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Occurrence of Antibiotics in Influent and Effluent from 3 Major Wastewater-Treatment Plants in Finland

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Abstract: Wastewater-treatment plants (WWTPs) are regarded as one of the main sources of antibiotics in the environment. In the present study, the concentrations of multiple antibiotics and their metabolites belonging to 5 antibiotic classes were determined in 3 major Finnish WWTPs. An online solid phase extraction–liquid chromatography–tandem mass spectrometry method was used for the extraction and analysis of the compounds. The method was fully validated using real and synthetic wastewaters. Seven antibiotics and 3 metabolites were found in the analyzed samples. Sulfonamides were removed most efficiently, whereas macrolides usually showed negative removal efficiency during the treatment, which means that the concentrations for individual antibiotics determined in the effluent samples were higher than in the influent samples. Sulfadiazine was found at concentrations up to 1018 ng/L, which was the highest concentration of any of the detected antibiotics in influent. In the effluent samples, the highest mean concentration was found for trimethoprim (532 ng/L). The measured mass loads of the antibiotics and metabolites to the receiving waters ranged from 2 to 157 mg/d per 1000 population equivalent. The evaluated environmental risk assessment showed that clarithromycin and erythromycin might pose a risk to the environment. The present study further underlines the importance of implementing technology for efficient removal of xenobiotics during wastewater treatment. *Environ Toxicol Chem* 2020;39:1774–1789. © 2020 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Multiresidue analyses; Wastewater; Antibiotics; Metabolites; Online SPE; Environmental risk assessment

INTRODUCTION

Large amounts of antibiotics are used worldwide to treat or prevent various diseases in human and veterinary medicine and as growth promoters in aquaculture and agriculture (Ventola 2015; Klein et al. 2018). In recent years, the occurrence of antibiotics in the environment has become a concern (O'Neill 2014). Many antibiotics are not fully metabolized in the human or animal body, and through excretion, the parent compounds, their transformation products, and their metabolites enter wastewater-treatment plants (WWTPs; Ribeiro et al. 2015). Modern WWTPs which apply tertiary treatment are capable of effectively reducing the concentrations of carbon and nitrogen as well as microbial pollution. However, very often the concentrations of some organic pollutants, such as some antibiotics, are not sufficiently eliminated during the treatment (Rizzo et al. 2013; Verlicchi et al. 2015); therefore, antibiotics are frequently found in effluent waters (Dinh et al. 2017). The elimination efficiency of antibiotics in WWTPs often depends on the treatment methods applied (Krzeminski et al. 2019). Some antibiotics have a low tendency to bind to activated sludge, and because of that, their microbial degradation might not be completed within the hydraulic retention time of the WWTPs (Besha et al. 2017). Therefore, antibiotics are frequently found in effluent waters (Dinh et al. 2017). Discharge from WWTPs is one of the main routes for organic compounds, such as antibiotics, to enter the environment, and the presence of antibiotics in the environment has been proven worldwide (Tran et al. 2018).

Antibiotics are regarded as "pseudopersistent" contaminants because of their continuous introduction into ecosystems (Lindberg et al. 2005). Antibiotics are usually excreted either unchanged or transformed via the urine and feces of humans (Ribeiro et al. 2015). The degree of metabolization of antibiotics in humans and animals varies both between and within chemical classes. For instance, the degree of human metabolism of tetracyclines (TETR) and macrolides is <20%, whereas >80% of sulfonamides are metabolized (Hirsch et al. 1999). The degree of metabolization depends mainly on the species subjected to the medication and its mode of application (Kümmerer 2009).

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One major issue caused by the presence of antibiotics in the environment is that they might foster the development of antibiotic resistance (Kraemer et al. 2019). Even at relatively low concentration levels (ng to low μg/L or g) in recipient waters and sediments, antibiotics are considered potentially hazardous for the aquatic biota, impacting their survival behavior and fostering the development of antibiotic resistance genes (Gullberg et al. 2014; Wang et al. 2014; Liu et al. 2018). For example, bacteria isolated from sewage bioreactors have exhibited resistance to some antibiotics, including trimethoprim (TMP), erythromycin (ERY), and TETR (Costanzo et al. 2005); and antibiotic-resistant bacteria have been detected in sediments of rivers that have been contaminated with antibiotics (Sabri et al. 2018). Antibiotic-resistant bacteria may also be found in the sludge that may eventually be used as a fertilizer on agricultural fields (Kümmerer 2009). The occurrence of antibiotic-resistant bacteria in the environment may be linked to the increased frequencies of the use of antibiotics in the treatment of human infections (Kümmerer 2009), and antibiotics in the aquatic environment thus pose a health hazard for humans (Pruden et al. 2013).

The objective of the present study was to determine the occurrence of the most commonly used antibiotics in Finland as well as 3 of their major metabolites in both the influents and effluents of 3 major municipal WWTPs in Finland. The selected antibiotics belong to 5 different antibiotic classes: tetracyclines (TETR, doxycycline [DOXY], oxytetracycline [OXY]), ß-lactams (benzylpenicillin [BPEN], phenoxymethylpenicillin [PHPEN], cloxacillin [CLOXA], ampicillin [AMP], cephalexin [CEPH]), sulfonamides (sulfadiazine [SDZ], sulfamethoxazole [SMX], *N*-acetyl sulfadiazine [*N*-SDZ], and *N*-acetyl sulfamethoxazole [*N*-SMX]), diaminopyrimidine (TMP), and macrolides (ERY, roxithromycin [ROXI], clarithromycin [CLARI], tylosin [TYL], and ERY enol ether [ERY-EE]). An environmental risk assessment (ERA) based on the calculated risk quotient (RQ) for the maximum mean concentrations detected in effluent waters for 3 trophic levels and 6 antibiotics was carried out. This is the second peer-reviewed study on the occurrence of antibiotics and their metabolites in any WWTPs in Finland and the first study on these particular WWTPs. The studied WWTPs are 3 out of the 4 largest in Finland, based on the treated amount of wastewater per year (Laitinen et al. 2014). The study provides comprehensive and valuable information about the occurrence of the most commonly used antibiotics in Finnish wastewaters, which could be used for decision-making and improving wastewater-treatment processes. The data in the present study were obtained using the advanced online solid-phase extraction (SPE) method, which provides several advantages over traditional SPE methods: 1) the sample preparation is restricted to centrifugation for samples containing particles, 2) it requires small sample volumes, and 3) the analysis time is reduced (Meierjohann et al. 2017). The developed fast and reliable analytical methods were applied to quantify multiple antibiotics among 5 different classes. The developed method which combines online SPE and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis can be used for multiple purposes, such as rapid screening analysis or laboratory quality control routines.

MATERIALS AND METHODS

Chemicals and materials

Analytical standards of CLARI, SDZ, SMX, TMP, ERY, tartrate (TYL), sodium salt (CLOXA), and carbamazepine (CBZ) were purchased from Sigma-Aldrich. Analytical standards of DOXY (hyclate), ERY-EE, ROXI, penicillin V (PHPEN), *N*-SDZ, *N*-SMX, monohydrate (CEPH), dehydrate (OXY), potassium salt, penicillin G (BPEN), and the internal standards cephalexin-D_5 hydrate, sulfadiazine-D_5, TMP-D_3, carbamazepine-D_8, 4-epitetracycline-D_6, and benzylpenicillin-D_7 potassium salt were purchased from Toronto Research Chemicals. The analytical standard for TETR (hydrochloride) was purchased from Amresco, and the standard for AMP (sodium salt) was purchased from AppliChem (Table 1).

Stock solutions of 1 mg/mL were prepared in methanol and stored at –20 °C. For further dilutions of the antibiotics, acetonitrile and ultrapurified water were used. The methanol used was of high-performance liquid chromatography grade. Liquid chromatography-MS-grade acetonitrile was used for dilutions and as mobile-phase solution. All water was purified using an ELGA Purelab Ultra water system. Formic acid for analysis (>98%) was used as a mobile-phase additive. Prior to use, all glassware was washed with hot water, rinsed with distilled water and acetone, and dried at 150 °C for 2 h. After that, the glassware was washed using a saturated ethylenediaminetetraacetic acid disodium salt dihydrate (VWR Chemicals) solution in methanol according to the procedure described by Hamscher et al. (2002) to prevent the binding of TETR to free silanol groups.

Sample collections and site description

Sewage influent and effluent samples (2 L) were collected in polypropylene bottles as 24-h composite samples and stored in the dark at –18 °C. Each of the composite samples consisted of 24 individual grab samples taken in intervals of 1 h. Influent samples were collected from waters received by WWTPs before any treatment, and effluent samples were collected from outgoing waters that are released to the receiving waters after final treatment. Samples were collected from 3 major municipal WTTPs in Finland—Turku (A), Tampere (B), and Helsinki (C)—during 3 consecutive days in the beginning of August 2014 (Figure 1). Samples were frozen until analysis. Two replicates of each sample were collected for the laboratory analysis, and each sample was analyzed in triplicate.

The treated amount of sewage in the studied WWTPs varied from 65 000 to 278 000 m^3/d. WWTP A serves approximately 300 000 people and WWTP B approximately 220 000 people. Based on the load, WWTP C is the largest in the Nordic countries, serving approximately 800 000 people. The investigated WWTPs combine biological, mechanical, and chemical processes for sewage treatments, including screening, ferrous

TABLE 1: Physical–chemical properties and consumption data from the year 2013 of the studied antibiotics (SRC PhysProp Database 2015)

Compound class	Compound	CAS number	MW (g/mol)	Water solubility (mg/L)	logK_{OW}	pK_a, 25 °C	Henry's law constant (atm m^3/mol)	Excreted unchanged (%)	Consumption in Finland (kg/yr; Fimea 2013)
Sulfonamides	Sulfadiazine	68-35-9	250.3	77	–0.09	6.36	1.58×10^{-10}	30–44[g]	319
	N-Acetyl sulfadiazine	127-74-2	292.3	150	0.39	—	5.13×10^{-13}	Metabolite	—
	Sulfamethoxazole	144-82-1	270.3	1050	0.54	2.1[c]	2.63×10^{-14}	60[b]	315
	N-Acetyl sulfamethoxazole	21312-10-7	295.3	1220	1.21	—	3.10×10^{-15}	Metabolite	—
Diaminopyrimide	Trimethoprim	738-70-5	290.3	400	0.91	7.12	2.39×10^{-14}	45–56[b]	788
Macrolides	Erythromycin	0114-07-08	733.9	1.4	3.06	8.88	5.42×10^{-29}	>60[a]	207
	Erythromycin enol ether	33396-29-1	715.9	—	—	—	—	Metabolite	—
	Roxithromycin	80214-83-1	837.1	0.02	2.75	12.45–9.08[c]	4.97×10^{-31}	>60[a]	136
	Clarithromycin	81103-11-9	748	0.34	3.16	8.99	1.73×10^{-29}	25[f]	276
	Tylosin	1401-69-0	916.1	5	1.63	7.73	5.77×10^{-38}	50–100[e]	Veterinary drug
ß-Lactams	Benzylpenicillin (G)	61-33-6	334.4	210.4	1.83	2.74	1.16×10^{-14}	50–70[a]	284
	Fenoxymetylpenicillin (V)	1987-08-01	350.4	101.1	2.09	2.79	4.42×10^{-15}	~40[a]	1029
	Cloxacillin	61-72-3	436.9	13.9	2.48	2.78	1.89×10^{-17}	—	276
	Ampicillin	69-53-4	349.4	10 100	1.35	3.24–7.44[c]	2.39×10^{-17}	30–60[a]	79
	Cephalexin	15686-71-2	347.4	1789	0.65	3.45–7.44[c]	2.77×10^{-17}	—	9579
Tetracyclines	Tetracycline	60-54-8	444.5	231.1	–1.3	3.3	4.66×10^{-24}	80–90[a]	1498
	Doxycycline	564-25-0	444.5	630	–0.02	–2.2 to 7.75[c]	4.66×10^{-24}	>70[a]	518
	Oxytetracycline	79-57-2	460.4	313	–0.9	3.27	1.70×10^{-25}	>80[a]	Veterinary drug
Anticonvulsant	Carbamazepine	298-46-4	236.3	112	2.45	–19.76	1.08×10^{-10}	85–90[d]	3311

[a]Hirsch et al. (1999).
[b]McEvoy (2004).
[c]Drugbank (2015).
[d]Heberer and Feldmann (2005).
[e]Kim et al. (2011).
[f]Zuccato et al. (2005).
[g]Rongkavilit et al. (2010).
CAS = Chemical Abstracts Service; MW = molecular weight.

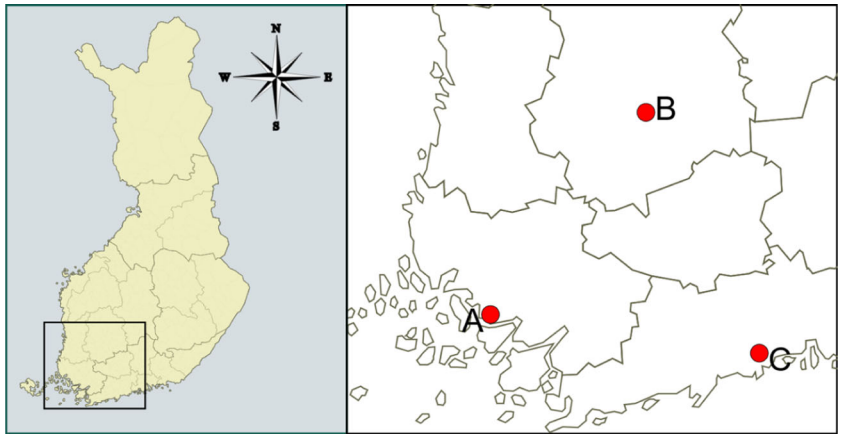

FIGURE 1: The investigated wastewater-treatment plants in Finland: A = Turku; B = Tampere; C = Helsinki.

salt addition, grit removal, sedimentation, aeration, activated sludge, denitrification, nitrification, conventional activated sludge, polymer addition, sand filtration, and/or biological filtration in the primary and/or secondary treatments (Table 2). Wastewater treatment plants A and C applied a tertiary treatment—sand filtration and biological filter, respectively. No disinfection step (such as chlorine, ozone, or ultraviolet treatment) was applied in any of the investigated WWTPs (Helsinki Region Environmental Services Authority n.d.; Tampereen Seudun Keskuspuhdistamo Oy n.d.; Turun seudun puhdistamo Oy n.d.).

In mechanical treatment processes, large objects and particles are removed from the influent, allowing the solid particles to settle. Chemical treatment involves chemical addition to precipitate ferrous sulfate and nutrients by flocking. This treatment is often performed in an aeration tank in several steps, some of which may be anaerobic. After aeration, there is a secondary sedimentation stage such as biological filtration, where the purification of the water is completed before it is released into the environment. The formed sludge is further treated and refined to be used as, for example, earth filling materials (Von Sperling 2007). The hydraulic retention time (hours) in the investigated WWTPs varied from 9.6 to 20 h. The solids retention time (days) varied from 11 to 20 d (Table 2).

Sample preparation

Before analysis, frozen samples were thawed, and 9.9 mL of the thawed samples were pipetted into 12-mL glass vials. The samples were divided into triplicate. The sample preparation consisted of centrifugation (FP-510 Centrifuge; Labsystems Oy) at 4500 rpm for 30 min of 9.9 mL of influent or effluent sample spiked with 100 μL of mixture of internal standards to reach the final concentration of 10 ng/mL. After that, the liquid part of the sample was transferred to 6-mL vials without septum. The pH values of the wastewater samples varied from 6.8 to 7.4.

Online SPE LC-MS/MS method

For MS/MS analysis, an Agilent 6460 triple-quadrupole mass spectrometer equipped with an Agilent Jetspray electrospray ionization source was used in dynamic multiple reaction monitoring mode. Two transitions were monitored for each compound, and the compounds were analyzed in positive

TABLE 2: Information about the studied wastewater-treatment plants

WWTP	Influent flow (m^3/d)	Population served	SRT (d)	HRT (h)	Preliminary treatment	Secondary treatment	Tertiary treatment	Recipient environment
A Turku	90 000	300 000	19	17.5	pre-S + fine S + Fe + G + Sed	Fe + AS (DN/N) + Sed	SF	Baltic Sea (Linnanaukko Harbor basin, Archipelago Sea)
B Tampere	65 000	220 000	11	20	pre-S + fine S + Fe + G + Sed	CAS + Pol + Sed	None	Lake Pyhäjärvi
C Helsinki	278 000	800 000	10	9.6	S + G + Fe + pre-Aer + Sed	AS (DN/N) + Fe + Sed	MeOH + BF	Baltic Sea (Gulf of Finland)

Aer = aeration; AS = activated sludge; BF = biological filter; CAS = conventional activated sludge; DN = denitrification; Fe = ferrous salt addition; G = grit removal; HRT = hydraulic retention time; N = nitrification; Pol = polymer addition; S = screening; Sed = sedimentation; SF = sand filtration; SRT = solids retention time; WWTP = wastewater-treatment plant.

TABLE 3: List of compounds with precursor and product ions, as well as fragmentor voltage collision energy retention time used for the dynamic multiple reaction monitoring method

Target compound	Precursor ion MS1	Product ions	Fragmentor (V)	Collision energy (V)	Retention time (min)
Ampicillin	350.1	159.9	110	12	5.47
		106.2		20	
Benzylpenicillin (G)	335.1	176.1	100	9	6.73
		160		9	
Carbamazepine	237.3	194	130	17	6.78
		179		37	
Cephalexin	348.1	174.1	95	9	5.42
		158		5	
Clarithromycin	748.5	158.1	165	29	6.84
		83.1		60	
Cloxacillin	436.1	277.1	100	9	7.34
		160.1		9	
Doxycycline	445.2	428.1	125	17	6.11
		98.1		53	
Erythromycin enol ether	716.5	558.4	165	13	6.82
		158.1		29	
Phenoxymethylpenicillin (V)	351.1	229.2	180	13	6.87
		160		8	
Erythromycin	734.5	576.2	160	16	6.45
		158		32	
N-Acetyl sulfadiazine	293.1	134.1	110	21	5.53
		65.1		49	
N-Acetyl sulfamethoxazole	296.1	134.1	110	25	6.24
		65.1		49	
Oxytetracycline	461.2	443.2	120	9	5.77
		426.1		17	
Roxithromycin	837.5	679.4	190	17	6.90
		158.1		37	
Sulfadiazine	251.1	156	105	13	5.41
		92.1		25	
Sulfamethoxazole	254.1	156	105	13	6.13
		92.1		25	
Tetracycline	445.2	427	110	8	5.62
		410		16	
Trimethoprim	291.2	230.1	130	21	5.39
		123.1		25	
Tylosin	916.5	174	215	44	6.60
		101.1		56	
4-Epi-tetracycline-D6	451.2	416.2	135	17	5.54
Benzylpenicillin-D7	342.1	183.1	90	9	5.71
Sulfadiazine-D4	255	96.1	110	25	5.40
Trimethoprim-D3	294	123.1	145	25	5.38
Cephalexin-D5	353	158	95	5	5.56
Carbamazepine-D8	245	202.1	105	20	6.75

electrospray ionization mode (Table 3). The internal standard (IS) method was used for quantification. Six isotope-labeled ISs were matched to the individual analytes by retention time and compound class (Table 4).

Nitrogen (99.5%) was used as a drying gas, sheath gas, nebulizer gas, and collision gas (purity of 99.999%). All the instrumental data, working principles of the online SPE system, and optimization results are presented in Meierjohann et al. (2017). Chromatographic separation was performed using an Agilent 1290 binary pump (pump 1). For loading and enrichment, an Agilent 1100 series binary pump (pump 2) coupled to an Agilent 1260 autosampler was used. A reusable Agilent Bond Elut online trapping column of spherical, rigid, macroporous polystyrene and divinylbenzene with the dimensions of 2.1 × 12.5 mm (15–20 µm) was used (Meierjohann et al. 2017).

A 1.8-mL aliquot of extract was injected onto an Agilent Poroshell HPH C_{18} column (2.1 × 50 mm, 2.7 µm) equipped with a guard column (Agilent, EC C_{18} precolumn, 2.7 µm, 2.1 × 15 mm). The eluents for both pumps 1 and 2 were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B).

Data quantification and method validation

A calibration curve of 7 concentrations (1, 5, 10, 50, 100, 500, and 1000 ng/L) was used for the quantification of antibiotics with a good linearity, >0.99.

Recoveries of the 19 target compounds were determined by 10 injections by the standard addition method at 10 and 100 ng/L. Limits of detection (LODs) of the antibiotics and

TABLE 4: Method validation results of the online solid-phase extraction liquid chromatography tandem mass spectrometry method

Compound class	Target compound	LOD (ng/L)	LOQ (ng/L)	ME (%)	Repeatability (RSD, %)		Linearity (r^2)	q/Q ratio	IS
					10 ng/L	100 ng/L			
Sulfonamides	Sulfadiazine	0.75	5	76	10	3	0.993	0.77	Sulfadiazine-D_4
	N-Acetyl sulfadiazine	0.75	1	65	5	4	0.992	0.68	
	Sulfamethoxazole	0.1	1	72	6	11	0.994	0.9	
	N-Acetyl sulfamethoxazole	0.75	1	69	14	7	0.993	0.69	
Diaminopyrimidine	Trimethoprim	0.5	1	73	4	1	0.992	0.74	Trimethoprim-D_3
Macrolides	Erythromycin	0.25	1	75	16 ($n = 9$)	19	0.997	0.62	Trimethoprim-D_3
	Erythromycin enol ether	2.5	5	135	24	12	0.996	0.95	
	Roxithromycin	5	10	122	20 ($n = 9$)	18	0.992	0.68	4-Epi-tetracycline-D_6
	Clarithromycin	2.5	5	116	11	10	0.992	0.34	
	Tylosin	0.25	2.5	128	14	14	0.997	0.52	
ß-lactams	Benzylpenicillin	0.5	1	61	4	1	0.995	0.87	Benzylpenicillin-D_7
	Phenoxymethylpenicillin	0.5	1	79	4	7	0.990	0.74	
	Cloxacillin	0.5	1	65	6	2	0.992	0.98	
	Ampicillin	0.5	1	62	7	5	0.992	0.51	Cephalexin-D_5
	Cephalexin	1	1	63	10	18	0.997	0.56	
Tetracyclines	Tetracycline	0.75	1	67	11	3	0.995	0.65	4-Epi-tetracycline-D_6
	Doxycycline	0.75	1	79	6	8	0.991	0.3	
	Oxytetracycline	0.1	1	68	19	3	0.990	0.49	
Anticonvulsant	Carbamazepine	0.25	1	81	6	6	0.998	0.55	Carbamazepine-D_8

IS = internal standard; LOD = limit of detection (ng/L); LOQ = limit of quantification (ng/L); ME = matrix effect obtained in synthetic wastewater (%); RSD = relative standard deviation.

metabolites were determined as the lowest concentrations resulting in a signal-to-noise ratio of 3. Limits of quantification (LOQs) were calculated with a signal-to-noise ratio of 10 (Table 4). To check for background contamination, a standard blank of ultrapurified water was analyzed between every sample. To determine the matrix effect, 5 injections of the analytes and ISs (at a concentration of 100 ng/L) in pure water and synthetic wastewater matrix (Table 5) were performed. The matrix effect was determined by calculating the ratios of the peak areas of the analytes in the synthetic matrix and in clean water. Synthetic wastewaters were used in the method validation tests to ensure that no traces of antibiotics were present in the matrix. In addition, isotope-labeled ISs were used after rigorously testing their behavior and extraction efficiency in the wastewater matrix for the quantification and for compromising the matrix effect.

ERA

The ERA calculations were based on the RQ for each compound. The RQ value was calculated by considering the worst possible scenario according to the European guidelines (Commission of the European Communities 2003), that is, by assuming the highest concentration detected in the wastewater as the measured environmental concentration (MEC). Environmental risk was assessed by computing the RQ for 3 trophic levels: algae, invertebrates, and fish. The RQ was calculated as a ratio between the MEC and predicted-no-effect concentration (PNEC),

TABLE 5: Composition of the synthetic wastewater[a]

Synthetic wastewater		Nutrient solution	
Component	Concentration (mg/L)	Component	Concentration (g/L)
$NaHCO_3$	328.2	EDTA	10.0
Yeast extract	209.7	$FeCl_3 \times 6\ H_2O$	1.50
Pepton	184.7	$MnCl_2 \times 4\ H_2O$	0.19
$CH_3COONa \times 3\ H_2O$	130.8	KI	0.18
$CaCl_2 \times 2\ H_2O$	70.0	H_3BO_3	0.15
$MgSO_4 \times 7\ H_2O$	60.9	$CoCl_2 \times 6\ H_2O$	0.15
NH_4Cl	38.2	$ZnSO_4 \times 7\ H_2O$	0.12
KH_2PO_4	35.1	$(NH_4)_6Mo_7O_{24} \times 4\ H_2O$	0.04
		$CuSO_4 \times 5\ H_2O$	0.03

[a]The final synthetic wastewater was prepared through addition of 0.3 mL nutrient solution per liter of synthetic wastewater.
EDTA = ethylenediaminetetraacetic acid.

divided by an assessment factor (usually 1000; equation 1: RQ = predicted-effect concentration [PEC]/PNEC). The PNEC toxicity data were collected from various studies. In addition, the corresponding dilution factor (DF) from the "National Annual Media Dilution Factor" calculated for each country by Keller et al. (2014) was applied to the calculation (Equation 2: PEC = MEC/DF). The approach suggested by Keller et al. (2014) combines the ratio between the volume of freshwater available and the domestic sewage discharge. However, following the European guidelines (Commission of the European Communities 2003), a default DF of 100 was applied; and the results were compared. Experimental toxicity data for the target antibiotics was available in the literature, and the PNEC calculated from these data was used. The RQ was evaluated according to commonly used risk-ranking criteria (Commission of the European Communities 2003): RQ < 0.1 is considered low environmental risk, 0.1 < RQ < 1 is considered moderate environmental risk, 1 < RQ < 10 is considered high environmental risk.

RESULTS AND DISCUSSION

Method performance and quality control

In most cases, a complex wastewater-like matrix requires a separate sample preparation step, such as SPE, for sample concentration and cleanup, prior to the analysis. Therefore, to develop a method that will combine both quick sample preparation and advanced analysis might be challenging. The developed online SPE LC-MS/MS method for analyzing multiple antibiotics proved to be fast (total analysis time of 7.5 min) and reliable, performing well in method validation.

The LODs obtained in the test varied from 0.75 to 5 ng/L. For all compounds, the LOD and LOQ depended rather on the individual compounds than on the group of the compounds (or the compound class). For CEPH, ROXI, and CLARI, the LOD was <1 ng/L. The LOQs ranged from 1 to 10 ng/L. Although the LOQs of TETR, β-lactams, and all sulfonamides except for SDZ (LOQ = 5 ng/L) were 1 ng/L, macrolides tended to have slightly higher detectability. In the case of macrolides, the LOQ was 1 ng/L for ERY; and for ERY-EE, ROXI, CLARI, and TYL, the LOQs were 5, 10, 5, and 2.5 ng/L, respectively (Table 4). Achieving good sensitivity (low LODs and LOQs) plays an important role, especially in analyzing environmental samples where the concentrations might be low.

The matrix effect for all compounds varied from 61 to 178%. For sulfonamides, the matrix caused a suppression of ionization that ranged from 15 to 36% (mean 20%). Antibiotics belonging to the macrolide class showed matrix enhancement in most cases (besides ERY, 75%) ranging from 116 to 135% (Table 4).

The repeatability of the method was assessed by calculating the relative standard deviation (RSD, percentage) for the 19 target compounds for 10 replicate injections at concentrations of 10 and 100 ng/L. The RSDs for 10 and 100 ng/L of the studied antibiotics were 4 to 24% and 1 to 19%, respectively. Repeatability at 10 ng/L of ERY-EE and ROXI was 24 and 20% ($n = 9$), respectively. This might be because the LOQ values for these compounds were relatively close to the tested concentrations for the repeatability.

The calculated standard deviation (SD) for all the analyzed compounds was <20%. No group-specific patterns of SD were observed. The linear response of the method proved to be good over the calibration range, with an r^2 value in excess of 0.99 for all compounds. From all the studied antibiotic groups, macrolides proved to be analytically challenging, the matrix effect rising to 135%, compared to other compounds with higher RSD and higher LOQ values.

Occurrence of antibiotics in influent and effluent samples

In the present study, the presence of antibiotics in the 3 WWTPs (A, B, and C) on 3 consecutive days was determined (Table 6). The mean removal efficiency (percentage) was calculated based on the mean concentrations of antibiotics in influent and effluent of the 3 consecutive days (Table 6). The mean concentrations of the consecutive days of influent and effluent in 3 WWTPs are presented in Figure 2. The mean removal efficiencies in all investigated WWTPs are presented in Figure 3. In all the investigated WWTPs, treatment was based on phosphorus precipitation by ferrous sulfate. Plant B applied conventional activated sludge treatment and polymer addition for the removal of biologically degradable organic matter, whereas the other 2 WWTPs applied an activated sludge process together with denitrification to enhance the nitrogen removal. A long solids retention time was found to have a positive effect on the removal of several compounds that are mainly removed by biodegradation, such as TMP (Leu et al. 2012).

Sulfonamides. All 4 investigated sulfonamides (SDZ, SMX, and the metabolites *N*-SDZ and *N*-SMX) were found in all WWTPs (Table 6). In general, sulfonamides were the most abundant antibiotics in wastewater (Figure 2), with SDZ being the major antibiotic in all influent water collected from the WWTPs (326–1069 ng/L). In WWTPs B and C, the concentrations of the metabolite *N*-SDZ were in the same range as those of SDZ. In contrast, the concentrations of *N*-SDZ in the influent of WWTP A were almost 5 times lower than the concentrations of SDZ. The concentrations of SMX in the influent samples were lower (ranging 20–71 ng/L) than the concentrations of SDZ. The metabolite *N*-SMX was not found in the influent samples from WWTP A, whereas its concentrations in WWTPs B and C were in a similar range to the concentrations of the parent compound.

In effluent waters, the concentrations of SDZ were lower than concentrations found in the influent samples (Table 6). Concentrations of SDZ in the effluent samples were <194 ng/L. Its mean removal rate in all WWTPs was approximately 83%. Concentrations of the metabolite *N*-SDZ were lower than those of SDZ (101–173 ng/L). The mean removal efficiency of the metabolite *N*-SDZ in all the WWTPs was lower than that of SDZ (~62%). The SMX concentration was below the detection limit (0.1 ng/L) in WWTP A, but in the effluent samples of WWTPs B and C, the compound was detected at a concentration <10 ng/L. The metabolite *N*-SMX was below the LOD in all the effluent samples.

TABLE 6: Concentrations of target compounds in influent and effluent from 3 consecutive days of 3 wastewater-treatment plants

			Concentration (ng/L)														
			A					B					C				
Compound class	Target compound	Sample	1	2	3	±SD	MR (%)	1	2	3	±SD	MR (%)	1	2	3	±SD	MR (%)
Sulfonamides	Sulfadiazine	Inf	913	1072	1069	91	83	326	363	414	44	65	500	549	386	83	91
		Eff	194	180	160	17		109	125	151	21		47	41	46	3	
	N-Acetyl sulfadiazine	Inf	192	382	222	102	58	408	528	511	65	77	581	237	290	185	55
		Eff	117	101	117	9		114	106	112	4		173	154	166	9	
	Sulfamethoxazole	Inf	38	20	59	19	100	29	57	61	18	85	71	50	47	13	93
		Eff	<LOQ	<LOQ	<LOQ	—		6	6	9	2		4	4	5	1	
	N-Acetyl sulfamethoxazole	Inf	<LOQ	<LOQ	<LOQ	—	—	23	30	29	3	100	96	48	53	27	100
		Eff	<LOQ	<LOQ	<LOQ			<LOQ	<LOQ	<LOQ	—		<LOQ	<LOQ	<LOQ	—	
Diaminopyrimide	Trimethoprim	Inf	210	294	220	46	13	170	254	226	43	–145	490	338	259	118	4
		Eff	265	218	147	59		563	500	531	31		322	355	370	25	
Macrolides	Clarithromycin	Inf	216	58	241	100	–21	57	68	50	9	–114	327	160	173	93	34
		Eff	214	189	219	16		130	113	131	10		141	140	157	9	
	Erythromycin	Inf	16	5	682	388	85	5	9	5	2	–457	27	11	13	9	–214
		Eff	25	24	54	17		40	34	31	4		51	52	54	1	
	Erythromycin enol ether	Inf	41	13	1424	806	89	16	22	11	5	–93	71	32	35	22	–274
		Eff	51	42	63	11		33	32	30	1		189	169	157	16	
	Roxithromycin	Inf	52	12	57	25	12	7	8	7	1	–203	145	60	63	48	26
		Eff	40	33	32	4		21	19	26	4		79	59	61	11	
ß-Lactams	Cloxacillin	Inf	<LOQ	<LOQ	<LOQ	—	—	<LOQ	<LOQ	<LOQ	—	—	<LOQ	<LOQ	<LOQ	—	—
		Eff	<LOQ	<LOQ	<LOQ			30	26	27	2		21	0	15	11	
Anticonvulsant	Carbamazepine	Inf	104	47	107	34	–186	130	165	161	19	–53	186	113	83	53	–55
		Eff	248	224	266	21		245	219	234	13		210	189	193	11	

A = Turku; B = Tampere; C = Helsinki; Eff = effluent; Inf = influent; <LOQ = below the limit of quantification; MR = mean removal; SD = standard deviation; — = negative mean removal, means that the concentrations were higher in effluent than in influent; 1, 2, 3 = sample in consecutive days.

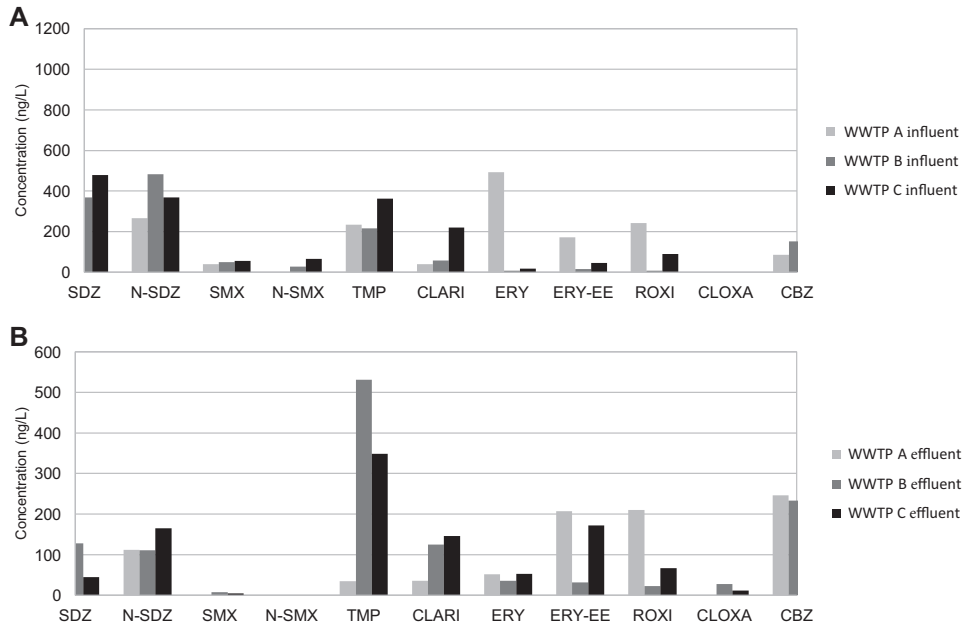

FIGURE 2: Mean concentrations of antibiotics in (**A**) influent and (**B**) effluent of the 3 studied wastewater-treatment plants. CBZ = carbamazepine; CLARI = clarithromycin; CLOXA = cloxacillin; ERY = erythromycin; ERY-EE = erythromycin enol ether; N-SDZ = *N*-acetyl sulfadiazine; N-SMX = *N*-acetyl sulfamethoxazole; ROXI = roxithromycin; SDZ = sulfadiazine; SMX = sulfamethoxazole; TMP = trimethoprim; WWTP = wastewater treatment plant.

N-Acetylated products are the major metabolites of sulfonamides, which are biologically inactive while entering sewage (Davis 1943). During wastewater treatment, the metabolites can transform back to the active parent compounds (Göbel et al. 2005), which can lead to negative removals of these compounds or to underestimation of their removal efficiencies. This process might be one of the reasons for the high variations in removal rates presented in the literature (Birošová et al. 2014; Yuan et al. 2014; Papageorgiou et al. 2016).

In a previous study, SDZ was detected in a Greek WWTP at concentrations up to 846 and 194 ng/L in influent and effluent, respectively, only during the spring season (Papageorgiou et al. 2016). According to the literature, SMX is one of the most commonly detected sulfonamides in WWTPs worldwide (Hanna et al. 2018). In another WWTP in Finland, SMX was found at mean concentrations of 202 and 130 ng/L in influent and effluent, respectively (Ngumba et al. 2016). In Slovakia, SMX was detected at concentrations of 103 and 88 ng/L in influent

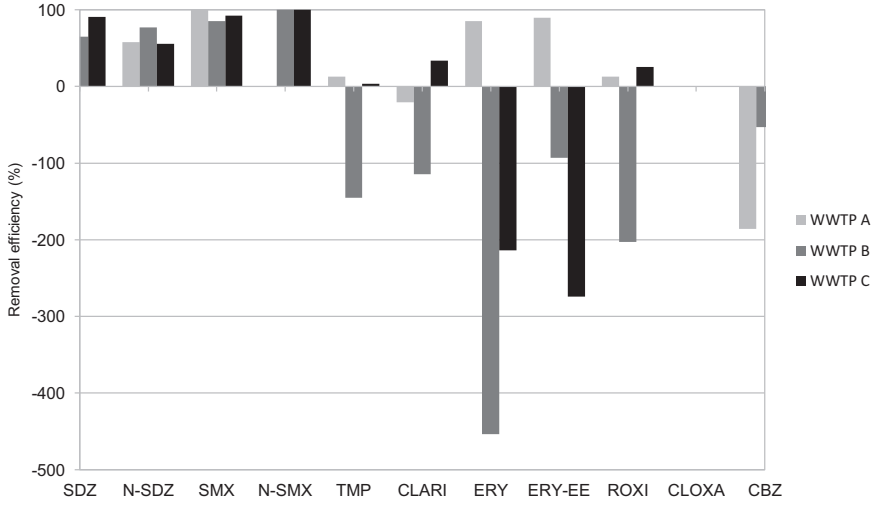

FIGURE 3: Mean removal efficiencies of the studied antibiotics in the wastewater-treatment plants. CBZ = carbamazepine; CLARI = clarithromycin; CLOXA = cloxacillin; ERY = erythromycin; ERY-EE = erythromycin enol ether; N-SDZ = *N*-acetyl sulfadiazine; N-SMX = *N*-acetyl sulfamethoxazole; ROXI = roxithromycin; SDZ = sulfadiazine; SMX = sulfamethoxazole; TMP = trimethoprim; WWTP = wastewater treatment plant.

and effluent, respectively (Birošová et al. 2014). Another study reported SMX in a WWTP in Greece at average concentrations of 88 and 17 ng/L in influent and effluent, respectively (Papageorgiou et al. 2016).

TMP (diaminopyrimidine). Trimethoprim was detected in all investigated WWTPs in both the influent and effluent samples (Table 6). In influent, TMP was found to occur at concentrations ranging from 170 to 490 ng/L. Concentrations of TMP in effluent waters were not considerably lower than in influent samples, but in WWTP B, the concentration in the effluent was approximately twice as high as in the influent water. Possibly, an effective deconjugation of TMP metabolites takes place in this treatment plant, resulting in an enhanced concentration of the parent compound in the effluent samples (Dinh et al. 2017). Previous studies have shown that TMP is metabolized to glucuronide conjugates that are cleaved by bacteria during the wastewater-treatment processes (Nordholm and Dalgaard 1984).

According to the consumption data published by the Finnish Medicines Agency (Fimea n.d.), TMP is used more than any of the analyzed sulfonamides. However, the concentrations of TMP were lower than those of the sulfonamides. This could indicate that TMP is metabolized to a greater degree than the sulfonamides (McEvoy 2004; Rongkavilit et al. 2010; Table 1). In human medicine TMP is commonly used in combination with sulfonamides at a ratio of 1:5 (Göbel et al. 2005). A ratio of 1:4 in influent from WWTP A between TMP and SDZ was noticed. However, the combination ratio between TMP and SDZ could not be observed in any other samples. On the other hand, the concentration of SMX in every influent sample was approximately 6 times lower than the concentration of TMP, which indicates that the use of SDZ in combination with TMP in Finland was preferred and that TMP is used as the synergist for other sulfonamides besides SDZ or SMX. The presence of TMP might imply that at least 5 times as much SMX is consumed. A review evaluating the SMX/TMP ratio as a potential marker in raw wastewater has concluded that hospital effluents and WWTP influents presented similar SMX/TMP footprints, whereas livestock effluents showed higher SMX/TMP ratios, mostly because of the use of SMX alone (Thiebault 2020).

In another study in Finland, TMP was reported to be present at a mean concentration of 558 ng/L in the influent and 517 ng/L in the effluent of one WWTP (Ngumba et al. 2016). In Italy, TMP was reported at concentrations of 59 and 40 ng/L in influent and effluent, respectively (Verlicchi et al. 2014). A study from Slovakia reported TMP at concentrations of 100 and 87 ng/L in influent and effluent, respectively (Birošová et al. 2014).

Macrolides. All the investigated macrolides (CLARI, ERY, ROXI, TYL, and the metabolite ERY-EE) were found in the influent samples with the exception of TYL, which could not be found in any of the analyzed samples (Table 6). The macrolide CLARI was detected in all influent waters (50–327 ng/L), and ERY was found at concentrations <27 ng/L in all influent samples, with the exception of the sample collected on the third sampling day from WWTP A, where the concentration was close to 700 ng/L. The concentration of the metabolite ERY-EE was also increased (~1400 ng/L) in the third sample from WWTP A, whereas in the samples from the first 2 d, the concentration of ERY-EE was significantly lower (≥41 ng/L). In a duplicate of the sample from the same day the results were similar. In addition, the samples were analyzed several times, and the results were in agreement. No obvious explanation for the extreme concentrations in this sample could be found. The samples were stored and treated in the same way as all other samples, and all the quality precautions were performed correctly. In addition, the sample preparation includes only 2 simple steps: addition of internal standards and centrifugation of the sample. Thus, transformation to another molecule was very unlikely. Previous studies showed that ERY is unstable under strong acidic condition and will be converted into ERY-EE (Hirsch et al. 1999; Zhang and Li 2011). The variations in the concentrations of ERY and its metabolite ERY-EE during the sampling campaign were on average 53 times higher on the third day than on the other 2 d. To better understand the occurrence of the antibiotics, more sampling events through the whole year would be necessary. According to the study performed by Marx et al. (2015) for antibiotics that show fluctuating input loads, 30 to 40 samples through the year are needed for representative determination of the concentrations in WWTPs. In influent waters, ROXI was found at concentrations similar to those of other macrolides (besides CLARI; commonly <70 ng/L). The highest concentration of ROXI was observed in the samples from WWTP C (60–145 ng/L).

In WWTPs A and B, the concentration of CLARI was higher in the effluent samples than in the influent samples, providing negative removal efficiency (Table 6). In WWTP C, the mean removal rate for CLARI was 34%. Also, the concentrations of ERY and the metabolite ERY-EE were higher in effluent samples from WWTPs B and C, and they thus showed negative removal efficiencies. No extraordinarily high concentrations of ERY and ERY-EE were detected in the effluent sample corresponding to the influent sample from the WWTP A sample, where these compounds were detected at extremely high concentrations. The removal rate of ROXI was 12 and 26% at WWTPs A and C, respectively. All macrolides were found to have negative removal efficiencies in WWTP B, which does not apply a tertiary treatment process; and this might be an explanation for the negative removal efficiencies.

In addition, the negative removal rate of some compounds, such as ROXI, might be related to their low measured concentrations, which makes them more susceptible to sampling errors or analytical errors. In other cases, the negative removal rate might result from persistence of the compound, a stronger matrix effect in the influent sample (Rossmann et al. 2015), or the transformation of conjugates back to the parent compounds during the wastewater-treatment processes (Göbel et al. 2005).

Three of the macrolides, CLARI, ERY, and azithromycin (not included in the present study), have been added to the European Union's watch list of potential priority substances, which was adopted in 2015 (Water Framework Directive 2013, Directive on Environmental Quality Standards [EQS]; in Decision 2015/495 [European Commission 2015]). The substances

were selected based on data on their occurrence, available treatment applied in WWTPs, and drinking water production. To our knowledge, no decision has yet been made on whether EQS should be set for these substances under the Water Framework Directive.

The results of previous studies on macrolides have been comparable to ours. In Slovakia, CLARI was found at high concentrations of 1646 ng/L in influent samples and 1260 ng/L in effluent samples, with a mean removal efficiency of 22% (Birošová et al. 2014). In the same study, ERY was detected at concentrations of 82 and 14 ng/L in influent and effluent, respectively, with a mean removal efficiency of 84% (Birošová et al. 2014). The concentrations of ROXI found in Italy were 65 and 290 ng/L in influent and effluent, respectively, with a negative removal efficiency (Verlicchi et al. 2014).

β-Lactams. Although β-lactams are very frequently used in Finland (Table 2), none of them could be detected in the influent samples, and only low concentrations of CLOXA (<30 ng/L) could be observed in some effluent samples (WWTPs B and C; Table 6).

With a high resistance to the β-lactamase enzymes, CLOXA is consequently more stable than the other β-lactams studied (O'Callaghan and Muggleton 1967). In addition, the β-lactam ring is easily opened by chemical hydrolysis and by the bacterial β-lactamase enzymes, resulting in inactive compounds. Because of their instability, β-lactams are not considered to pose a threat to the environment (Cha et al. 2006).

Even though some studies have reported the presence of a few β-lactams in influent waters, these compounds tend to be removed significantly in wastewater-treatment processes (Van den Meersche et al. 2016). For example, >96% of CEPH (from ~2000 ng/L in influent to 78 ng/L in effluent) was removed in a conventional WWTP in Australia (Costanzo et al. 2005). In another study, from Canada, CLOXA was found at concentrations of 19 and 12 ng/L in influent and effluent, respectively (Guerra et al. 2014), and PHPEN was detected at concentrations of 50 and 14 ng/L in influent and effluent, respectively (Guerra et al. 2014). In Greece, AMP was found in a WWTP at average concentrations of 1243 and 150 ng/L in influent and effluent, respectively (Papageorgiou et al. 2016).

TETR. Despite the frequent detection of TETRs in other sewage-treatment plants worldwide and their high use in Finland, TETRs could not be detected in the present study. Previous studies have shown that TETRs sorb onto activated sludge, sediments, and particles to a greater extent than other antibiotics (Samuelsen et al. 1992) and that their concentrations might thus be below the LOD.

However, in a study of a smaller WWTP in Finland (Jyväskylä WWTP, which receives an average of 35 000 m^3 of wastewater per day and serves a population of 150 000), TETR was detected at mean concentrations of 37 ng/L in influent and 18 ng/L in effluent (Ngumba et al. 2016). In addition, in the same study, DOXY was found at the mean concentrations of 55 and 18 ng/L in influent and effluent, respectively (Ngumba et al. 2016). In one of the WWTPs in a Slovakian study, TETR was found at approximately 6 ng/L in both influent and effluent waters (Birošová et al. 2014). In Sweden, DOXY was detected at concentrations up to 2480 ng/L in influent and up to 915 ng/L in effluent (Lindberg et al. 2005). However, DOXY in a WWPT in Slovakia was detected at concentrations of 13 and 4 ng/L in influent and effluent, respectively (Birošová et al. 2014), and OXY was detected at concentrations of 8 ng/L in influent and <3 ng/L in effluent (Birošová et al. 2014).

CBZ. In the present study, CBZ was included as a tracer for wastewater contamination. Previous studies have demonstrated that CBZ is very stable and resistant to biodegradation, and thus the compound can be used as a marker for natural waters mixed with effluent-originated waters (Hajj-Mohamad et al. 2014; Tran et al. 2018).

Carbamazepine was detected in all effluent and influent samples (47–417 ng/L; Table 6). In all investigated WWTPs, CBZ had a negative removal efficiency, which is to say that in all cases the concentrations of CBZ in individual samples from effluent were higher than the concentrations in influent samples. This result is in accordance with the findings of previous studies (He et al. 2019). It has been argued that the negative removal rate of CBZ results mainly from the deconjugation of the glucuronide-*N*-carbamazepine during digestion by bacteria in wastewater-treatment processes (Maggs et al. 1997). Multiple studies have reported high concentrations of CBZ in the aquatic environment (Brumovský et al. 2017; Björlenius et al. 2018), and thus, CBZ has received growing attention as an emerging contaminant based on its potential threat to aquatic organisms (Aguirre-Martínez et al. 2013; Tsiaka et al. 2013; Hampel et al. 2014).

Calculations of measured mass loads

The concentrations of antibiotics in the influent and effluent of the three 24-h composite sampling intervals were averaged, and the average concentrations were then used to calculate average daily mass fluxes to the WWTPs and receiving waters (mg/d/1000 population equivalent). The average mass fluxes of each antibiotic were calculated by multiplying the measured average concentrations of antibiotics (ng/L) by the average influent flow (m^3/d) and dividing by the average population served (Table 7). For comparison reasons, the results were normalized to 1000 population equivalent in Table 7.

Because the sampling was performed during summer, no extra loads from melting snow contributed to the overall flow rate. During the sampling time, no considerable rain was noticed. The individual loads of the studied antibiotics, ranging between 0.3 and 0.35 g/d, were similar in all the studied WWTPs because of their similarity in size.

The biggest mass loads were noticed for sulfonamides, ranging from 8 to 305 mg/d per 1000 population equivalent to WWTPs and from 2 to 53 mg/d per 1000 population equivalent to the environment (Table 7). The mass loads of TMP ranged from 64 to 126 mg/d per 1000 population equivalent to WWTPs and from 63 to 157 mg/d per 1000 population equivalent to the environment. The mass loads of macrolides ranged

TABLE 7: Daily mass loads of antibiotics to wastewater treatment plants (WWTPs) and to the receiving waters per 1000 capita

	Mass loads of antibiotics to WWTPs (mg/d/1000 PE)										
WWTP	SDZ	*N*-SDZ	SMX	*N*-SMX	TMP	CLARI	ERY	ERY-EE	ROXI	CLOXA	CBZ
A	305	80	12	n.a.	72	52	70	148	12	n.a.	26
B	109	143	14	8	64	17	2	5	2	n.a.	45
C	166	128	19	23	126	76	6	16	31	n.a.	44
	Mass loads of antibiotics to receiving waters (mg^{-1} d^{-1} 1000 PE^{-1})										
Receiving waters	SDZ	*N*-SDZ	SMX	*N*-SMX	TMP	CLARI	ERY	ERY-EE	ROXI	CLOXA	CBZ
Archipelago Sea	53	34	n.a.	n.a.	63	62	10	16	11	n.a.	74
Lake Pyhäjärvi	38	33	2	n.a.	157	37	10	9	7	8	69
Gulf of Finnland	16	57	2	n.a.	121	51	18	60	23	4	69

A = Turku; B = Tampere; C = Helsinki; CBZ = carbamazepine; CLARI = clarithromycin; CLOXA = cloxacillin; ERY = erythromycin; ERY-EE = erythromycin enol ether; n.a. = estimated calculations not available, concentration of target antibiotic <LOQ; *N*-SDZ = *N*-acetyl sulfadiazine; *N*-SMX = *N*-acetyl sulfamethoxazole; PE = population equivalent; ROXI = roxithromycin; SDZ = sulfadiazine; SMX = sulfamethoxazole; TMP = trimethoprim.

from 2 to 148 mg/d per 1000 population equivalent to WWTPs and from 7 to 62 mg/d per 1000 population equivalent to the environment. The mass loads of CLOXA to WWTPs and to the environment were negligible. The mass loads of CBZ through the WWTPs ranged from 26 to 45 mg/d per 1000 population equivalent to WWTPs and from 69 to 74 mg/d per 1000 population equivalent to the environment (Table 7).

To compare, in another study, the mass loads of SMX and TMP into Ryaverket WWTP (situated in Gothenburg, Sweden) were observed to range from 10 to 70 mg/d per 1000 population equivalent and from 10 to 130 mg/d per 1000 population equivalent, respectively. The WWTP served approximately 628 000 to 726 000 people, and the flow rates ranged from 2.99 to 7.24 m^3/s during the study (Paxéus et al. 2016). The mass loads of SMX in the studied Swedish WWTP were smaller compared to the WWTPs investigated in the present study, whereas the mass loads of TMP were comparable to WWTP C (Paxéus et al. 2016) The studied Swedish WWTP and WWTP C were comparable in size. The authors of the study in Sweden reported decreased mass loads of TMP and SMX over approximately 9 yr, originating from the recommendations of the Swedish Strategic Program for Rational Use of Antimicrobial Agents and Surveillance of Resistance to limit the use of antibiotics.

Another study, from Switzerland, reported the mass loads of several antibiotics in the effluent waters of 2 WWTPs (Göbel et al. 2005). The average mass loads of SMX and *N*-SMX were 121 and 302 mg/d per 1000 population equivalent, respectively. The mass load of TMP was calculated to be 76 mg/d per 1000 population equivalent. The mass loads of the macrolides CLARI, ROXI, and ERY-EE were 118, 10, and 32 mg/d per 1000 population equivalent, respectively. In the effluent, the mass loads ranged from 84 to 340 mg/d per 1000 population equivalent and from 8 to 120 mg/d per 1000 population equivalent for SMX and TMP, respectively. For ROXI and ERY-EE, the mass loads ranged from 4 to 12 mg/d per 1000 population equivalent and from 24 to 44 mg/d per 1000 population equivalent (Göbel et al. 2005). These mass loads are close to our results, although the Finnish WWTPs were approximately 3 times smaller (Göbel et al. 2005). Another study, from Italy, reported mass loads for SMX, 61 and 7 mg/d per 1000 population equivalent in the influent and effluent of one WWTP, respectively. The mass loads were, for TMP, 14 and 12 mg/d per 1000 population equivalent and, for ERY-EE, not detected and 11 mg/d per 1000 population equivalent in the influent and effluent, respectively (Lindberg et al. 2005). A study from Italy reported the mass loads of SMX into the receiving river to be on average 27 mg/d per 1000 population equivalent from 4 WWTPs (Spataro et al. 2019).

During the summer, when the sampling for the present study was performed, the mass loads of antibiotics to WWTPs and to receiving waters might be lower than during winter. Several studies have shown that the concentrations and the detection frequency of antibiotics are mostly higher in the winter (Golovko et al. 2014; Mohapatra et al. 2016). This might be for several reasons, such as increased antibiotic consumption during the winter associated with treatment of the common cold, respiratory tract infection, flu, and similar infections common during the winter. Another reason might be the higher water consumption during summer, which leads to antibiotics being more diluted.

ERA

The ERA based on the calculated RQ for the maximum concentrations detected in any WWTPs for 6 antibiotics and 3 trophic levels are presented in Table 8. The MEC was divided by a DF calculated by Keller et al. (2014; DF = 1702.28). It must be noted that according to the European guidelines (Commission of the European Communities 2003) on calculating PEC_{local}, the DF that is applied for calculation of the local concentration in surface water should not be >1000. In addition, it should be kept in mind that complete mixing of the effluent in the surface water can take a long time, especially in the Baltic Sea, where no tidal influences are present. The initial DF is usually approximately 10. A DF for discharges to a coastal zone of 100 may then tentatively be assumed, which seems to be representative of a realistic worst-case scenario.

When applying the calculated DF by Keller et al. (2014), the results showed that most of the RQ values were close to zero (RQ_1, Table 8). Only RQ_1 values for CLARI and ERY for algae

TABLE 8: Summary of the aquatic toxicity data, maximum concentration, and calculated risk quotient for the detected antibiotics

Antibiotics	Target species	Toxicity data (mg/L)	Reference	MEC (ng/L)	RQ1	RQ2
Sulfadiazine	*Microcystis aeruginosa*	EC50 = 0.135	Holten-Lützhøft et al. (1999)	178	0.00	0.01
	Daphnia magna	EC10 = 8.8	Wollenberger et al. (2000)	178	0.00	0.00
	Cirrhinus mrigala	EC50 = 200	Bundschuh et al. (2015)	178	0.00	0.00
Sulfamethoxazole	*M. aeruginosa*	EC50 = 0.55	Grinten et al. (2010)	7	0.00	0.00
	Daphnia magna	NOEC = 0.12	Lu et al. (2013)	7	0.00	0.00
	Danio rerio	NOEC = 0.533	Madureira et al. (2012)	7	0.00	0.00
Trimethoprim	*Pseudokirchneriella subcapitata*	EC50 = 40	Yang et al. (2008)	210	0.00	0.00
	Brachionus koreanus	EC50 = 198.5	Rhee et al. (2012)	210	0.00	0.00
	Danio rerio	NOEC = 100	Blaise et al. (2006)	210	0.00	0.00
Clarithromycin	*P. subcapitata*	EC50 = 0.002	Isidori et al. (2005)	207	**0.06**	**1.04**
	Daphnia magna	EC50 = 25.72	Isidori et al. (2005)	207	0.00	0.00
	Oryzias latipes	LC50 >100	Kim et al. (2009)	207	0.00	0.00
Erythromycin	*P. subcapitata*	EC50 = 0.020	Isidori et al. (2005)	532	**0.02**	**0.27**
	Daphnia magna	EC50 = 22.45	Isidori et al. (2005)	532	0.00	0.00
	O. latipes	LC50 >100	Kim et al. (2009)	532	0.00	0.00
Roxithromycin	*Vibrio fisheri*	IC50 >1000	Choi et al. (2008)	146	0.00	0.00
	Daphnia magna	EC50 = 7	Choi et al. (2008)	146	0.00	0.00
	O. latipes	LC50 = 288	Choi et al. (2008)	146	0.00	0.00
Carbamazepine	*Chlorella vulgaris*	EC50 = 74	Moermond and Smit (2016)	246	0.00	0.00
	Daphnia magna	EC50 = 70	Moermond and Smit (2016)	246	0.00	0.00
	O. latipes	LC50 = 35	Moermond and Smit (2016)	246	0.00	0.00

EC10/EC50 = 10 and 50% effect concentrations, respectively; IC50 = 50% inhibition concentration; LC50 = 50% lethal concentration; MEC = measured environmental concentration; NOEC = no-observed-effect concentration; RQ = risk quotient; Bold in RQ1 = indicates elevated risk quotient, low environmental risk; Bold in RQ2 = indicates elevated risk quotient, moderate to high environmental risk.

showed that the value is <0.1, which is considered to be low environmental risk. It should not be forgotten that the RQ value only takes the parent compound into consideration, although the metabolites show similar pharmacological activity to the parent molecule (Besse et al. 2008). In addition, the risk was calculated for each compound individually, not including a mixture of multiple antibiotics and other substances that are simultaneously released to the environment. Therefore, the risk for the environment and humans might actually be higher. The low impact of antibiotics to the environment in the present study is in agreement with other studies worldwide (Faleye et al. 2019; Rodriguez-Mozaz et al. 2020).

If the default DF of 100 is used, the calculated RQ_2 for CLARI in algae showed $1 < RQ$, which is considered to be high risk. For ERY at algae level the RQ was $0.1 < RQ < 1$, which is considered to be moderate risk. The environmental risk of antibiotics in regard to direct toxicity can thus not be ignored and might need to be considered besides the commonly addressed antimicrobial resistance issue.

CONCLUSIONS

In the present study, a total of 18 antibiotics belonging to 5 different classes were investigated. Compounds representing 3 classes of antibiotics were detected in the 3 major WWTPs in Finland. No compounds belonging to the β-lactam or TETR class were found in any of the samples collected from influent waters. Macrolides and sulfonamides were the most frequently detected groups of antibiotics out of the investigated classes. Overall, the highest antibiotic concentrations were detected in WWTP A, where SDZ, *N*-SDZ, SMX, TMP, CLARI, ERY, ERY-EE, and ROXI were detected. Besides the antibiotics detected in WWTP A, *N*-SMX and CLOXA were detected in WWTPs B and C. The removal efficiencies of antibiotics were shown to be higher in WWTP A than in the other 2 WWTPs. In contrast, the lowest removal efficiencies were found in WWTP B, which is the only one that does not apply a tertiary treatment process. Many of the compounds were not removed during the wastewater-treatment processes, and some antibiotics, especially macrolides, were present at higher concentrations in the effluent than in the influent. This might be attributable to the possible conversion of the conjugated metabolites to the parent compound by enzymatic processes in the treatment plant. In addition, the occurrence and removal rates of antibiotics might depend on many factors such as the structure and initial concentration of antibiotics in the influent, wastewater composition, treatment process, and the season of the year.

From the investigated antibiotic classes, sulfonamides had the highest removal rate in WWTPs. Even though some of the antibiotics were not detected in either the influent or the effluent, they might still have been present in those samples at concentrations lower than the LODs. The assessed environmental risk proved to be negligible for most of the compounds and trophic levels. Depending on the applied DF, CLARI and ERY can pose an insignificant to moderate risk for the aquatic environment, especially at the algae level. However, it should be remembered that the estimations are based only on the parent compound and no mixtures of xenobiotics being simultaneously released to the environment.

The results obtained in the present study are in agreement with previously published data on antibiotics in wastewater. The detected concentrations as well as mass loads were compared to those observed by previous studies. Determination of the trend of the occurrence of the antibiotics in the receiving waters is necessary for protecting aquatic life. When comparing

the solids retention time and hydraulic retention time for individual WWTPs, no significant differences in removal of the antibiotics were noticed.

The online SPE method was shown to be fast and reliable for the analyses of multiple antibiotics in wastewater matrices. The sample preparation time and steps are reduced, which diminishes the possibility of mistakes during sample treatment and reduces analytical variation. In addition, the method can be applied to various matrices and applications in the future when automation will become vital.

The issues associated with antibiotic resistance are becoming of increasing concern to the public and decision makers. In future, this concern might lead to the need for continuous surveys of antibiotics, especially macrolides and sulfonamides, in WWTPs around the globe.

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