Supplementary Materials

1. Supplementary Tables

Table S1. List of samples collected in this study. DNA samples were collected in dates in bold.

Site	Sampling day at WWTP	Sampling day at sea	Marine station coordinates
Francavilla al Mare	28 April 2020	**28 April 2020**	14.275°E; 42.464°N
	29 May 2020	**29 May 2020**	
	17 June 2020	17 June 2020	
	21 July 2020	21 July 2020	
	28 August 2020	28 August 2020	
	22 September 2020	**22 September 2020**	
Zadar	**30 July 2019**	**30 July 2019**	15.236°E; 44.087°N
	13 September 2019	**13 September 2019**	
	7 November 2019	**7 November 2019**	
	22 April 2020	22 April 2020	
	18 May 2020	18 May 2020	
	18 June 2020	**18 June 2020**	
	27 July 2020	27 July 2020	
	26 August 2020	26 August 2020	
	29 September 2020	**29 September 2020**	
Katalinića Brig	**31 July 2019**	**31 July 2019**	16.453°E; 43.490°N
	27 February 2020	27 February 2020	
	23 April 2020	**23 April 2020**	
	28 May 2020	**28 May 2020**	
	26 June 2020	26 June 2020	
	22 July 2020	22 July 2020	
	21 September 2020	21 September 2020	
Stobreč	**31 July 2019**	**31 July 2019**	16.518°E; 43.482°N
	27 February 2020	**27 February 2020**	
	23 April 2020	**23 April 2020**	
	28 May 2020	28 May 2020	
	26 June 2020	**26 June 2020**	
	22 July 2020	22 July 2020	
	21 September 2020	21 September 2020	

Table S2. Primer sets used for qPCR.

Target	Primer name	Primer sequence (5'- 3')	Amplicon size (bp)	Annealing T (°C)	Reference
16SrDNA	Bact1369F	CGGTGAATACGTTCYCGG	142	55	[51]
	Prok1492R	GGHTACCTTGTTACGACTT			
ermB	ermB Fw	CCGAACACTAGGGTTGCTC	139	55	[52]
	ermB Rev	ATCTGGAACATCTGTGGTATG			
*tet*A	tetA Fw	GCTACATCCTGCTTGCCTTC	210	64	[55]
	tetA Rev	CATAGATCGCCGTGAAGAGG			
*sul*II	sulII Fw	TCCGGTGGAGGCCGGTATCTGG	191	60	[56]
	sulII Rev	CGGGAATGCCATCTGCCTTGAG			
qnrS	qnrS Fw	GACGTGCTAACTTGCGTGAT	118	62	[57]
	qnrS Rev	TGGCATTGTTGGAAACTTG			
$bla_{\text{CTX-M}}$	blaCTX-M Fw	CTATGGCACCACCAACGATA	103	60	[58]
	blaCTX-M Rev	ACGGCTTTCTGCCTTAGGTT			
mcr-1	mcr-1 qF1	ACACTTATGGCACGGTCTATG	120	63	[59]
	mcr-1 qR1	GCACACCCAAACCAATGATAC			
bla_{TEM}	blaTEM Fw	TTCCTGTTTTTGCTCACCCAG	112	60	[60]
	blaTEM Rev	CTCAAGGATCTTACCGCTGTTG			
bla_{OXA}	Oxa-rt Fw	AGGCACGTATGAGCAAGATG	189	60	[61]
	Oxa-rt Rev	TGGCTTGTTTGACAATACGC			

Supplementary Table S3. qPCR parameters for gene quantification. Melting peaks, efficiencies, and r^2 are the mean of the whole data produced for all runs for each gene ± standard deviation.

Target	**Melting peak**	**Efficiency** (%)	**r^2**	**Standard curve** (copies μl^{-1})	**LOQ** (copies μl^{-1})
16SrDNA	85.3 ± 0.7	100.1 ± 2.7	0.998 ± 0.001	4.23×10^5 4.23×10^4 4.23×10^3 4.23×10^2	430
*sul*II	90.7 ± 0.3	98.7 ± 1.1	0.998 ± 0.001	3.36×10^5 3.36×10^4 3.36×10^3 3.36×10^2 3.36×10^1	34
*tet*A	89.5 ± 0.2	97.0 ± 0.4	0.999 ± 0.001	1.23×10^5 1.23×10^4 1.23×10^3 1.23×10^2 1.23×10^1	13
bla_{OXA}	83.5 ± 0.0	90.0 ± 0.04	0.999 ± 0.000	3.05×10^5 3.05×10^4 3.05×10^3 3.05×10^2 3.05×10^1	31
mcr-1	82.8 ± 0.3	101.7 ± 3.8	0.999 ± 0.001	8.91×10^4 8.91×10^3 8.91×10^2 8.91×10^1 8.91×10^0	9
bla_{TEM}	81.9 ± 0.4	99.2 ± 2.0	0.999 ± 0.001	4.10×10^5 4.10×10^4 4.10×10^3 4.10×10^2 4.10×10^1	41
bla_{CTX-M}	84.6 ± 0.03	99.2 ± 1.9	0.999 ± 0.001	1.37×10^5 1.37×10^4 1.37×10^3 1.37×10^2 1.37×10^1	14
*erm*B	81.6 ± 0.04	100.5 ± 0.4	1.000 ± 0.001	2.45×10^5 2.45×10^4 2.45×10^3 2.45×10^2 2.45×10^1	25
*qnr*S	82.5 ± 0.4	100.2 ± 2.4	0.998 ± 0.001	2.29×10^5 2.29×10^4 2.29×10^3 2.29×10^2 2.29×10^1	23

2. Supplementary figures

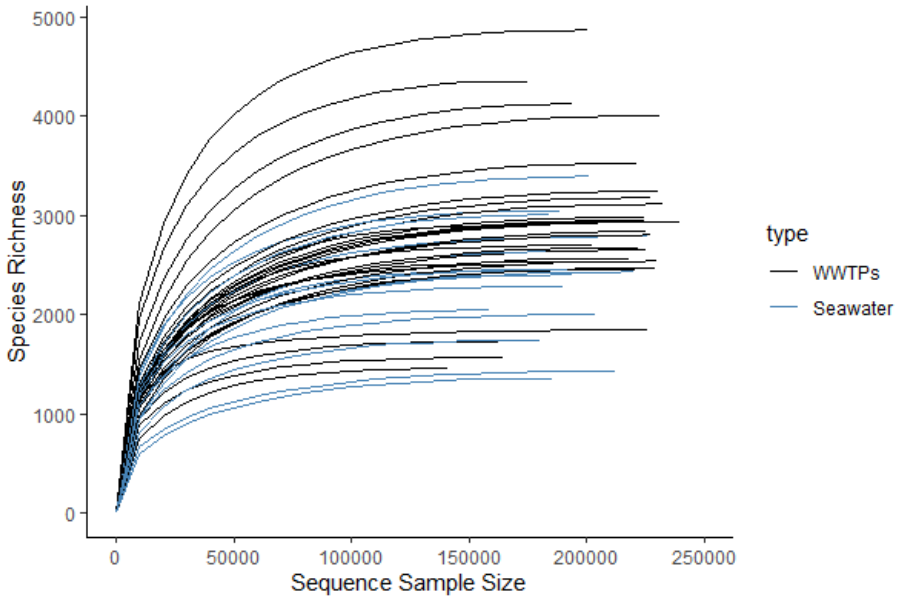

Figure S1. Rarefaction curves of treated sewage (WWTPs) and sweater communities based on 16S rDNA amplicon sequencing. Curves were built before performing any filtration.

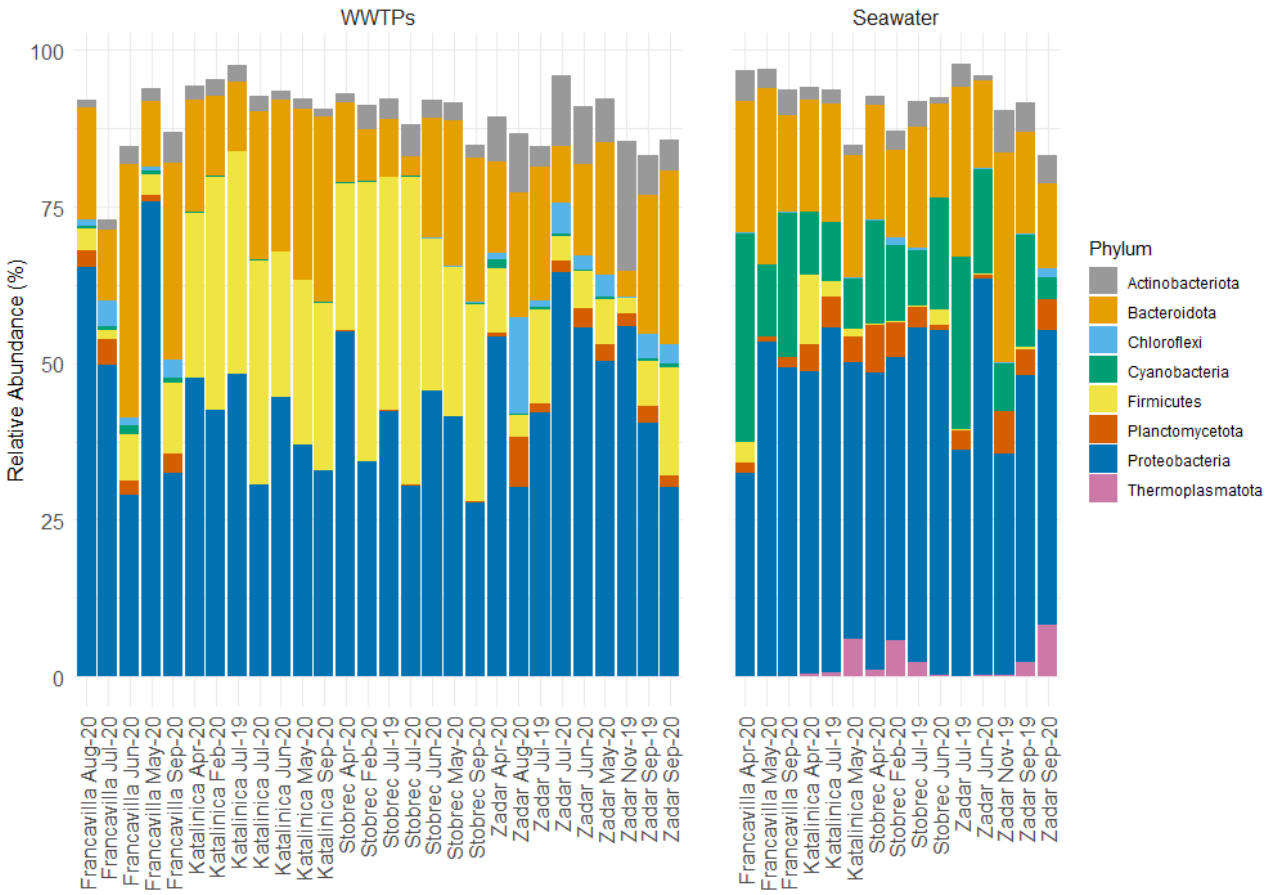

Figure S2. Bacterial community composition at phylum level of treated sewage (WWTPs) and sweater samples based on 16S rDNA amplicon sequencing. Only top phyla are depicted.

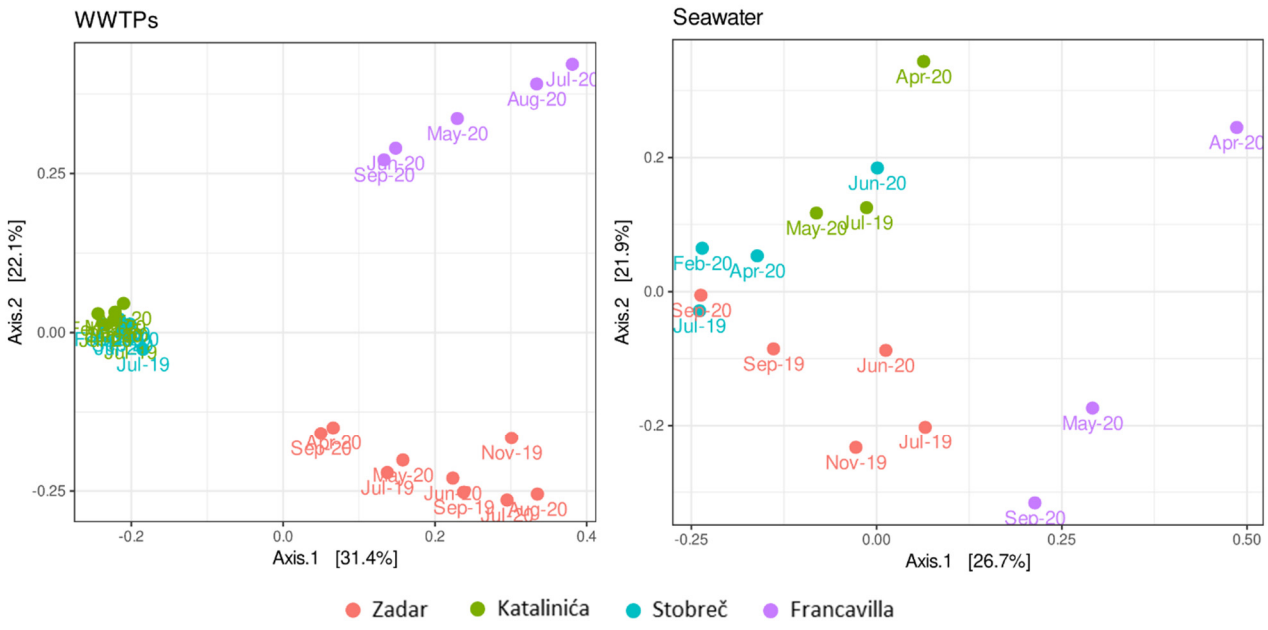

Figure S3. Sample ordination by PCoA of Bray-Curtis distances. In Katalinića and Stobreč WWTPs, sewage undergoes only basic mechanical treatments (i.e., "primary treatments"). Zadar and Francavilla WWTPs perform activated sludge treatment subsequently to primary treatments. Only in the case of Francavilla WWTP treated sewage undergoes a final disinfection step.

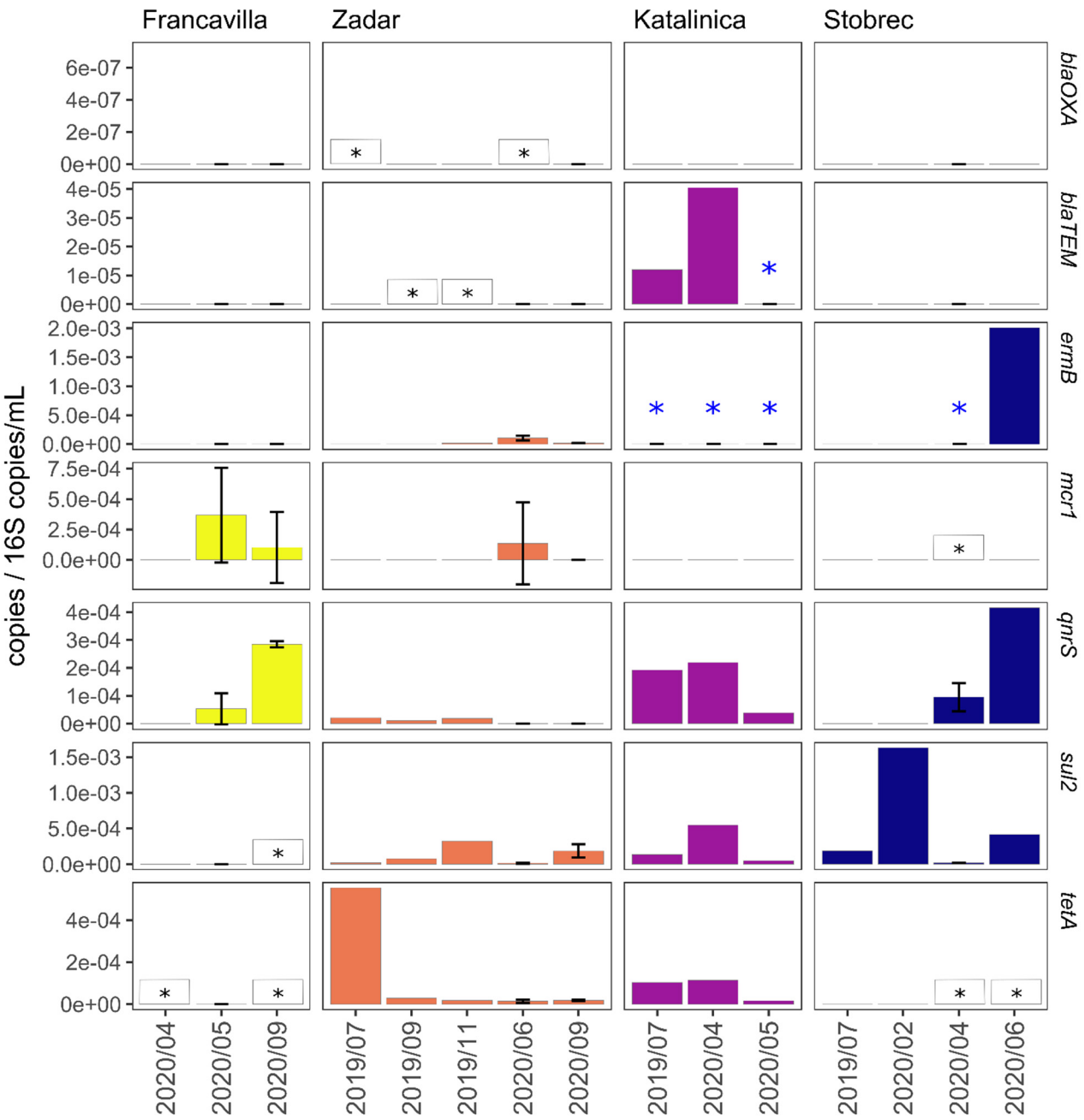

Figure S4. Content of ARGs in seawater samples over time. Black stars in white boxes are <LOQ values. Blue stars indicate not quantifiable samples due to primer-specificity issues. Error bars show the standard deviation between biological replicates (n.2).