Supplementary Materials

Investigation of the Correlation between the Use of Antibiotics in Aquaculture Systems and their Detection in Aquatic Environments: A Case Study of the Nera River Aquafarms in Italy

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Table S1. Summary of information on sampling points and related aquafarms with specification of quantity and type of production.

Sampling Points	Coordinates (N, E)	Upstream Aquafarms[1] (n°)	Fattening Aquafarms[1] (n°)	Prefattening Aquafarms[1] (n°)	Juvenile Aquafarms[1] (n°)	No-Prescription Aquafarms[1] (n°)
P1	42.90717, 13.03294	4	3	–	1	1
P2	42.87999, 12.99158	5	3	1	1	1
P3	42.81428, 12.91549	4	–	–	–	1
P4	42.71214, 12.82946	2	2	–	–	1
P5	42.58246, 12.75803	–	–	–	–	–

P1: Molini; P2: Pontechiusita; P3: Borgo Cerreto; P4: Scheggino; P5: Casteldilago. [1]All the farms' information were extracted from the italian national database (Banca Dati Nazionale—BDN). Accesible at: https://www.vetinfo.it/.

Table S2. List of antibiotics and their abbreviations with the relevant limits of detection (LODs) included in the method developed for river waters (64 compounds) and for sediments (56 compounds).

Analyte	Abbreviation	Class	LOD Waters (ng/L)	LOD Sediments (ng/g)
Florfenicol amine (florfenicol metabolite)	FFA	Amphenicols (3)	1	1
Florfenicol	FF		1	1
Thiamfenicol	TMF		1	1
Amoxicillin	AMX	Beta Lactams (17)	100	-
Ampicillin	AMP		10	10
Cefacetrile	CEF		10	-
Cefalexin	LEX		1	10
Cefalonium	CLM		10	-
Cefapirin	CFP		1	10
Cefazoline	CZL		10	10
Cefoperazone	CPR		10	10
Cefquinome	CQM		10	-
Ceftiofur	CFT		1	10
Cloxacillin	CLX		10	-
Desacetycefapirin	DES		10	10
Dicloxacillin	DIC		10	-
Nafcillin	NAF		10	10
Oxacillin	OXA		10	10
Penicillin G	PEN-G		10	10
Penicillin V	PEN-V		10	-
3-O-Acetyltylosin	A_TYL	Macrolides (11)	10	1
Erythromycin A[1]	ERY		1	1

Gamithromycin	GAM		1	1
Neospiramycin	NEO		10	1
Spiramycin	SPI		1	1
Tildipirosin	TLD		10	-
Tilmicosin	TLM		1	1
Tylosin A	TYL		1	1
Tylvalosin	TLV		10	1
Tulathromycin	TUL		10	-
Tulathromycin marker	TUL_m		1	1
Lincomycin	LIN	Others (5)	1	1
Tiamulin	TIA		1	1
Rifaximin	RFX		10	1
Valnemulin	VAL		10	1
Trimethoprim	TMP		1	1
Oxolinic Acid	OXO	Quinolones (10)	1	1
Nalidixic Acid	NAL		1	1
Ciprofloxacin	CIP		1	1
Danofloxacin	DAN		1	1
Difloxacin	DIF		1	1
Enrofloxacin	EFX		1	1
Flumequine	FLU		1	1
Marbofloxacin	MRB		1	1
Norfloxacin	NOR		1	1
Sarafloxacin	SAR		1	1
Sulfaquinoxaline	SQX	Sulfonamides (11)	1	1

Sulfadiazine	SDZ		1	1
Sulfadimethoxine	SDM		1	1
Sulfaguanidine	SGN		1	1
Sulfamerazine	SMR		1	1
Sulfamethazine	SMT		1	1
Sulfamethoxazole	SMS		1	1
Sulfamonomethoxine	SMM		1	1
Sulfanilamide	SNM		1	1
Sulfapyridine	SPD		1	1
Sulfathiazole	STZ		1	1
Chlortetracycline[2]	CTC	Tetracyclines (7)	10	1
Doxycycline	DOX		1	1
4-Epichlortetracycline	4-CTC		10	1
4-Epioxytetracefalocycline	4-OTC		10	1
4-Epitetracycline	4-TC		10	1
Oxytetracycline[2]	OTC		1	1
Tetracycline[2]	TC		1	1

[1]Erythromycin A is measured as the sum of erythromycin A and its anhydrous form.[2]Chlortetracycline, oxytetracycline and tetracycline are reported as sum of parent drug and their epimer.

Table S3. DDDA and DCDA for antibiotics prescribed on the selected aquafarms.

AS	Antimicrobial Class	Route of Administration	DDDA[2] (mg/kg)	DCDA[3] (mg/kg)
FF	Amphenicols	Oral	10	100
OTC	Tetracyclines	Oral	75	750
SDZ	Sulfonamides_TMP[1]	Oral	24	199.1
TMP	Trimethoprim _SUL[1]	Oral	4.8	39.8
FLU	Quinolones	Oral	12	84

[1]Sulfonamide in association with trimethoprim. [2]DDDA: The assumed average dose per kg animal per species per day. [3]DCDA: The assumed average dose per kg animal per species per treatment course.

Table S4. Summarised concentrations (ng/L) and frequencies (%) of detected antibiotics in water samples from the five sites. P1: Molini, P2: Pontechiusita, P3: Borgo Cerreto, P4: Scheggino, P5: Casteldilago.

Antibiotic Concentrations in Water Samples (ng/L)												
AS	P1				P2				P3			
	Frequency	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**
AMX	17	ND – 100	8.9	ND	0	ND	ND	ND	0	ND	ND	ND
CFP	0	ND	ND	ND	8	ND – 2	0.2	ND	0	ND	ND	ND
CFT	0	ND	ND	ND	8	ND – 2	0.2	ND	0	ND	ND	ND
CIP	17	ND – 1	0.2	ND	8	ND – 2	0.2	ND	0	ND	ND	ND
DOX	8	ND – 2	0.2	ND	0	ND	ND	ND	0	ND	ND	ND
ERY	33	ND – 18	2.1	ND	50	ND – 14	3.1	1	8	ND – 1	0.1	ND
FF	67	ND – 192	34.8	12.5	100	1 – 81	32.1	23	75	ND – 11	4.2	4
FFA	42	ND – 18	3	ND	67	ND – 5	2.1	2	0	ND	ND	ND

	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med
FLU	100	ND – 892	77.2	2	100	1 – 1083	93.6	4	33	ND – 76	6.6	ND
LIN	0	ND	ND	ND	8	ND – 1	0.1	ND	0	ND	ND	ND
MRB	0	ND – 6	ND	ND	0	ND	ND	ND	0	ND	ND	ND
NAL	8	ND – 7	0.6	ND	0	ND	ND	ND	8	ND – 3	0.3	ND
NOR	0	ND	ND	ND	8	ND – 1	0.1	ND	0	ND	ND	ND
OTC	75	ND – 477	73.9	3	100	2 – 1139	200	15.5	42	ND – 19	3.9	ND
OXO	0	ND	ND	ND	8	ND – 1	0.1	ND	8	ND – 1	0.08	ND
RFX	8	ND – 1	0.1	ND	0	ND	ND	ND	0	ND	ND	ND
SDM	8	ND – 17	1.4	ND	50	ND – 7	2.2	0.5	0	ND	ND	ND
SDZ	100	ND – 509	164.9	91	100	27 – 571	230.7	195.5	100	6 – 109	39.2	29.5

	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med
SMM	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
SMS	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
SMT	0	ND	ND	ND	17	ND – 5	0.5	ND	0	ND	ND	ND
SPD	0	ND	ND	ND	8	ND – 1	0.1	ND	0	ND	ND	ND
TC	8	ND – 6	0.5	ND	25	ND – 5	1.1	ND	0	ND	ND	ND
TLD	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
TYL	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
TUL	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
TMF	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
TMP	83	ND – 122	38.5	21	100	5 – 180	67.3	57.5	100	2 – 12	5.7	4

ND = Not detected.

Table S4. Summarised concentrations (ng/L) and frequencies (%) of detected antibiotics in water samples from the five sites. P1: Molini, P2: Pontechiusita, P3: Borgo Cerreto, P4: Scheggino, P5: Casteldilago (continued).

Antibiotic Concentrations in Water Samples (ng/L)								
AS	P4				P5			
	Frequency	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**
AMX	0	ND	ND	ND	0	ND	ND	0
CFP	0	ND	ND	ND	0	ND	ND	0
CFT	0	ND	ND	ND	0	ND	ND	0
CIP	8	ND – 6	0.5	ND	0	ND	ND	0
DOX	0	ND	ND	ND	0	ND	ND	0
ERY	8	ND – 2	0.2	ND	0	ND	ND	0
FF	75	ND – 108	18.6	4.5	67	ND – 75	14.1	3

	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med
FFA	25	ND – 7	1.4	ND	25	ND – 5	1	0
FLU	50	ND – 33	3.7	0.5	42	ND – 13	1.7	0
LIN	8	ND – 1	0.1	ND	0	ND	ND	0
MRB	8	ND – 5	0.4	ND	0	ND	ND	0
NAL	8	ND – 1	0.1	ND	0	ND	ND	ND
NOR	0	ND	ND	ND	0	ND	ND	0
OTC	67	ND – 3	1.1	1	33	ND – 3	0.5	0
OXO	0	ND	ND	ND	0	ND	ND	0
RFX	0	ND	ND	ND	8	ND – 3	0.3	0
SDM	8	ND – 1	0.1	ND	0	ND	ND	0

AS	Frequency	Range	Mean	Med	Frequency	Range	Mean	Med
SDZ	100	4 – 553	185.7	102.5	100	3 – 295	104.8	71.5
SMM	0	ND	ND	ND	8	ND – 1	0.1	0
SMS	0	ND	ND	ND	8	ND – 1	0.1	0
SMT	0	ND	ND	ND	0	ND	ND	0
SPD	0	ND	ND	ND	0	ND	ND	0
TC	0	ND	ND	ND	0	ND	ND	0
TLD	0	ND	ND	ND	0	ND	ND	0
TYL	8	ND – 37	3.1	ND	8	ND – 35	2.9	0
TUL	0	ND	ND	ND	0	ND	ND	0
TMF	17	ND – 19	1.7	ND	0	ND	ND	0
TMP	100	2 – 119	36.8	13.5	100	1 – 76	24.2	8.5

ND = Not detected.

Table S5. Summarised concentrations (ng/g d.w.) and frequencies (%) of detected antibiotics in sediment samples from the five sites. P1: Molini, P2: Pontechiusita, P3: Borgo Cerreto, P4: Scheggino, P5: Casteldilago.

Antibiotics Concentrations in Sediment Samples (ng/g d.w.)												
AS	P1				P2				P3			
	Frequency	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**
CIP	83	ND – 32.8	7.1	6	58	ND – 14.9	3.5	1.3	17	ND – 2.4	0.3	ND
DOX	8	ND – 1.2	0.1	ND	0	ND	ND	ND	0	ND	ND	ND
ERY	8	ND – 2.9	0.2	ND	0	ND	ND	ND	0	ND	ND	ND
FLU	100	1.3 – 187	25.3	8.1	100	ND – 133	22.8	12	8	ND – 1	0.1	ND
OTC	100	5.3 – 394	112.7	49	100	50.7 – 892	269.9	128.5	92	ND – 4.9	2.4	2.2
OXO	83	ND – 10.5	4.4	3.6	92	ND – 10.3	3.9	3.5	17	ND – 1.3	0.2	ND
RFX	25	ND – 3.9	0.7	ND	0	ND	ND	ND	0	ND	ND	ND
SDZ	58	ND – 10.5	3.7	3	83	ND- 9	2.9	2.4	0	ND	ND	ND
TC	33	ND – 4.3	0.9	ND	42	ND – 9.1	2.2	ND	0	ND	ND	ND
TMP	50	ND – 49.5	11.8	4.3	92	ND – 31.4	8.7	4.5	0	ND	ND	ND

ND = Not detected.

Table S5. Summarized concentrations (ng/g d.w.) and frequencies (%) of detected antibiotics in sediment samples from the five sites. P1: Molini, P2: Pontechiusita, P3: Borgo Cerreto, P4: Scheggino, P5: Casteldilago (continued).

Antibiotics Concentrations in Sediment Samples (ng/g d.w.)								
AS	**P4**				**P5**			
	Frequency	**Range**	**Mean**	**Med**	**Frequency**	**Range**	**Mean**	**Med**
CIP	33	ND – 1.6	0.4	ND	92	ND – 5.3	2.5	2.3
DOX	0	ND	ND	ND	0	ND	ND	ND
ERY	0	ND	ND	ND	0	ND	ND	ND
FLU	33	ND – 1.6	0.4	ND	58	ND –1.8	0.8	1
OTC	92	ND – 17.1	6	5.6	92	ND – 14.5	4.	3.2
OXO	0	ND	ND	ND	0	ND	ND	ND
RFX	0	ND	ND	ND	0	ND	ND	ND
SDZ	0	ND	ND	ND	0	ND	ND	ND
TC	0	ND	ND	ND	0	ND	ND	ND
TMP	0	ND	ND	ND	0	ND	ND	ND

ND = Not detected.

Table S6. Prescribed DDDA × 10^4 in farms located in the areas between the evaluated sampling points. A1: upstream Molini, A2: Molini–Pontechiusita, A3: Pontechiusita–Borgo Cerreto, A4: Borgo Cerreto–Scheggino.

	pDDDA × 10^4 (mg/kg)																							
	A1						A2						A3						A4					
	ERY	**FF**	**FLU**	**OTC**	**SDZ**	**TMP**	**ERY**	**FF**	**FLU**	**OTC**	**SDZ**	**TMP**	**ERY**	**FF**	**FLU**	**OTC**	**SDZ**	**TMP**	**ERY**	**FF**	**FLU**	**OTC**	**SDZ**	**TMP**
Jan	0	0	0	0	0	0	0	0	0	0	25	25	0	0	0	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	40	40	0	0	0	0	35	35
Mar	0	10.6	0	0	0	0	0	15.8	0	0	12	12	0	0	0	0	0	0	0	0	0	0	40	40
Apr	0	12	0	4	26	26	0	27	0	28	5	5	0	2	0	0	6	6	0	0	0	0	0	0
May	0	0	0	0	145	145	0	34	10	64	12	12	0	0	0	0	48	48	0	0	0	0	70	70
Jun	0	5.2	0	0	98	98	0	32.8	0	0	0	0	0	0	0	0	0	0	0	8	0	0	20	20
Jul	5.3	3.8	3	27	30	30	0	20.5	0	40	10	10	0	0	0	0	0	0	0	11	0	0	0	0
Aug	0	4.4	0	0	67.3	67.3	0	5	0	0	33	33	0	0	0	0	0	0	0	0	0	0	30	30
Sept	0	7	40	0	90	90	0	14	0	0	21	21	0	0	0	0	0	0	0	9	0	0	2	2
Oct	0	1.6	0	0	22	22	0	22	0	0	18	18	0	0	0	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	50	50	0	1	0	0	8	8	0	0	0	0	0	0	0	0	0	0	45	45

Dec	0	11	0	28	33	33	0	0	0	0	8	8	0	0	0	0	0	0	0	0	0	0	0	0
Tot	5.3	55.6	43	59	561.3	561.3	0	172.1	10	132	152	152	0	5	0	0	94	94	0	28	0	0	242	242
Range	0 – 5.3	0 – 12	0 – 40	0 – 28	0 – 145	0 – 145	0	0 – 34	0 – 10	0 – 64	0 – 33	0 – 33	0	0 – 3	0	0	0 – 48	0 – 48	0	0 – 11	0	0	0 – 70	0 – 70
Mean	0.4	4.6	3.6	4.9	46.8	46.8	0	14.3	0.8	11	12.7	12.7	0	0.4	0	0	7.8	7.8	0	2.3	0	0	22	22
Med	0	4.1	0	0	31.5	31.5	0	14.9	0	0	11	11	0	0	0	0	0	0	0	0	0	0	20	20

Table S7. Summary of the number of prescribed DDDA × 10^4 (mg/kg) of antibiotics of the evaluated aquafarms by type of production.

	pDDDA × 10^4 (mg/kg)					
Type of production	**ERY**	**FF**	**FLU**	**OTC**	**SDZ**	**TMP**
Juvenile fish	0	184.1	0	0	1.5	1.5
Prefattening	0	10	0	74	87	87
Fattening	0	194.1	0	74	88.5	88.5
Total	0	388.2	0	148	177	177