SUPPLEMENTARY MATERIAL

Screening of commonly used antibiotics in fresh and saltwater samples impacted by aquacultures: analytical methodology, occurrence and environmental risk assessment

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Table S1. List of the surveyed antibiotics with their physicochemical properties and therapeutic use.

Antibiotic	Category	Elemental Formula	M.W.	Solubility, S (mg L^{-1}, 25 °C)	pK_a	LogK_{ow}	Treatment/ Therapeutic use
Oxytetracycline	Tetracycline	$C_{22}H_{24}N_2O_9$	460.148	1399	3.27	-0.9	Bacterial infections (Vibrio anguillarum etc.) (Akiyama et al., 2020)
Oxolinic acid	Quinolone	$C_{13}H_{11}NO_5$	261.230	8007	5.58	0.94	Bacterial infections (Enterobacter, Shigella, Vibrio etc.) (Touraki et al., 2012)
Erythromycin	Macrolide	$C_{37}H_{67}NO_{13}$	733.461	2000	8.88	3.06	Bacterial infections (Streptococcus, Lactococcus garvieae etc.) (Di Salvo et al., 2013)
Sulfamethoxazole	Sulfonamide	$C_{10}H_{11}N_3O_3S$	253.078	3942	1.97 / 6.16	0.89	Bacterial infections (Aeromonas, Pseudomonas etc.) (Phu et al., 2015)
Trimethoprim	Benzylpyrimidine	$C_{14}H_{18}N_4O_3$	290.318	2334	7.12	0.91	Bacterial infections (Aeromonas, Pseudomonas etc.) (Phu et al., 2015)
Florfenicol	Amphenicol	$C_{12}H_{14}Cl_2FNO_4S$	357.000	5936	9.03	-0.04	Bacterial infections (Streptococcus, Salmonella etc.) (Zeng et al., 2019)

Table S2. Physicochemical properties values of water samples depending on their origin.

Physicochemical parameters	Distilled water	River water	Seawater*
TDS (mg L^{-1})	3.00	225-10500	52400
Conductivity (mS cm^{-1})	2.20	325-11620	51600
Salinity (‰)	0	0 -9.5	36.8
pH	7.14	6.73*	7.81

* Sampling points mean values of the physicochemical parameters.

Table S3. Gradient elution programs for positive (PI) and negative ionization (NI).

Positive			Negative		
Time (minutes)	**A%**	**B%**	**Time (minutes)**	**A%**	**B%**
0.00	90	10	0.00	90	10
2.00	50	50	1.00	90	10
8.00	5	95	9.00	0	100
8.10	90	10	9.10	90	10
9.00	90	10	10.0	90	10

Table S4. Detection parameters for SIM MS/dd-MS^2 analysis of the selected antibiotics.

Antibiotics	t_R (min)	Molecular ion[a]	Theoretical Mass (m/z)	Mass accuracy (ppm)	Fragment ions[b] (m/z)
TMP	2.73	$C_{14}H_{19}N_4O_3^+$	291.1452	-1.98	230.1170
SMX	2.86	$C_{10}H_{11}N_3O_3S^+$	254.0594	-1.77	188.0806
OTC	3.04	$C_{22}H_{24}N_2O_9^+$	461.1555	-2.99	443.1448
OXO	3.98	$C_{13}H_{11}NO_5^+$	262.0710	-2.74	244.0613
ERY-H_2O	6.05	$C_{37}H_{65}NO_{12}^+$	716.4580	-1.62	588.3606
FFC	2.64	$C_{12}H_{14}Cl_2FNO_4S^-$	355.9932	-0.75	335.9857

[a]Positive ionization for all compounds except FFC for which Negative ionization was applied

[b]Normalized Collision Energy CID 35%

Table S5. Operational parameters of LTQ- Orbitrap HRMS instrument in positive and negative ionization respectively.

Parameters	**Positive**	**Negative**
Spray voltage	3.5V	3.5V
Sealth gas	30au	14au
Auxiliary gas	7au	12au
Capillary voltage	40V	-35V
Capillary temperature	330^{o}C	330^{o}C
Tube lens voltage	110V	-90V
Resolution	60000	60000

Table S6. Acute and chronic RQ values obtained after the potential risk assessment for each identified antibiotic in river and sea samples "based on the worst-case scenario" at the three trophic levels.

	RQ					
	Fish		Invertebrate		Algae	
Antibiotic	**acute**	**chronic**	**acute**	**chronic**	**acute**	**chronic**
TMP (river)	2.69804E-05	0.000001000	0.000304507	8.01282E-06	0.000362	0.00000200
TMP (sea)	0.000175912	0.000006520	0.001985384	5.22436E-05	0.002362	0.00001304
OTC (river)	0.005105263	0.000000388	0.215555556	0.002155556	0.228235	0.01552000
OTC (sea)	0.011105263	0.000000844	0.468888889	0.004688889	0.496471	0.03376000
SMX (sea)	4.58043E-05	9.38086E-05	0.023809524	0.000416667	0.185185	0.00500000
FFC (sea)	6.85832E-06	4.36364E-06	0.000300000	3.15789E-05	0.001846	0.00003200

Table S7. Acute toxicity data (EC50, mg/L) of antibiotics on fish, invertebrates and algae (**lower values** indicated in bold font)

	Fish			Invertebrates			Algae		
Antibiotics	**EC50 (mg/l)**	**Test organismç**	**Reference**	**EC50 (mg/l)**	**Test organism**	**Reference**	**EC50 (mg/l)**	**Test organism**	**Reference**
TMP	795	*O. latipes*	(Gros et al., 2010)	120.7	*D. magna*	(Ginebreda et al., 2010)	40	*P.subcapitata*	(Valcárcel et al., 2011)
	92.66	*P.reticulata*	(De Liguoro et al., 2012)	123	*D. magna*	(Grung et al., 2008)	80.3 (72h)	*S.capricornutum*	(Eguchi et al., 2004)
	>100	*O. latipes*	(Kim et al., 2007)	**8.21**	*D. magna*	(De Liguoro et al., 2012)	83.8	*P. subcapitata*	(De Liguoro et al., 2012)
				189.5 (24h)*	B. koreanus	(X. Wang et al., 2022)	16 (96h)		(Ginebreda et al., 2010; Gros et al., 2010)
				>100	H. vulgaris	(Quinn et al., 2008)	11	*A.variabilis*	(Ando et al., 2007)
				92	D. magna	(Park & Choi, 2008)	90.86	*C. vulgaris*	(Borecka et al., 2016)
				100	*D. magna*	(Kolar et al., 2014)	**6.9**	*M. aeruginosa*	(Carvalho & Santos, 2016)
							53	*Nostoc sp.*	(Ando et al., 2007)
							>9	*P. subcapitata*	(Carvalho & Santos, 2016)
OTC	62.5 (24h)	*M. saxatilis*	(Kovalakova et al., 2020)	40.13	*H.vulgaris*	(Pro et al., 2003)	0.32	*A. cylindrica*	(Ando et al., 2007)
	215 (48h)	*O.latipes*	(Park & Choi, 2008)	46.2 (48h)	*D. magna*	(Wollenberger et al., 2000)	**0.17**	*S. capricornutum*	(Pro et al., 2003)
	110 (96h)	*O. latipes*	(Park & Choi, 2008)	22.64 (24h)	*D. magna*	(Isidori et al., 2005)	4.5 (72h)	*P. subcapitata*	(Kovalakova et al., 2020)
	>200 (24h)	*S. namaycush*	(Park & Choi, 2008)	21.8 (48h)	P.lividus	(X. Wang et al., 2022)	17.25 (96h)	*T. suecica*	(X. Wang et al., 2022)
	7.6 (48h)*	*P.mesopotamicus*	(Carraschi et al., 2011)	>300 (24h)*	B.koreanus	(X. Wang et al., 2022)	1.73 (96h)	*P.tricornutum*	(X. Wang et al., 2022)
	127.6 (72h)	*D. rerio*	(Oliveira et al., 2013)	114	D. magna	(Kołodziejska et al., 2013)	6.43 (96h)	*I. galbana*	(X. Wang et al., 2022)

	139.5	*D. rerio*	(Pro et al., 2003)	>250	Moina m.	(Ji et al., 2012)	11.18 (96h)	*T. chui*	(X. Wang et al., 2022)
	597 (96h)*	*M.saxatilis*	(X. Wang et al., 2022)	126.7	Moina m.	(Pro et al., 2003)	39 (96h)	*T. chui*	(X. Wang et al., 2022)
				1.87	B.calyciflorus	(Pro et al., 2003)	0.23	*M. aeruginosa*	(Ando et al., 2007)
				0.18	C. dubia	(Ji et al., 2012)	0.35	*M. wesenbergii*	(Ando et al., 2007)
				7.4	D. magna	(Ji et al., 2012)	0.6	*P. subcapitata*	(Q. Li et al., 2020)
				25 (24h)*	T. platyurus	(Ji et al., 2012)	0.92	*P. subcapitata*	(Magdaleno et al., 2017)
							0.342 (72h)	*P. subcapitata*	(Kovalakova et al., 2020)
FFL	>1000 (48h)*	*P.mesopotamicus*	(Carraschi et al., 2011)	337	*D. magna*	(Kołodziejska et al., 2013)	9.03 (96h)*	T. suecica	(X. Wang et al., 2022)
	>780 (48h)*	*O. mykiss*	(Carraschi et al., 2011)	**8 (96h)**	*I. galbana*	(X. Wang et al., 2022)	215 (96h)	*C. pyrenoidosa*	(Q. Li et al., 2020)
	>830 (48 h)*	*L. macrochirus*	(Carraschi et al., 2011)	>64	*L.vannamei*	(X. Wang et al., 2022)	**1.3 (96h)**	*T. chui*	(X. Wang et al., 2022)
	349.94 (96h)	*O. niloticus*	(Mattioli et al., 2020)				9.4	*C.pyrenoidosa*	(Q. Li et al., 2020)
							14.25	*C. pyenoidosa*	(Z. Wang et al., 2016)
							18	*C. fusca*	(Q. Li et al., 2020)
							2.3	*P. subcapitata*	(Q. Li et al., 2020)
							11.18 (96h)	*T. chuii*	(Ferreira et al., 2007)
SMX	562.5	*O. latipes*	(Gros et al., 2010)	25.2	*D. magna*	(Gros et al., 2010)	**0.027**	*S. leopoliensis*	(Gros et al., 2010)
	>750 (48h)	*O. latipes*	(Kim et al., 2007)	26	*B. calyciflorus*	(Grung et al., 2008)	0.146	*P. subcapitata*	(Grung et al., 2008)
	109.16	*D. rerio*	(L. Li et al., 2019)	15.5	*C. dubia*	(Grung et al., 2008)	2.4	*C. meneghiniana*	(Grung et al., 2008)
				9.63	*B.calyciflorus*	(Grung et al., 2008)	0.57	*P. subcapitata*	(Grung et al., 2008)
				177.3	*D. magna*	(Q. Li et al., 2020)	0.03 (96h)	*S. leopolensis*	(W. Li et al., 2012)
				15.51 (48 h)	*C. dubia*	(W. Li et al., 2012)	1.54	*S. Vacuolatus*	(Białk-Bielińska et al., 2011)

	276.1 (24h)*	B.koreanus	(X. Wang et al., 2022)	0.146	*P. subcapitata*	(Ferrari et al., 2004)
	0.21	C.dubia	(Isidori et al., 2005)	1.54	*S. vacuolatus*	(Białk-Bielińska et al., 2011)
	75.3	D. magna	(Osorio et al., 2016)	0.98	*C. vulgaris*	(Borecka et al., 2016)
	3.3	D. magna	(Q. Li et al., 2020)	0.55	*M. aeruginosa*	(Q. Li et al., 2020)
	7.2 (96h)	D. magna	(Yazdanbakhsh et al., 2020)	0.268	*S.leopolensis*	(Q. Li et al., 2020)
	1.29	D.magna	(L. Li et al., 2019)	0.12	*S.obliquus*	(Q. Li et al., 2020)

* indicates LC50 (mg/L) value

Table S8. Chronic toxicity data (NOEC, mg/L) of antibiotics on fish, invertebrates and algae (**lower values** indicated in bold font)

Compound	Fish			Invertabrates			Algae		
	NOEC (mg/L)	**Test organism**	**Reference**	**NOEC (mg/L)**	**Test organism**	**Reference**	**NOEC (mg/L)**	**Test organism**	**Reference**
TMP	**25**	*P. reticulata*	(De Liguoro et al., 2012)	**3.12**	*D. magna*	(De Liguoro et al., 2012)	**12.5**	*P. subcapitata*	(De Liguoro et al., 2012)
				6	*D. magna*	(Park & Choi, 2008)	25.5	S. capricornutum	(Eguchi et al., 2004)
				124.4	*Brachionus koreanus*	(X. Wang et al., 2022)			
				20 (21 days)*	*D. magna*	(Kovalakova et al., 2020)			
SMX	>8	*D. rerio*	(Ferrari et al., 2004; Straub et al., 2016)	25	*B. calyciflorus*	(Ferrari et al., 2004)	0.09	*P. subcapitata* / *S. leopoliensis*	(Ferrari et al., 2004), (Straub et al., 2016)
	0.533	*D. rerio*	(L. Li et al., 2019)	0.25	*C. dubia*	(Ferrari et al., 2004)	1.25	*C. meneghiniana*	(Ferrari et al., 2004)
				0.21*	*C. dubia*	(Grung et al., 2008)	**0.01**	*L. gibba*	(Grung et al., 2008)
				9.63	*B. calyciflorus*	(Isidori et al., 2005)	0.0059	*S. leopolensis*	(Ferrari et al., 2004)
				197.5	*B. koreanus*	(X. Wang et al., 2022)	0.5	*P. subcapitata*	(Nie et al., 2013)
				0.12	*D.magna*	(Lu et al., 2013)	0.52	*P. subcapitata*	(Isidori et al., 2005)
				5	*H. vulgaris*	(Quinn et al., 2008)			
				5	*H.attenuata*	(Straub et al., 2016)			
OTC	**1000**	*D. rerio*	(Isidori et al., 2005)	100*	*Daphnia spp.*	(Wollenberger et al., 2000)	0.1	*P.tricornutum*	(X. Wang et al., 2022)
				0.18	*D. dubia*	(Isidori et al., 2005)	0.183 (72h)	*P. subcapitata*	(Ji et al., 2012)

				27.7	*M. macrocopa*	(Ji et al., 2012)	0.05*	*I. galbana*	(X. Wang et al., 2022)
				50	*H.attenuata*	(Quinn et al., 2008)	3.5 (72h)	*C. vulgaris*	(Kovalakova et al., 2020)
				1.87	*B. calyciflorus*	(Isidori et al., 2005)	0.17	*P. subcapitata*	(Isidori et al., 2005)
				0.3	*C. silvestrii*	(Freitas et al., 2018)	0.25	*M. wesenbergii*	(Ando et al., 2007)
							0.031 (144h)	*M.aeruginosa*	(Ando et al., 2007)
							0.025 (144h)	*Anabeana sp.*	(Ando et al., 2007)
FFL	34.99	*O. niloticus*	(Mattioli et al., 2020)	1.5	D. magna	(Staveley et al., 2013)	**0.75 (96 h)**	P. subcapitata	(Staveley et al., 2013)
	5.5	P. promelas	(Staveley et al., 2013)	0.76	B. calyciflorus	(Staveley et al., 2013)	3	*T. chuii*	(Ferreira et al., 2007)
				25	C. riparius	(Staveley et al., 2013)			
				0.27	C.silvestrii	(Freitas et al., 2018)			
				7.6	*D. magna*	(Martins et al., 2013)			

* indicates LOEC (mg/L) value

Figure S1. Sampling locations in N.W. Greece (S refers to saltwater sampling points and R to the river ones; R1 to R4 sampling points are shown in the enlarged map area of Louros river, sampling point R5 that is the river estuary lies southern of R4 and it is not included in the map).

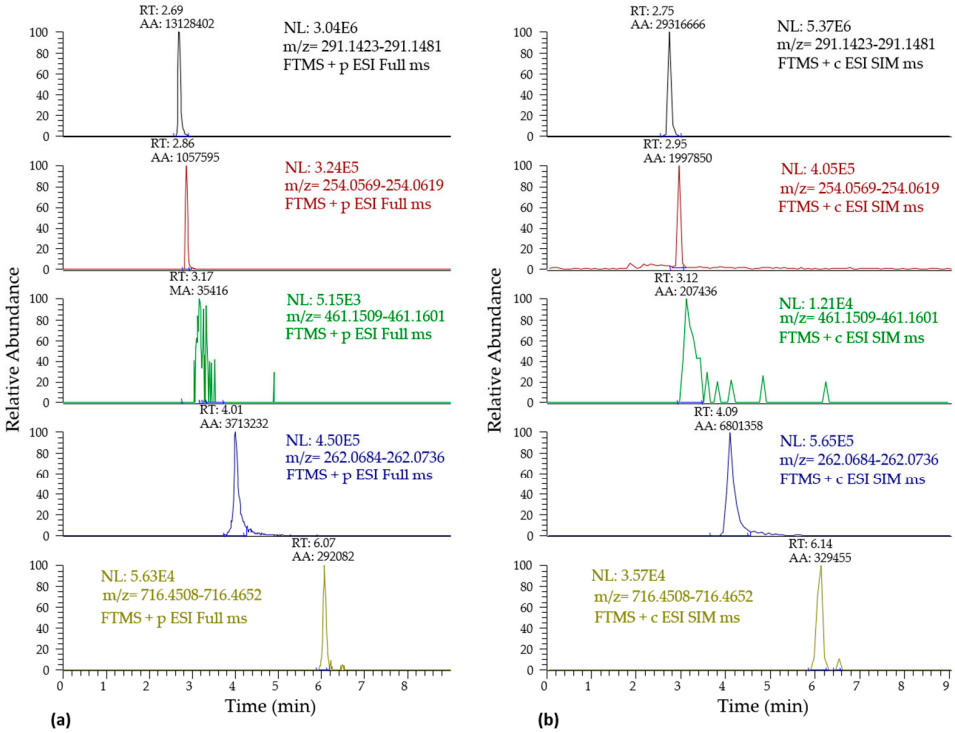

Figure S2. Comparative depiction of the SIM - LC-LTQ/Orbitrap MS chromatograms of the selected antibiotics in positive ionization at concentration 50 μg L^{-1} with: (a) full scan acquisition mode and (b) time-scheduled SIM acquisition mode (all other parameters were the same).

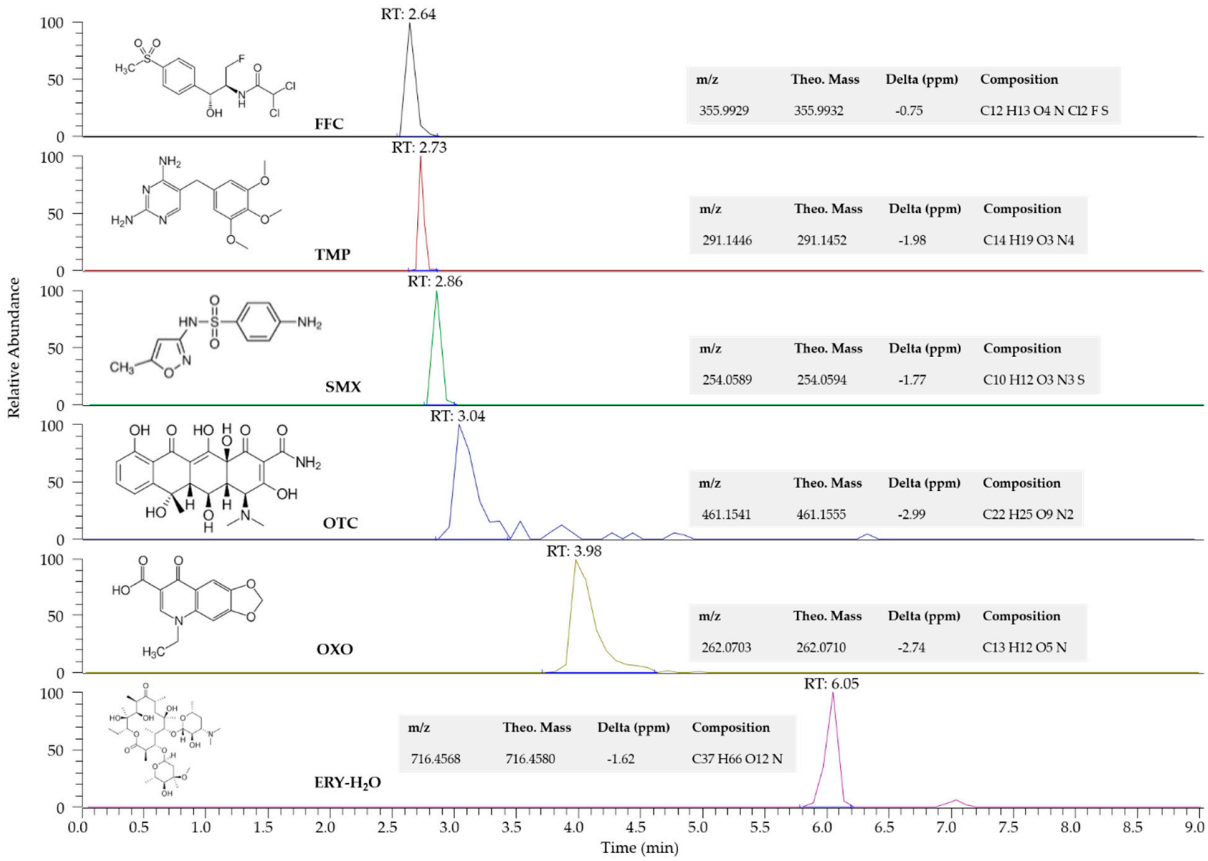

Figure S3. SIM-LC-LTQ/Orbitrap MS chromatogram of the selected antibiotics matrix-matched solution at concentration 25 μg L^{-1}. Peaks are: (a) florfenicol (FFC), (b) trimethoprim (TMP), (c) sulfamethoxazole (SMX), (d) oxytetracycline (OTC), (e) oxolinic acid (OXO), (f) erythromycin-H_2O (ERY-H_2O)

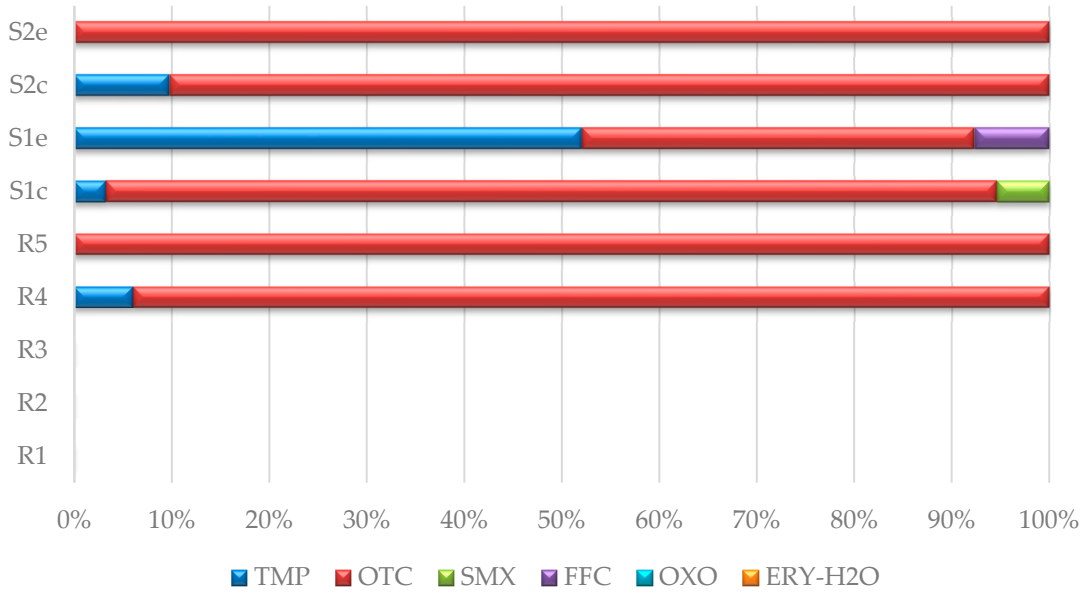

Figure S4. The selected antibiotics' concentration levels expressed as percent (%) occurrence detected in each sampling point (R1-5 and S1-2 exit and center) of surface waters impacted by aquaculture facilities.

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