me Labs output acts as the normalization factor, the read counts per ARG were directly used as abundance values for downstream analyses, eliminating the need for additional normalization methods, such as ARG copies per gene, ARG copies per 16S rRNA gene, or relative abundances. The ABRicate tool, together with the PlasmidFinder database, was used to identify plasmids (Carattoli et al., 2014). Through PlasmidFinder, instances where the same read IDs matched ARGs detected by Resfinder were identified. Such co-detection strongly indicates that the ARGs are probably plasmid-borne. For additional analyses, custom databases were incorporated into ABRicate. Metal resistance genes were identified using the Megares v3.0 database, while MGEs were detected using databases such as ISfinder (Siguier, 2006). Metagenomes are publicly available through the National Center for Biotechnology Information's BioProject ID PRJNA1233187.

2.2.3. Biomeme qPCR analysis

Triplicate samples were collected from Mekjarvik (each month, May 2023–January 2024), and Reykjavik (each month, April 2023–March 2024, except for November 2023) (Table S1), to monitor ARGs using the Franklin™ Real-Time PCR Thermocycler (Biomeme). Composite samples for qPCR were prepared by pooling triplicates with equal DNA mass, using the same approach as for ONP analysis. If ONP was performed, the same aliquots used for qPCR analysis. Standard curves for the analyzed genes (Table S2) were established using gBlocks Gene Fragments (Integrated DNA Technologies, IDT, Coralville, IA, USA). All reactions were conducted using the standard Biomeme LyoDNA program, with an annealing temperature of 60 °C, a primer concentration of 500 nM, and 5 μL of DNA. Samples were analyzed using LyoGreen™ 2.0 Master Mix (Biomeme). Positive controls constituted reactions performed using Mastemix LyoDNA™ 2.0 + IPC Master Mix to identify any potential inhibition effects. The cycle threshold (CT) was set to 35 for ensuring a high detection rate, and the mean value of the replicates was used for analysis, if detected at least in two replicates.

2.2.4. High-throughput qPCR

Wastewater samples were collected four times (April, May, August, September) from Mariehamn (Åland, Finland) (Table S1) to monitor ARGs using the SmartChip Real-Time PCR system (Takara Bio, Mountain View, CA, USA), analyzed by Resistomap Oy (Helsinki, Finland). The nucleic acid concentration from each sample was diluted to 10 ng/μl with nuclease-free water and 30 μl of each sample was shipped in cool boxes with ice within 24 h. The samples were fitted on 48 sample-based chips, together with a negative control, one NTC, and one positive control (Majlander et al., 2021; Sarekoski et al., 2024). Reagents and qPCR cycling conditions were done as as reported earlier (Sarekoski et al., 2024). To minimize the possibility of false-positive or false-negative results, each sample was run in three replicates, and amplification in at least two out of the three replicates was considered positive (Sarekoski et al., 2024). As recommended by the developer, the CT-value was set to 27 and the mean value of the replicates was used for analysis.

Thirty-six assays (31 ARGs, five taxonomic) targeting 21 clinically relevant ARGs and five taxonomic markers were used in the SmartChip qPCR system (Table S3). ARGs included those conferring resistance to wide range of β-lactams (*bla*$_{CMY}$, *bla*$_{MOX}$, *bla*$_{DHA}$, *bla*$_{ACT}$, *bla*$_{OXA10}$, *bla*$_{VEB}$, *bla*$_{CTX-M}$, *bla*$_{GES}$, *bla*$_{OXA58}$, *bla*$_{OXA48}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{OXA23}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{IMI}$, *bla*$_{SME}$), vancomycin (*vanA, vanB*), tetracycline (tetO_1), and methicillin (*mecA*). Taxonomic assays targeted crAssphage, *A. baumannii, K. pneumoniae, Staphylococci* spp. and the total bacterial 16S rRNA gene. The *crAss56* assay served as a positive control, and the 16S rRNA gene was used to normalize CT values for each ARG, determining their relative abundance ($2^{-\Delta CT}$, where ΔCT = CT_targeted gene – CT_16S rRNA gene) (Majlander et al., 2021; Sarekoski et al., 2024).

2.3. Antibiotic residue monitoring

2.3.1. Sample preparation and suspect screening analysis

Antibiotic residues were analyzed in influent and effluent from Mekjarvik and Reykjavik (Table S1). Duplicate samples (100 mL influent, 150 mL effluent) were spiked with a mixture of deuterated standards, including azithromycin-d_3, ofloxacin-d_8, and sulfamethoxazole-d_4 (Toronto Research Chemicals, Canada), at a final concentration of 300 ng/L. Samples were then processed using solid-phase extraction (SPE), as previously described (Gros et al., 2012).

Forty-five antibiotics (Table S4) were screened using a modified suspect screening method in a liquid chromatography system coupled to an Orbitrap Exploris 240 high-resolution mass spectrometer (Jaén-Gil et al., 2019) (Thermo Fisher Scientific). Chromatographic separation was performed on a ZORBAX Eclipse Plus C18 (150 mm × 2.1 mm, 3.5 μm; Agilent Technologies) at 0.350 mL/min for a 20-min run. The mobile phases selected were: (A) water/methanol (98:2, v/v) with 5 mM ammonium formate and 0.01 % formic acid and (B) methanol/water (98:2, v/v) with the same additives. The gradient started at 95 % A (1 min), decreased to 5 % A (11 min), held (2 min), returned to 95 % A (1 min), and held (5 min).

The Orbitrap Exploris 240, equipped with a heated electrospray ionization source (HESI-II), operated in positive mode (Jaén-Gil et al.,

2019). Samples were acquired in data dependent acquisition (DDA) mode with full-scan (*m/z* 100 to 1000) at 60,000 FWHM, followed by a second scan (*m/z* 50 to 1000) at 30,000 FWHM. The top five most intense ions from the inclusion list of tentative antibiotic exact masses (Table S5) were selected and fragmented using high-collision dissociation (HCD) at 40 eV with a 2 Da isolation width. The system was controlled via Thermo Scientific Xcalibur (v4.4.16.14).

2.3.2. Computational data treatment and semi-quantification

Compound Discoverer 3.3 SP2 (Thermo Fisher Scientific) identified suspected antibiotic residues in recorded files. Automatic data processing selected MS spectra (*m/z* 100–1000, 1–14 min) with a peak intensity threshold at 1.5 signal-to-noise ratio (S/N). Antibiotics from the inclusion list (Table S4) were set as expected compounds, detected using a ±5 ppm mass tolerance, a minimum peak intensity of 1000 counts, and a 1.5 chromatographic S/N threshold. Compounds were grouped at a 0.3 min retention time tolerance and ±5 ppm mass tolerance. Fragment spectra were analyzed using FISh scoring (S/N $\geq$ 3, ±5 ppm mass tolerance error). Identifications were manually reviewed and compared with internal standards (when available). Semi-quantification used the most structurally and chromatographically similar internal standard as previously described (Čelić et al., 2021).

2.4. Microplastic analysis

2.4.1. Samples collection

For MPs monitoring, five technical replicates of influent and effluent were collected with a autosampler on a single day from Mekjarvik (December 2023) and Reykjavik (October 2023) WWTPs (Table S1). Water samples were pumped into a custom-designed device with removable laser-cut stainless-steel filters (300 μm and 10 μm) using a peristaltic Oberdorfer bronze rotary head pump. Each sampling event lasted 0.5–2 h, depending on suspended material, with a maximum flow rate of 8–10 L/min. The filtered water volume was recorded, and filters were removed, placed in a pre-cleaned glass Petri jar, and stored at +4 °C in the dark for analysis. Before the sampling, the sampler was thoroughly cleaned by rinsing with filtered deionized water, washing with a lab-grade detergent, rinsing again with filtered water, and then kept covered to prevent contamination.

2.4.2. Sample preparation and analysis

The material trapped on the filter was dried at 60 °C for 72 h. To remove natural organic and inorganic matter without damaging polymer structures, samples underwent enzymatic digestion followed by iron-catalyzed oxidation (Gomiero et al., 2024). Technical-grade enzymes (Sigma Aldrich, Germany) were filtered (0.2 μm) before use. Purification began with 50 mL of sodium dodecyl sulfate (SDS, 5 % w/v) per gram of dry weight (DW) sample, incubated at 50 °C for 6 h in a pre-cleaned 2L crystallization dish. SDS-incubated samples were vacuum-filtered on a pre-muffled 47 mm, 10 μm mesh filter. The digestates were vacuumed off, while the remaining material trapped on the stainless-steel filter was rinsed with 50 mL of GF/A (1.2 μm) filtered ultrapure water and the filter placed on a pre-cleaned 50 mL beaker.

Vicozyme and cellulase mixture and protease enzymes were sequentially used following Gomiero et al. (2024), modified by replacing the glass crucible filter units with a stainless-steel filter introduced in this work (Gomiero et al., 2024). The residual filter-trapped organic material after multi enzymatic incubations was further resuspended using ultrasonication and oxidized using an iron (II)-catalyzed hydrogen peroxide (H_2O_2) reaction (Fenton). H_2O_2 was added to a final concentration of 250 g/L, along with iron (II), 2.5 g/L (Simon et al., 2018). The filter in the beaker was kept in a water bath to maintain a temperature between 30 and 40 °C. The oxidized material was vacuumed off, and the residual iron catalyzer was washed from the stainless-steel filter surface by flushing with 100 mL of 0.5 M HCl solution, followed by 50 mL of Milli-Q water. The obtained sample was transferred to a pre-cleaned separator funnel, and MPs particles were density-separated from the residual material using a $ZnCl_2$ ($\rho = 1.75$ g/cm^3) solution for 10 days. Floating particles were filtered and resuspended in a 5 mL ethanol/-water (50:50) solution, and three 0.5 mL aliquots were deposited on a 13 mm Ø Anodisk filter for μ-FTIR scanning by Nicolet iN 10 MX (Thermofisher, Germany). The entire surface of each Anodisk filter was scanned. The scan images and the associated IF spectra were recorded and compared with the polymer database spectra using "SiMPLE - Systematic Identification of MicroPlastics in the Environment" software (Primpke et al., 2020). Each MP was detected and all research scores in % were noted but only polymers matching with a threshold greater than 85 % to the reference spectra present in the SiMPLE database were considered. Each identified particle will be, characterized by the software for its major, minor, and Ferret dimension as well as the estimated volume and total weight. Three technical replicates were run for each sample; measurements were performed in reflection mode.

2.5. SOS-*E. coli* analyses

SOS-*E. coli* analysis was performed on wastewater influent and effluent samples from Mekjarvik and Reykjavik (Table S1). The assay evaluates genotoxicity and the stress response of *E coli,* a cellular mechanism triggered by DNA damage, including repair proteins and stress-related genes. *E. coli* expressing the *recA*-GFP reporter system (Horizon Discovery, PEC3876-202384786) was cultured overnight in Luria-Bertani (LB) medium supplemented with 25 μg/mL kanamycin (Andersson et al., 2022). The culture was diluted in LB and grown to an OD600 of ~0.4, then further diluted to OD600 0.1. Water samples were mixed with the bacteria at a 1:1 (v/v) ratio and incubated at 37 °C with shaking at 50 rpm for 1, 2, and 4 h (Andersson et al., 2022). Fluorescence was measured using a Victor reader (485/530 nm). All experiments were performed in biological triplicates and technical quadruplicates.

2.6. Statistical analysis

Differences in ARG abundance and SOS-*E. coli* fluorescence were analyzed using the Mann-Whitney *U*-test in SPSS to assess spatial and influent/effluent variations. To compare resistome profiles across samples, ARG abundances were standardized as a percentage of total ARGs per sample, and a Bray-Curtis resemblance matrix was generated in Primer-E (version 6.1.13). Non-metric multidimensional scaling (NMDS), and permutational multivariate analysis of variance (PERMANOVA) were used to analyze beta diversity, assessing differences in microbial communities and antimicrobial resistance genes (Morris et al., 2014; Tiwari et al., 2021). NMDS visualized community variation using Bray-Curtis metrics, and PERMANOVA tested statistical significance among three treatment plants, and influent vs. effluent (Morris et al., 2014). ARGs were considered attenuated if their read counts were $\geq$2.5 times higher in the influent than the effluent or exceeded three in the influent but were undetected in the effluent. Conversely, ARGs were classified as amplified if their read counts were $\geq$2.5 times higher in the effluent or exceeded three in the effluent but were undetected in the influent.

3. Results

3.1. Microbial diversity revealed by Oxford Nanopore sequencing

Microbial reads and taxonomic diversity (genera, species, and classes) for each sample of influent and effluent from three WWTPs are shown in Table 1. A total of 1,466,295 microbial reads were recorded, 98.8 % of which were bacterial. Mekjarvik samples accounted for over half of the total reads, while Mariehamn contributed only about 10 % (Table 1).

The beta diversity plot shows significant differences in microbial communities across the three WWTPs (NMDS, PERMANOVA $R^2 = 0.49$,

Table 1
Summary of microbial reads and total number of microbial and bacterial species, genera and classes identified in the samples. Percentage for the total number of microbial and bacterial reads are calculated out of the grand total of reads in all studied samples.

WWTP name	Sample name	Total number of microbial reads	Bacterial reads	Total number of microbial/ bacterial species	Total number of microbial/ bacterial genera	Total number of microbial/ bacterial classes
Mekjarvik (Norway)	Influent_May	182,790 (12.5 %)	182,324 (12.6 %)	3598/3398	1224/1086	107/66
	Influent_June	104,447 (7.1 %)	104,203 (7.2 %)	2488/2381	917/836	91/56
	Influent_July	101,624 (6.9 %)	101,378 (7.0 %)	2629/2506	955/865	90/58
	Influent Total	**388,861 (26.5 %)**	**387,905 (26.8 %)**	**5094/4812**	**1567/1390**	**124/77**
	Effluent_May	152,373 (10.4 %)	144,481 (10.0 %)	4787/4510	1469/1302	112/68
	Effluent_June	68,664 (4.7 %)	64,292 (4.4 %)	2940/2791	1097/983	101/60
	Effluent_July	175,124 (11.9 %)	174,752 (12.1 %)	3231/3080	1084/982	91/55
	Effluent Total	**396,161 (27.0 %)**	**383,525 (26.5 %)**	**6126/5749**	**1744/1535**	**123/75**
Reykjavik (Iceland)	Influent_May	89,744 (6.1 %)	89,553 (6.2 %)	2539/2449	894/818	75/52
	Influent_June	76,892 (5.2 %)	76,738 (5.3 %)	2445/2357	865/794	83/52
	Influent_July	138,343 (9.4 %)	138,131 (9.5 %)	2892/2796	991/913	86/57
	Influent Total	**304,979 (20.8 %)**	**304,422 (21.0 %)**	**4412/4216**	**1382/1245**	**104/67**
	Effluent_May	123,614 (8.4 %)	123,409 (8.5 %)	2982/2871	1029/937	87/56
	Effluent_June	54,892 (3.7 %)	54,752 (3.8 %)	2367/2296	820/757	80/53
	Effluent_July	48,492 (3.3 %)	48,373 (3.3 %)	2030/1960	718/660	76/50
	Effluent Total	**226,998 (15.5 %)**	**226,535 (15.7 %)**	**4219/4037**	**1319/1188**	**104/67**
Mariaehamn (Åland-Finland)	Influent _ May	53,553 (3.7 %)	53,124 (3.7 %)	3062/2925	1127/1029	102/68
	Influent _ Aug	55,452 (3.8 %)	54,958 (3.8)	3300/3136	1198/1097	101/70
	Influent _ Sept	20,612 (1.4 %)	20,213 (1.4 %)	2258/2129	968/880	101/64
	Influent Total	**129,617 (8.8 %)**	**129,617 (8.9 %)**	**5193/2050**	**1652/1479**	**132/86**
	Effluent_May	7,877 (0.5 %)	7348 (0.5 %)	2195/2050	924/812	96/65
	Effluent_Aug	6,739 (0.5 %)	6411 (0.4 %)	2344/2201	989/885	103/69
	Effluent_Sept	5,063 (0.3 %)	4854 (0.3 %)	1490/1401	716/649	81/54
	Effluent Total	**19,679 (1.3 %)**	**18,613 (1.4 %)**	**4259/3990**	**1471/1294**	**120/79**
	Grand Total	**1,466,295**	**1,449,294**	**8434/7709**	**2195/1813**	**152/93**

Note: Microbial reads primarily comprised bacterial reads but also included bacteriophages, viruses, and archaea.

$p < 0.001$). Influent and effluent communities were distinct but showed some overlap (NMDS, PERMANOVA $R^2 = 0.16$, $p = 0.028$) (Fig. 1). The R^2 value represents the proportion of variance in the microbial community composition, with higher R^2 values indicating stronger differences between groups.

Across all WWTPs, 8434 species (91.4 % bacterial), 2195 genera (82.6 % bacterial), and 152 classes (61.2 % bacterial) were identified. Mekjarvik WWTP had the highest taxonomic diversity (measured in microbial species, genera and classes), followed by Mariehamn, and then Reykjavik (Table 1). The Chao 1 and Shannon indices at each WWTP are shown in Figure S1. Chao1 index in influent samples was highest in Mekjarvik (5005.4 ± 663.4), followed by Mariehamn (4908.3 ± 661.4) and Reykjavik (4312.0 ± 416.3), while in effluent, it was Mekjarvik (5877.7 ± 820.1), Reykjavik (4200.4 ± 755.7), and Mariehamn (4186.4 ± 454.5). For the Shannon index, influent values were highest in Mariehamn (5.02 ± 0.21), followed by Reykjavik (4.88 ± 0.07) and Mekjarvik (4.59 ± 0.03), while in effluent, they were highest in Mariehamn (5.84 ± 0.45), Reykjavik (4.97 ± 0.11), and Mekjarvik (4.83 ± 0.23).

Among the microbial taxonomic classes, *Gammaproteobacteria* dominated all samples, with the highest relative abundance in Mekjarvik influent (75.2 % of reads) and lowest in Mariehamn effluent (37.5 %). *Betaproteobacteria* ranked second, followed by *Alphaproteobacteria* (Fig. 2). The relative abundance of *Alphaproteobacteria* increased post-treatment (4.3 %–9.1 % in Mekjarvik, 2.3 %–3.3 % in Reykjavik, and 2.5 %–7.5 % in Mariehamn). At the genus level, the top three genera in Mekjarvik influent and effluent were *Acinetobacter* (33.8 % in influent, 22.6 % in effluent), *Aeromonas* (27.5 %, 23.3 %), and *Bacteroides* (6.9 %, 9.6 %). In Reykjavik, *Acinetobacter* (34.5 % in influent, 31.9 % in effluent), *Aeromonas* (13.5 %, 12.7 %), and *Pseudomonas* (9.9 %, 8.0 %) were the most abundant. Mariehamn influent was dominated by *Acinetobacter* (51.8 %), *Psychrobacter* (5.7 %), and *Bacteroides* (5.6 %), while the effluent contained members from the genera *Escherichia* (15.5 %), *Salmonella* (7.6 %), and *Polynucleobacter* (4.8 %). Among *Acinetobacter*, the most abundant species were *A. baumannii*, *A. johnsonii*, and *A. lwoffii*. *Aeromonas,* a major genus in both Mekjarvik and Reykjavik was strongly dominated with *A. caviae*, *A. veronii*, and *A. hydrophila*. In Reykjavik wastewater, *Pseudomonas* ranked among the top three genera, mostly dominated by *P. aeruginosa*, *P. fragi*, and *P. lundensis*.

3.1.1. Detection of ARGs by Oxford Nanopore sequencing

In total, 39,285 high-quality metagenomic ARGs sequencing reads were detected in this study. The diversity of ARGs and distribution differed across the three WWTPs (NMDS, PERMANOVA $R^2 = 0.45$, $p < 0.001$), but differences in diversity and distribution between influent and effluent were not significant (NMDS, PERMANOVA $R^2 = 0.07$, $p = 0.21$) (Fig. 1). Overall, wastewater samples from Reykjavik showed the highest ARG read recovery, both in influent (mean ± SD: 4486.7 ± 1757.5) and effluent (3757.0 ± 3075.3). These were followed by samples from Mekjarvik, both for influent (2541.7 ± 1066.9) and effluent (1998.7 ± 1062.8). The lowest ARG recovery was observed in Mariehamn, influent (305.7 ± 111.1) and effluent (5.3 ± 4.7) samples. The chao 1 and Shannon indices at each WWTP are shown in Figure S1. Chao1 index in influent and effluent samples was 88.4 ± 6.6 and 88.0 ±

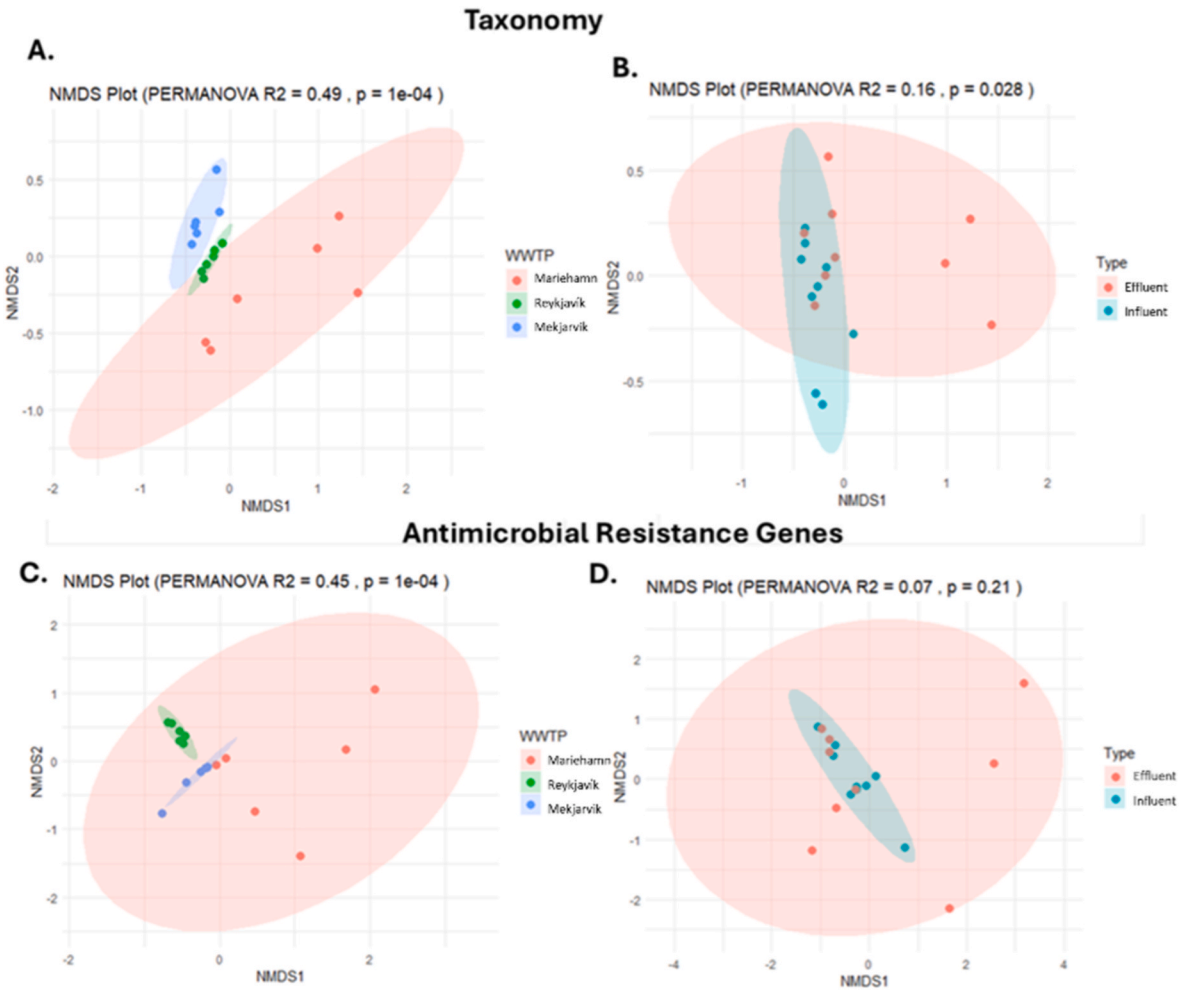

Fig. 1. Nonmetric multidimensional scaling of Oxford Nanopore data across three treatment plants, influent, and effluent, using the Bray-Curtis index. (A) Bacterial taxonomic reads combining influent and effluent. (B) Influent and effluent reads combined from three treatment plants. (C) Antimicrobial resistance gene reads combining influent and effluent. (D) Antimicrobial resistance genes in influent and effluent, combined from three treatment plants. Taxonomic level for (A) and (B): genus.

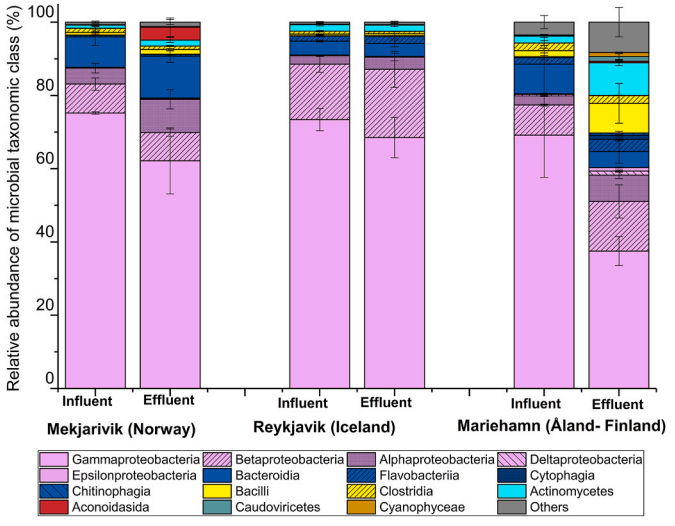

Fig. 2. Taxonomic composition of microbial communities presented as relative abundance of classes in wastewater samples. Error bars represent standard deviations across replicates.

28.1 in Reykjavik, 85.4 ± 20.8 and 67.2 ± 30.1 in Mekjarvik, and 19.1 ± 2.7 and 3 ± 0 in Mariehamn, respectively. For the Shannon index, influent and effluent values were 2.26 ± 0.24 and 2.64 ± 0.37 in Mariehamn, 1.91 ± 0.23 and 1.85 ± 0.25 in Reykjavik, and 2.07 ± 0.43 and 0.75 ± 0.10 in Mekjarvik, respectively.

A total of 193 ARGs were found in our study, with the highest number (80) detected in the influent samples from a Mekjarvik sample, and the lowest in Mariehamn effluent samples (two). In general, a greater ARGs diversity was recorded in influent samples compared to respective effluent. In the influent samples, the average number of ARG types was highest in Reykjavik (66.3 ± 4.1), followed by Mekjarvik (61.3 ± 14.1) and Mariehamn (18.0 ± 2.2). However, for effluent samples, the average ARG count per sample was highest in Mekjarvik (55.0 ± 16.9), followed by Reykjavik (53.3 ± 12.5), and Mariehamn (2.3 ± 0.5).

In Mekjarvik samples, a total of 125 ARGs were detected, with 50 appearing in only one sample and including 45 singletons. A total of 20 ARGs were present across all Mekjarvik samples, including tetracycline resistance genes (*tet(39)*, *tet(C)*, *tet(E)*, *tet(Q)*, *tet(W)*), aminoglycoside resistance genes (*ant(2″)-Ia*, *aph(3″)-Ib*, *aph(3′)-Via*, *aph(6)-Id*), nitroimidazole resistance genes (*nimA*, *nimD*, *nimE*), macrolides (*erm(F)*, *mph(E)*), macrolide-streptogramin B (*msr(E)*), carbapenemase (bla_{AER-1}), β-lactamase ($bla_{OXA-129}$), chloramphenicol (*cat*), quinolone (*qnrS2*), and sulfonamide (*sul2*). Notably, *mph(E)* (576 ± 308), *msr(E)* (491 ± 258), and *tet(39)* (417 ± 232) exhibited the highest ARG read counts.

In Reykjavik samples, 141 ARGs were detected, with half found in only one sample, mostly as singletons. A total of 21 ARGs were consistly present across all influent and effluent samples, including aminoglycoside resistance genes (*aac(6′)-Im*, *aadA1*, *aadA11*, *aadA2*, *aadA6*, *ant(2″)-Ia*, *ant(3″)-Ia*, *aph(2″)-Ib*, *aph(3″)-Ib*, *aph(3′)-IIIa*, *aph(3′)-VIa*, *aph(3′)-Ia*, *aph(6)-Id*), β-lactamase genes (bla_{ACI-1}, bla_{AER-1}), and carbapenemase genes ($bla_{CARB-10}$, $bla_{CARB-14}$, bla_{CARB-4}, and bla_{LCR-1}). Among these, *aph(3′)-VI* (1683 ± 1258), *aph(3′)-IIIa* (870 ± 532), and *aph(3′)-Ia* (764 ± 470) had the highest read prevalence.

A total of 36 ARGs were detected in the Mariehamn samples, half of them detected in only one sample, with 6 being singletons. No genes were universally present across all samples, *tet(39)* was detected in five samples, and *tet(Q)* in four samples. Among the ARGs, *tet(39)* was the most universal ARG, detected in all three WWTPs (17/18 samples), missing only in one Mariehamn effluent. Next, *tet(Q)* was found in 16 samples, and absent in two Mariehamn effluents. ARGs, *mph(E)*, *msr(E)*, and *tet(W)* appeared in 15 samples, except in all Mariehamn effluents.

3.1.2. ARGs conferring resistance to different antibiotic classes

We calculated the relative abundance of ARGs conferring resistance to various antibiotic classes in influent and effluent samples from the three WWTPs (Fig. 3). Tetracyclines, macrolides, beta-lactams, multidrug resistance (MDR), and aminoglycosides were the most prevalent resistance classes. Mekjarvik, having both primary and secondary treatment, showed a reduction in tetracycline, macrolide, and MDR in the effluent, suggesting partial ARG removal. However, beta-lactamase and aminoglycoside resistance slightly increased. Reykjavik, having only primary treatment, had a high proportion of quinolone resistance in the influent, which decreased in the effluent, while macrolide resistance remained dominant. Quinolone resistance was significantly higher in Reykjavik than in the two other WWTPs. Mariehamn, with both primary and secondary treatment, showed a notable shift in the effluent, with a higher proportion of beta-lactam and tetracycline resistance. Overall, the beta-lactam resistance increased in plants having a secondary treatment unit, while macrolide and MDR decreased across all sites.

Table S6 shows potential attenuated and amplified genes per sample based on total read counts. Potential attenuated ARGs generally outnumbered amplified ones, except in two cases. In July at Mekjarvik, the trend reversed, with numerous aminoglycoside, macrolide, and tetracycline genes potentially amplified, while only two ESBL and carbapenem genes were attenuated. In May at the Reykjavik WWTP, some aminoglycoside, β-lactam, macrolide, and tetracycline genes appeared amplified, while others in the same classes were likely attenuated. Ten ARGs, mainly targeting β-lactams, carbapenems, aminoglycosides, macrolides, chloramphenicol, and sulfonamides, had higher read counts in effluent than influent samples.

3.1.3. Plasmid types and ARGs

A total of 13 plasmid types associated with ARGs were detected with ONP. Majors were *Col440, IncQ1, IncQ2,* and *ColRNAI,* and *IncP6,* carrying genes that confer MDR. These were the most prevalent, found in many wastewater samples from nearly all WWTPs, highlighting their role in HGT (Table S7). Plasmid-associated ARGs conferred resistance to multiple antibiotic classes (Figure S2), with a high prevalence in quinolones, tetracyclines, beta-lactams, aminoglycosides, macrolides, and sulfonamides across all WWTPs, regardless of the presence or absence of secondary treatment. Plasmid-associated ARG diversity was higher in Mekjarvik (with secondary treatment), spanning all antibiotic classes (e.g., *aph*(3″)-Ib, bla_{TEM}, *qnr*, *aph*, *sul*2, *tet*(C)). Reykjavik (with only primary treatment) predominantly had quinolone resistance genes (*qnr*-variants) but also *aph*(3″)-Ib, bla_{OXA}, and *tet*(C) (Table S7). Despite having a secondary operation unit, the Mariehamn WWTP had fewer ARGs, conferring resistance to macrolides, lincosamides, and streptogramins (Table S7). Among plasmid-mediated ARGs, *qnr*B19, *qnr*S2, and *tet*(C) were among the most widely distributed genes in the majority of samples in all WWTPs, in both influent and effluent.

3.1.4. Metal stress genes and possible co-selection pressure

With ONT metagenomic sequencing, a total of 966 metal stress reads were recorded, with the majority related to mercury (64.9 %), followed by multimetal (23.7 %), copper (6.4 %), biocide and metal (2 %), nickel (1.8 %), and others, including zinc, tellurium, and chromium. As shown in Figure S3, influent samples resulted in a higher number of metal stress reads than effluent samples. Among the three WWTPs, most reads

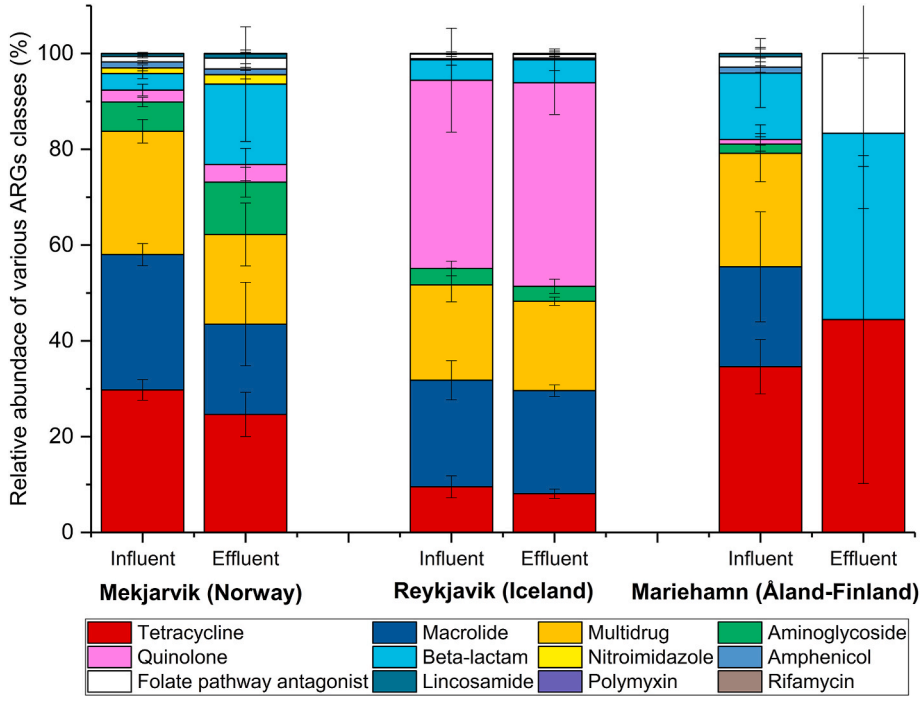

Fig. 3. Relative abundance of ARGs conferring resistance to various antibiotic classes detected in influent and effluent of three wastewater treatment plants using Oxford-Nanopore high-throughput metagenomic sequencing of total nucleic acids.

associated with metal resistance were recorded in the Mekjarvik and Reykjavik treatment plants.

Of the total reads, 722 (74.7 %) were associated with heavy metal resistance proteins, 222 (23 %) with heavy metal stress regulator proteins, and 17 (1.8 %) with ABC efflux pumps. The remaining ~0.5 % were linked to RND efflux pumps, ABC efflux regulators, MFS efflux pumps, and MFS efflux regulators. Notably, most reads conferring nickel resistance were associated with ABC efflux pumps. Mercury, multimetal, and copper-related reads were universally detected across all WWTPs. However, biocide and metal-related reads were predominantly found in Reykjavik and Mariehamn samples, while chromium-related reads were mostly observed in Reykjavik samples.

3.2. Detection of ARGs with qPCR methods

The prevalence of ARGs in the Mekjarvik (Norway) and Reykjavik (Iceland) samples was monitored using a Biomeme qPCR platform. ARGs were monitored across 11 sampling events in Reykjavik and nine times in Mekjarvik WWTPs. Most of the targets had higher detection frequencies in effluent samples (Table S8). Among the 9 targeted ARGs, the gene copies of the *int1* and *sul1* genes exhibited the highest prevalence, while the *van* gene had the lowest prevalence in both WWTPs (Fig. 4, Table S8). In Mekjarvik, genes *int*1 and *kor*B showed a higher prevalence in effluent than in influent samples. Interestingly, in the Reykjavik WWTP, the prevalence of $bla_{CTX\text{-}M}$, bla_{CMY}, *int1*, and *van* was significantly higher in the effluent compared to the influent, while, in the Mekjarvik WWTP, *int1* and *korB* showed significantly a higher prevalence in the effluent than in the influent. Meanwhile, $bla_{CTX\text{-}M}$, *secE*, and *tetA* exhibited a higher prevalence in the influent than in the effluent (Fig. 4).

The prevalence of ARGs in the Mariehamn samples was monitored using a SmartChip qPCR system. Influent samples were collected four times, and effleut samples were collected three times. Out of 31 ARGs assyas 13 (bla_{GES}, bla_{NDM}, bla_{OXA48_2}, bla_{CTX_M}, $bla_{CTX\_M\_1}$, $bla_{CTX\_M\_2}$, $bla_{CTX\_M\_4}$, bla_{OXA10_1}, bla_{ACT}, bla_{CMY2}, *tetO_1*, *van*A, *van*B_1) were detected in all three influent and one effluent sample. Two assays (bla_{OXA58_2}, bla_{VEB}) were found in all influent but not in effluent samples. Five assays (bla_{OXA48}, $bla_{CTX\_M\_8}$, *amp*C_bla_{DHA}, bla_{CMY_1}, bla_{MOX}_bla_{CMY}) were detected in three influent samples but not in effluent samples. Two assays ($bla_{CTX\_M\_5}$, $bla_{CTX\_M\_6}$) were found in two samples, while three assays (bla_{IMI}, bla_{KPC_2}, bla_{SME}) were detected in only one influent sample. One assay (bla_{IMP_1}) was detected only in effluent, while five assays (bla_{KPC}, bla_{KPC_3}, bla_{OXA23}, bla_{VIM}, *mec*A) were not detected in any samples. Among the taxonomic assays, *crAss56* was detected in all influent

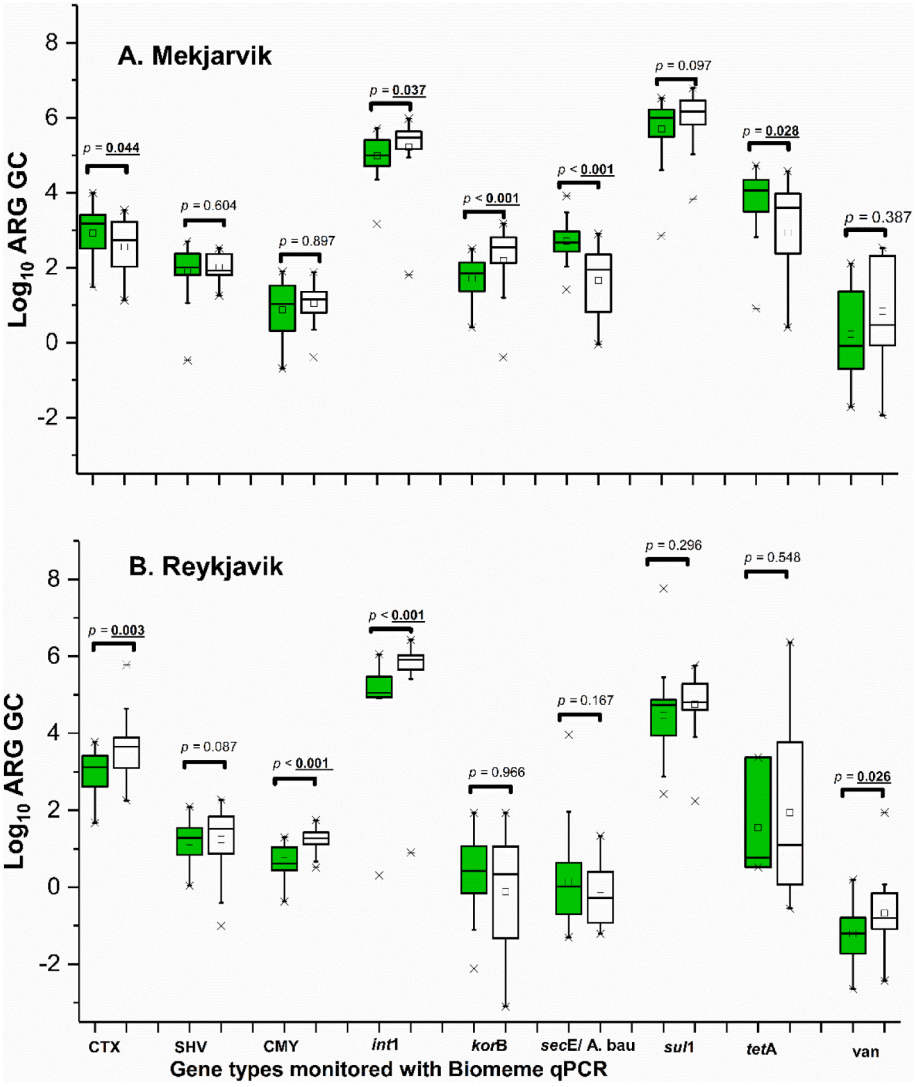

Fig. 4. Box plot of gene copy numbers (Log_{10} GC/mL) for targeted ARGs using the Biomeme qPCR method. The black line indicates the median, the square marker indicates the mean, and the box edges correspond to the third and first quartiles. Significance was tested using the **Mann-Whitney *U*-test.** Green boxes represent influent and white boxes show genes in effluent.

and effluent samples. *A. baumannii* was found in all influent samples, *K. pneumoniae* was present in three influent samples but *Staphylococci* was not detected in any sample. Although many ARGs had higher detection rates in influent, assays targeting carbapenemase genes (bla_{IMI}, bla_{KPC}, bla_{OXA-48}), the ESBL gene (bla_{CTX-M}), *amp*C β-lactamase genes (bla_{CMY2}, and bla_{ACT}) and vancomycin resistance genes (*van*A and *van*B) showed a higher prevalence in effluent (Fig. 5).

3.3. Prevalence of antibiotic residues

Of the 45 antibiotics screened (Table S3), only five met the sufficient confidence level for reporting (Fig. 6, Table S4): sulfamethoxazole, sulfapyridine, azithromycin, ciprofloxacin, and ofloxacin. Among these, sulfamethoxazole and sulfapyridine showed the highest concentration in both Mekjarvik and Reykjavik WWTPs, whereas azithromycin, ciprofloxacin, and ofloxacin were mostly below detection limits at both sites. Antibiotic concentrations exhibited marked temporal variation in Mekjarvik, peaking at ~2000 ng/L in April and averaging ~600 ng/L, with notable declines (~200 ng/L) in July, September, and October. In contrast, Reykjavík maintained consistently low concentrations (~200 ng/L) throughout the study, with only a modest peak of ~300 ng/L in December. These patterns indicate pronounced seasonal fluctuation in Mekjarvik but relative stability in Reykjavík over the monitoring period (Nov–Jan).

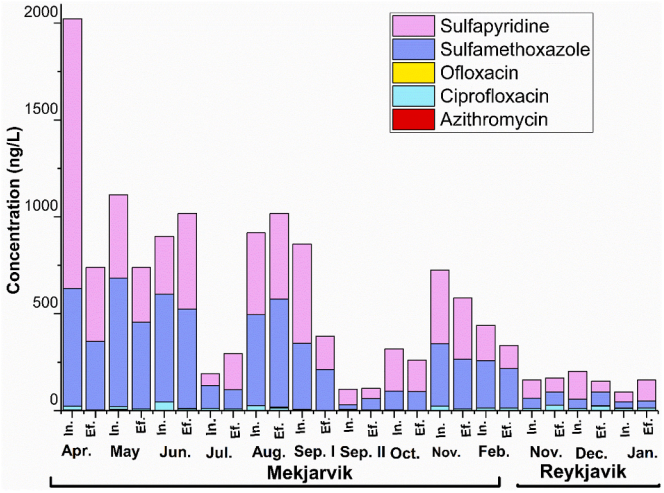

Fig. 6. Prevalence of antibiotic residues in Mekjarvik and Reykjavik wastewater treatment plant during the study period. (In. = influent, Ef. = effluent).

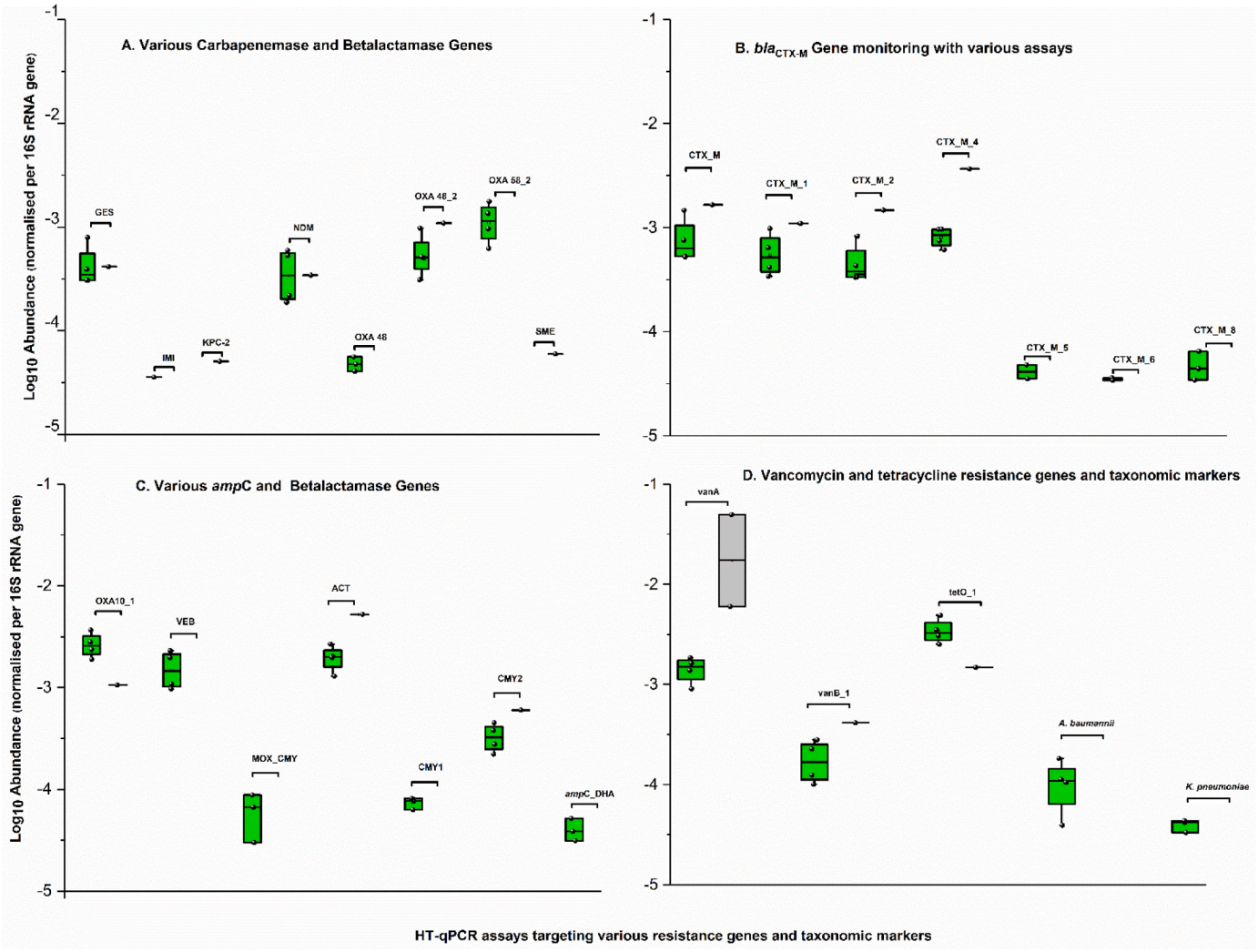

Fig. 5. Relative abundance of HT-qPCR SmartChip assay types targeting antimicrobial resistance and taxonomic genes in influent and effluent samples from Mariehamn. Green boxes indicate influent, and gray boxes represent effluent.

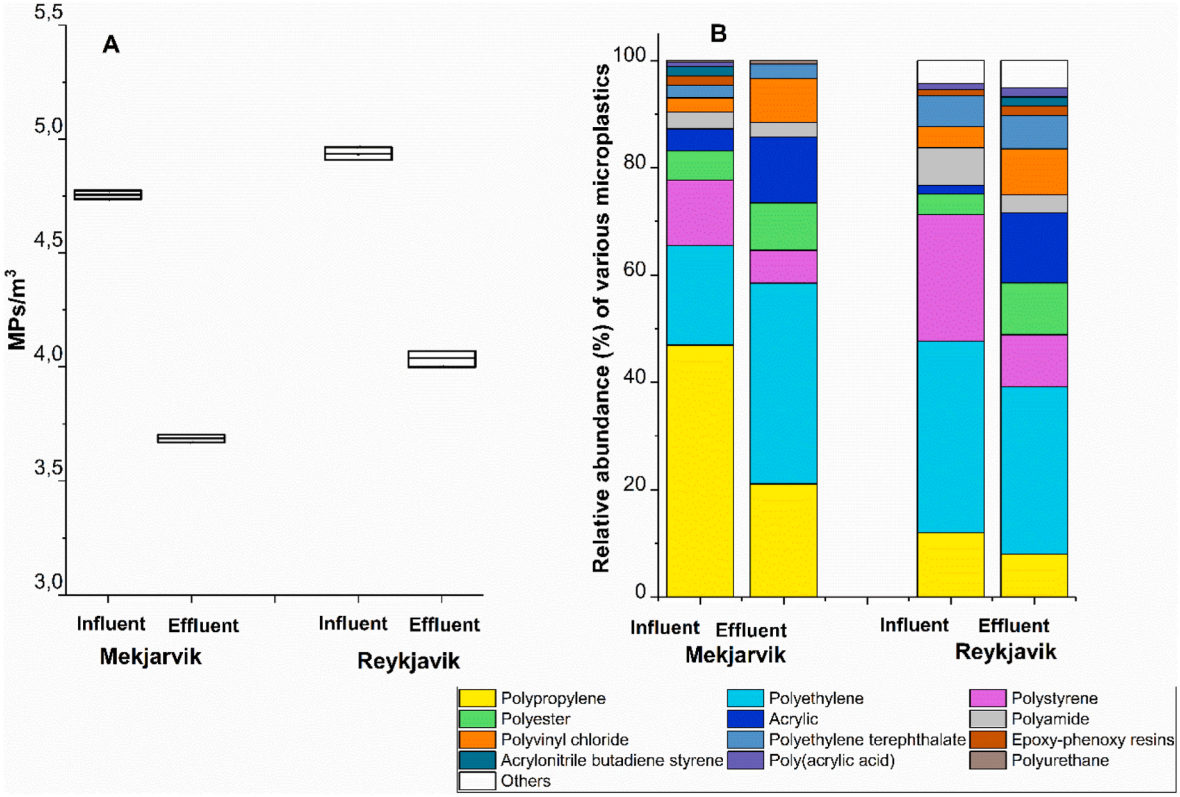

Fig. 7. Variation in the MP count and types in influent and effluent from Mekjarvik and Reykjavik WWTPs. (A) MP count. (B) MP types.

3.4. Prevalence of microplastics

MP particles were monitored only at Mekjarvik and Reykjavik WWTPs. The highest count was recorded in Reykjavik influent (8200 MP/m^3), followed by Mekjarvik influent (5900 MP/m^3). Reykjavik effluent had 1100 MP/m^3, while Mekjarvik effluent had the lowest at 750 MP/m^3 (Fig. 7). Polyethylene accounted for about one-third of total MPs in Reykjavik influent and effluent and in Mekjarvik effluent. However, in Mekjarvik influent, nearly half of the MPs were polypropylene. Polystyrene made up roughly one-fifth of MPs in Reykjavik influent wastewater. Other major MP distributions are shown in Fig. 7.

The distribution of MP size varied across wastewater types and locations. Mekjarvik influent samples predominantly contained the 101–150 μm fraction (~31 %), followed by the 151–200 μm (~23 %) and 201–300 μm (~14 %) sizes. In Reykjavik influent, the 50–100 μm (~30 %) and 101–150 μm (~30 %)sizes were also prominent. In both effluent samples, fine MPs increased while larger particles decreased after treatment, with the size fraction of 20–50 μm dominating the effluent water (~41 %) in the Mekjarvik wasterwater treatment plant and the 50–100 μm size fraction contributing about 40 % of the total in

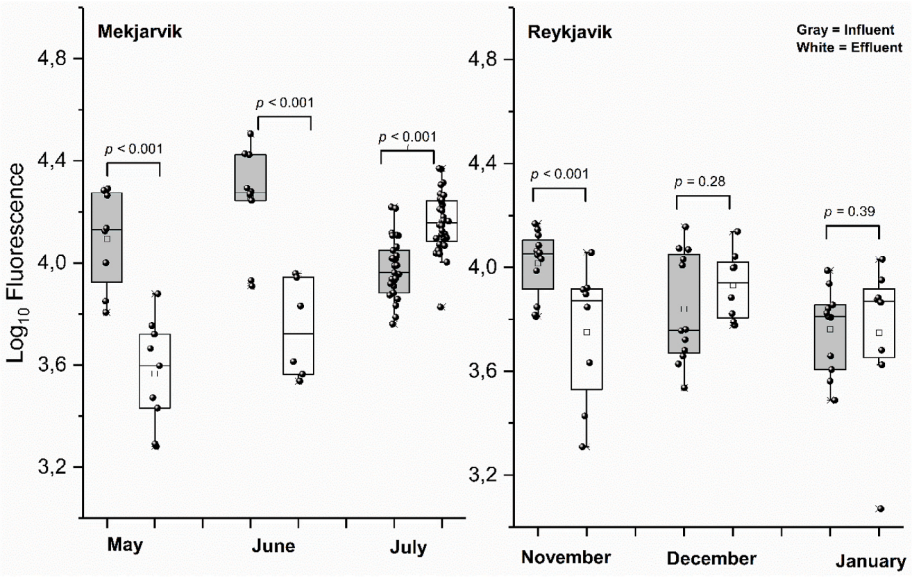

Fig. 8. SOS-*E. coli* fluorescence levels in influent and effluent samples from Mekjarvik and Reykjavik WWTPs across different sampling months. Each dot represents the measurement of sample replicates from each monitoring event. The Mann-Whitney *U*-test was used to test for significance.

the Reykjavik effluent (Figure S3).

3.5. SOS response: bacterial DNA damage repair

In Mekjarvik (with a secondary treatment unit), SOS-*E. coli* fluorescence levels were significantly higher in influent than effluent samples in May and June (Fig. 8). However, in July, effluent levels exceeded influent levels. Average fluorescence levels (±SE) for influent samples in May, June, and July were 4.09 ± 0.06, 4.25 ± 0.06, and 3.98 ± 0.02, respectively, while effluent averages were 3.57 ± 0.07, 3.74 ± 0.07, and 4.16 ± 0.02. In Reykjavik (with only a primary treatment unit and wastewater dilution using home-heated water), higher counts were demonstrated in influent only in November, with effluent levels exceeding influent levels in December and January, among the three consecutive months monitored. Average influent fluorescence levels (±SE) were 4.01 ± 0.04, 3.84 ± 0.06, and 3.76 ± 0.05, while effluent averages were 3.75 ± 0.09, 3.09 ± 0.04, and 3.75 ± 0.10, respectively, across November, December, and January (Fig. 8).

4. Discussion

This study investigated ARGs in influent and effluent from three Nordic WWTPs (Reykjavik, Mekjarvik, and Mariehamn), as well as antibiotic residues and MPs in Reykjavik and Mekjarvik. Our findings indicates tentative concordance between national antibiotic consumption patterns and the detected ARGs in wastewater, reinforcing WS as a potential tool for monitoring ARGs at the population level (ECDC, 2023; Palsdottir et al., 2020; Tiwari et al., 2024). The relatively higher tetracycline, fluoroquinolone/ciprofloxacin, and β-lactam consumption in Iceland compared to Finland and Norway, was indicated with an increased prevalence of corresponding ARGs in Reykjavik wastewater (ECDC, 2023; Tiwari et al., 2024). Tetracyclines, widely used in human and veterinary medicine in the Nordic region (ECDC, 2023), along with macrolides for respiratory and skin infections, and quinolones for urinary and respiratory tract infections, contribute to the spread of resistance (ECDC, 2023). Although we need to account for some variation in ARG results across platforms, the SmartChip Real-Time PCR system provides ARG data normalized to total 16S rRNA gene copies in wastewater samples, whereas metagenomics do not offer such normalization. Nevertheless, Still, qPCR and metagenomics showed some conconrdance in ARG detection, with β-lactamase and carbapenemase genes amplified during biological treatment. Further comparison between qPCR and metagenomics was not possible due to differences in monitoring approaches. In general, qPCR is a more targeted and sensitive approach compared to metagenomics, a broader and more exploratory approach (Luby et al., 2016; Tiwari et al., 2022a). The Biomeme platform may be more sensitive than the Resistomap approach, as it uses a higher amount of DNA template for amplification.

Our study further demonstrated the effectiveness of portable tools—ONP metagenomics and the Biomeme Franklin qPCR device—for detecting ARGs, microbial communities, and genetic elements, offering scalable solutions for real-time monitoring. These devices enabled onsite ARG monitoring and near-real-time visualization, serving as an early warning tool for AMR spread and wastewater treatment efficiency (Frimpong et al., 2023; Taxt et al., 2020; Zowawi et al., 2021).

Next, the detection of plasmid-mediated ARGs, metal stress genes, and DNA from potential opportunistic pathogens, *A. baumannii*, *P. aeruginosa*, *Aeromonas*, *Bacteroides*, *E. coli*, and *Salmonella,* in both influent and effluent suggests potential roles of WWTPs in ARB dissemination (Murray et al., 2022; Santajit and Indrawattana, 2016). *A. baumannii* and *P. aeruginosa*, key ESKAPE pathogens linked to hospital outbreaks and global AMR-related deaths, are on the WHO critical priority list requiring serious attention (Miller and Arias, 2024; Santajit and Indrawattana, 2016). Micropollutants such as pharmaceuticals, MPs, and heavy metals further drive co-selection, as metal resistance genes often co-occur with ARGs, enabling resistance in the absence of antibiotics (Junaid et al., 2022; L.-G. Li et al., 2017).

In WWTPs, microbial interaction and diversity impacts HGT mechanisms (conjugation, transduction, transformation), facilitating ARG transfer (Karkman et al., 2016; Lood et al., 2017). Biofilms and high bacterial densities in secondary treatment enhance gene exchange, promoting AMR dissemination (Karkman et al., 2016). MPs can promote biofilm formation (Karkman et al., 2018; Simon et al., 2018), and influence extracellular DNA and MGE dynamics, thereby altering ARG dissemination (Sivalingam et al., 2025). While ARG levels generally decrease (potentially due to reduced bacterial loads), many persist and may increase relative to total bacterial reduction (Karkman et al., 2016). Our SOS-*E. coli* analysis, evaluating genotoxicity, DNA damage, and stress responses to environmental contaminants (e.g., trace antibiotics, heavy metals), showed reduced SOS activity during treatment (Crane et al., 2021; Serment-Guerrero et al., 2020). In May and June, Mekjarvik WWTP removed stressors more effectively than Reykjavik WWTP, which had only primary treatment. Well-operated WWTPs with both primary and secondary treatment units appear more effective at reducing bacterial stressors (Macrì et al., 2024).

Interestingly, the relative abundance of some ARGs increased in Reykjavik's WWTP, which has only primary treatment. This may result from elevated sewage temperatures, as in Reykjavik, wastewater is collected, mechanically screened, and mixed with heated household water before discharge. Higher temperatures can enhance HGT, as enteric pathogens thrive in mesophilic conditions (Du et al., 2015; Shamsizadeh et al., 2024). Studies have demonstrated that ARG abundance and *intI*1 prevalence correlate with effluent temperature, shaping microbial communities and ARG spread (Jiao et al., 2018; Shamsizadeh et al., 2024). The optimal temperature for biological reactions is 15–35 °C, where higher temperatures may promote bacterial growth and ARG transfer (Du et al., 2015), while lower temperatures may reduce acetylation enzyme activity, affecting transcription and microbial growth (Jiao et al., 2018).

After treatment, the effluents still contained ESBL and carbapenem resistance determinants. Previous studies had also reported a significant prevalence of carbapenemase-producing bacteria and resistance genes in treated wastewater in countries such as Italy and Spain (Caltagirone et al., 2017; Monge-Olivares et al., 2025). The detection of ESBL, carbapenemase genes (bla_{TEM}, bla_{OXA}, bla_{KPC}, bla_{GES}) along with MLSB resistance genes (*ermB, ermF*) in effluent sample indicates a serious One Health concern —threatening human, environmental, and potentially animal health (Al-Mustapha et al., 2023; Tiwari et al., 2023b). ARGs in effluent pose pollution risks to receiving surface waters, making effluent monitoring vital for assessing public health threats (Hultman et al., 2018; Khan et al., 2019). While WWTPs reduce ARG prevalence, dominant ARGs often persist post-treatment, highlighting the inadequacy of current processes and the need for strategies to mitigate AMR spread (Khan et al., 2019; Macrì et al., 2024). Site variations likely reflect regional antibiotic use, treatment technologies, and environmental factors influencing ARG persistence (Tiwari et al., 2024). Major drivers for increasing multidrug resistance prevalence in Nordic countries are travel and hospitalizations abroad (Tiwari et al., 2024). In Reykjavik, broad-spectrum antibiotics such as azithromycin increase the AMR risk through environmental excretion, underscoring the need for prudent prescribing (Palsdottir et al., 2020).

4.1. Antibiotic residues in wastewater: sources and ecological fate

Antibiotic residues in wastewater depend on community use, fate, and decay rates. Of 45 antibiotics screened, only five were detected. Despite tetracyclines and beta-lactam penicillins being the most commonly used antibiotics in Norway and Iceland (ECDC, 2023; Palsdottir et al., 2020), their absence suggests rapid decay in the sewage system or effective metabolism in human and animal use. Penicillin and tetracyclines are unstable under environmental conditions (e.g. pH and temperatures (Wang et al., 2021). Dilution in sewers may also lower

concentrations below detection limits. One earlier study mentioned sulfonamides as commonly detected in wastewater (Samrot et al., 2023). However, the detection patterns of antibiotic residues in wastewater did not correspond with those of the respective ARGs.

4.2. Well-operated WWTPs remove larger-sized MPs

Well-operated wastewater treatment plants (WWTPs) have been shown to effectively remove a substantial proportion of MPs, particularly those of larger size fractions (e.g., >100 μm). Primary treatment processes, such as screening and sedimentation, are efficient in capturing macro- and larger MPs particles due to their physical characteristics. Subsequent secondary treatments, including activated sludge processes and biofilm-based systems, further enhance removal through mechanisms such as bioflocculation, settling, and entrapment in sludge. Advanced tertiary treatments—such as sand filtration, membrane filtration, or dissolved air flotation—can further clean the effluent, achieving removal efficiencies exceeding 99.7 % for larger MPs (Magnusson et al., 2016). As a result, the majority of large MPs are retained in sludge rather than being released into the aquatic environment, underscoring the importance of proper sludge management for preventing secondary pollution (Magnusson et al., 2016). However, this level of removal was not observed in a treatment plant with only a primary treatment unit (Iceland) (Magnusson et al., 2016).

5. Conclusion

Our study revealed that the influent resistome composition varied across the three WWTPs, reflecting region-specific ARG patterns probably shaped by local antibiotic use, healthcare practices, and travel-related factors. ARG removal efficiency differed according to both the resistance class and treatment plant, with some genes—especially those associated with mobile genetic elements (e.g., bla_{KPC}, bla_{OXA}, *ermB*)—persisting in effluents, indicating variability in treatment performance. Partial concordance was observed between metagenomics and qPCR approaches. While both detected key ARGs, differences in sensitivity, target range, and detection thresholds limited full comparability. Metagenomics captured broader ARG diversity, whereas qPCR provided sensitive quantification of selected targets.

No strong association was observed between antibiotic residues and corresponding ARGs in wastewater. This mismatch probably reflects the complex dynamics of ARG persistence, selection pressure, degradation rates, and historical antibiotic exposure. The treatment process also influenced MPs removal, with variations observed between Mekjarvik and Reykjavik. Nonetheless, MPs remained detectable in effluents, indicating incomplete removal and potential environmental risks.

The presence of clinically relevant ARGs, plasmid-mediated resistance, and pathogen markers (e.g., *A. baumannii*, *P. aeruginosa*) in effluents highlights the public health and environmental risks. Due to the risk of horizontal gene transfer and AMR spread, ongoing surveillance of influent and effluent is essential. Portable sequencing (e.g., ONP metagenomics) and Biomem qPCR proved effective for detecting ARGs, microbial communities, and genetic elements, offering scalable solutions for real-time monitoring. Overall, these findings underscore the need for continuous wastewater surveillance to inform risk-based interventions, support compliance with the recast EU Urban Wastewater Treatment Directive (UWWTD), and guide the development of coordinated AMR monitoring strategies across Nordic countries.

CRediT authorship contribution statement

Ananda Tiwari: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Adrián Jaén-Gil:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Anastasia Karavaeva:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Alessio Gomiero:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Ásta Margrét Ásmundsdóttir:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Maria João Silva:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Elisa Salmivirta:** Writing – review & editing, Resources, Project administration, Methodology. **Tam T. Tran:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation. **Anniina Sarekoski:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Jeremy Cook:** Writing – review & editing, Supervision, Software, Project administration, Methodology, Investigation. **Rolf Lood:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Tarja Pitkänen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Adriana Krolicka:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envres.2025.122629.

Data availability

Data will be made available on request.

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