

Supplementary material

Survey on Antibiotic Residues in Egg Samples in Italy

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Table S1. List of the 73 investigated analytes.

Class (Number of Analytes)	Analyte
Amphenicols (3)	Florfenicol
	Florfenicol amine
	Thiamfenicol
Cephalosporins (9)	Cefacetrile
	Cefalexin
	Cefalonium
	Cefazolin
	Cefoperazone
	Cefquinome
	Cephapirin
	Ceftiofur
	Desacetyl cephalpirin
Diaminopyrimidines (1)	Trimethoprim
Lincosamides (2)	Lincomycin
	Pirlimycin
Macrolides (11)	Erithromycin A
	Gamitromycin
	Neospiramycin
	Spiramycin I
	Tildipirosin
	Tilmicosin
	Tylosin A
	Tylosin-3-acetate
	Tulathromycin A
	Tulatromycin marker (CP 60,300)
	Tylvalosin
Penicillins (8)	Amoxicillin
	Ampicillin
	Cloxacillin
	Dicloxacillin
	Nafcillin
	Oxacillin
	Penicillin G
	Penicillin V
Pleuromutilins (2)	Tiamulin
	Valnemulin

Quinolones (10)	Ciprofloxacin
	Danofloxacin
	Difloxacin
	Enrofloxacin
	Flumequine
	Marbofloxacin
	Nalidixic acid
	Norfloxacin
	Oxolinic acid
	Sarafloxacin
Rifamycins (1)	Rifaximin
Sulfonamides (19)	Sulfacetamide
	Sulfachloropyridazine
	Sulfadiazine
	Sulfadimethoxine
	Sulfadoxin
	Sulfaguanidine
	Sulfamerazine
	Sulfameter
	Sulfamethizole
	Sulfamethoxazole
	Sulfamethoxypyridazine
	Sulfamonomethoxine
	Sulfamethazine
	Sulfamoxole
	Sulfanilamide
	Sulfapyridine
	Sulfaquinoxaline
	Sulfathiazole
	Sulfisoxazole
Tetracyclines (7)	4-Epichlortetracycline
	4-Epioxytetracycline
	4-Epitetracycline
	Chlortetracycline
	Doxycycline
	Oxytetracycline
	Tetracycline

Table S2. Individual stock solutions of analytes (storage temperature: -20°C).

Drug class (Number of Compounds)	Exceptions	Solvent	Concentration ($\mu\text{g mL}^{-1}$)	Storage Time (months)
Amphenicols (3)	-	MeOH	100	12 ¹
Cefalosporins (9)	-	Water/ACN 75/25, <i>v/v</i>	100	9 ²
	cefquinome			1 ¹
	desacetylcephapirin	DMF		8 ¹
	ceftiofur			9 ²
Diaminopyrimidines (1)	-	MeOH	100	24 ¹
Lincosamides (2)	-	MeOH	100	9 ²
Macrolides (11)	- erythromycin A, tilmicosin, tylosin A	MeOH	100	6 ¹ 9 ²
Penicillins (8)	- penicillin V	Water/ACN 75/25, <i>v/v</i>	100	9 ² 3 ¹
Pleuromutilins (2)	-	MeOH	100	12 ¹
Quinolones (10)	-	Water/MeOH 80/20, <i>v/v</i>	100	6 ³
	ciprofloxacin, enrofloxacin, nalidixic acid, norfloxacin, oxolinic acid	DMF		
Rifamycins (1)	-	MeOH/H ₂ O 50/50, <i>v/v</i>	100	6 ¹
Sulphonamides (19)	-	MeOH	100	24 ¹
Tetracyclines (7)	- 4-epioxytetracycline	MeOH	100	3 ¹ 7 days ¹

¹ Tested in-house, ² [18] ³ [19].

Table S3. UHPLC-Q-Orbitrap parameters of the 73 analytes and 7 ISs.

Peak	Analyte	RT (min)	Molecular Formula	Adduct	Monoisotopic Exact Mass (m/z)	CE	Fragment 1 1 (m/z)	Fragment 2 1 (m/z)
1	Sulfaguanidine	3.2	C ₇ H ₁₀ N ₄ O ₂ S	[M+H] ⁺	215.0597	20	108.1	156.0
2	Florfenicol amine	3.6	C ₁₀ H ₁₄ FN ₃ O ₃ S	[M+H] ⁺	248.0751	18	230.1	130.1
3	Sulfanilamide	3.6	C ₆ H ₈ N ₂ O ₂ S	[M+H-NH ₃] ⁺	156.0114	20	92.1	108.1
4	Desacetyl-cephapirin	5.7	C ₁₅ H ₁₅ N ₃ O ₅ S ₂	[M+H] ⁺	382.0526	30	152.0	112.0
5	Sulfacetamide	6.4	C ₈ H ₁₀ N ₂ O ₃ S	[M+H] ⁺	215.0485	25	108.1	156.0
6	Amoxicillin	7.3	C ₁₆ H ₁₉ N ₃ O ₅ S	[M+H] ⁺	366.1118	10	208.0	349.1
7	Sulfadiazine	7.3	C ₁₀ H ₁₀ N ₄ O ₂ S	[M+H] ⁺	251.0597	25	156.0	108.1
8	Tildipirosin	7.8	C ₄₁ H ₇₁ N ₃ O ₈	[M+3H] ⁺⁺⁺	245.5153	15	245.5	281.2
9	Sulfathiazole	7.9	C ₉ H ₉ N ₃ O ₂ S ₂	[M+H] ⁺	256.0209	25	156.0	108.1
10	Cephapirin	8.0	C ₁₇ H ₁₇ N ₃ O ₆ S ₂	[M+H] ⁺	424.0632	10	292.1	424.1
11	Sulfapyridine	8.2	C ₁₁ H ₁₁ N ₃ O ₂ S	[M+H] ⁺	250.0645	25	156.0	184.1
12	Cefquinome	8.5	C ₂₃ H ₂₄ N ₆ O ₅ S ₂	[M+2H] ⁺⁺	265.0698	25	134.1	199.1
13	Sulfamerazine	8.6	C ₁₁ H ₁₂ N ₄ O ₂ S	[M+H] ⁺	265.0754	25	156.0	190.0
14	Cefacetile	8.9	C ₁₃ H ₁₃ N ₃ O ₆ S	[M+Na] ⁺	362.0417	15	258.0	178.0
16	Cefalonium	9.2	C ₂₀ H ₁₈ N ₄ O ₅ S ₂	[M+H] ⁺	459.0791	35	152.0	123.1
17	Lincomycin	9.3	C ₁₈ H ₃₄ N ₂ O ₆ S	[M+H] ⁺	407.2210	20	407.2	126.1
18	Tulathromycin-marker	9.3	C ₂₉ H ₅₆ N ₂ O ₉	[M+2H] ⁺⁺	289.2066	13	289.2	420.3
19	Trimethoprim	9.4	C ₁₄ H ₁₈ N ₄ O ₃	[M+H] ⁺	291.1451	30	230.1	261.1
20	Sulfamoxole	9.4	C ₁₁ H ₁₃ N ₃ O ₃ S	[M+H] ⁺	268.0750	25	108.1	156.0
21	Thiamphenicol	9.4	C ₁₂ H ₁₅ Cl ₂ NO ₅ S	[M+NH ₄] ⁺	373.0386	13	308.0	338.0
22	4-Epitetracycline	9.4	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.1605	33	201.1	410.1
23	Marbofloxacin	9.5	C ₁₇ H ₁₉ FN ₄ O ₄	[M+H] ⁺	363.1463	15	320.1	363.1
24	Sulfameter	9.5	C ₁₁ H ₁₂ N ₄ O ₃ S	[M+H] ⁺	281.0703	25	108.1	156.0
25	Sulfamethizole	9.7	C ₉ H ₁₀ N ₄ O ₂ S ₂	[M+H] ⁺	271.0318	25	108.1	156.0
IS	Sulfamethazine- ¹³ C ₆	9.8	¹³ C ₆ H ₁₄ N ₄ O ₂ S	[M+H] ⁺	285.1112	-	-	-
26	Sulfamethazine	9.8	C ₁₂ H ₁₄ N ₄ O ₂ S	[M+H] ⁺	279.0910	25	124.1	204.0
27	4-Epioxytetracycline	10.1	C ₂₂ H ₂₄ N ₂ O ₉	[M+H] ⁺	461.1555	33	337.1	426.1
28	Sulfamethoxypyridazine	10.2	C ₁₁ H ₁₂ N ₄ O ₃ S	[M+H] ⁺	281.0703	25	156.0	108.0
29	Tulathromycin A	10.3	C ₄₁ H ₇₉ N ₃ O ₁₂	[M+3H] ⁺⁺⁺	269.5295	10	269.5	289.2
30	Tetracycline	10.3	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.1605	30	410.1	154.1
31	Norfloxacin	10.3	C ₁₆ H ₁₈ FN ₃ O ₃	[M+H] ⁺	320.1405	13	320.1	276.2
32	Cefalexin	10.5	C ₁₆ H ₁₇ N ₃ O ₄ S	[M+H] ⁺	348.1012	30	158.0	174.1
IS	Enrofloxacin-d5	10.6	C ₁₉ H ₁₇ D ₅ FN ₃ O ₃	[M+H] ⁺	365.2032	-	-	-
33	Enrofloxacin	10.6	C ₁₉ H ₂₂ FN ₃ O ₃	[M+H] ⁺	360.1718	40	203.1	245.1
34	Ciprofloxacin	10.6	C ₁₇ H ₁₈ FN ₃ O ₃	[M+H] ⁺	332.1405	35	332.1	245.1
35	Oxytetracycline	10.6	C ₂₂ H ₂₄ N ₂ O ₉	[M+H] ⁺	461.1555	30	337.1	426.1
36	Danofloxacin	10.7	C ₁₉ H ₂₀ FN ₃ O ₃	[M+H] ⁺	358.1561	40	358.2	255.1
37	Cefazolin	10.8	C ₁₄ H ₁₄ N ₈ O ₄ S ₃	[M+H] ⁺	455.0373	35	156.0	133.0
38	Sulfachloropyridazine	10.8	C ₁₀ H ₉ ClN ₄ O ₂ S	[M+H] ⁺	285.0208	25	156.0	108.0
39	Sulfamethoxazole	10.9	C ₁₀ H ₁₁ N ₃ O ₃ S	[M+H] ⁺	254.0594	25	156.0	254.0
40	Difloxacin	11.0	C ₂₁ H ₁₉ F ₂ N ₃ O ₃	[M+H] ⁺	400.1467	27	299.1	279.1
41	Ampicillin	11.1	C ₁₆ H ₁₉ N ₃ O ₄ S	[M+H] ⁺	350.1169	15	106.1	192.1
42	Sulfamonomethoxine	11.2	C ₁₁ H ₁₂ N ₄ O ₃ S	[M+H] ⁺	281.0703	25	156.0	108.1
IS	Florfenicol-d3	11.2	C ₁₂ H ₁₁ D ₃ Cl ₂ FNO ₄ S	[M+H] ⁺	361.0266	-	-	-
43	Florfenicol	11.2	C ₁₂ H ₁₄ Cl ₂ FNO ₄ S	[M+H] ⁺	358.0077	10	340.0	241.0
44	Cefoperazone	11.3	C ₂₅ H ₂₇ N ₉ O ₈ S ₂	[M+H] ⁺	646.1497	23	143.1	290.1
45	Sulfadoxin	11.3	C ₁₂ H ₁₄ N ₄ O ₄ S	[M+H] ⁺	311.0809	25	156.0	108.1

46	Sarafloxacin	11.3	C ₂₀ H ₁₇ F ₂ N ₃ O ₃	[M+H] ⁺	386.1311	45	299.1	386.1
47	Sulfisoxazole	11.4	C ₁₁ H ₁₃ N ₃ O ₃ S	[M+H] ⁺	268.0750	25	108.1	156.0
48	4-Epichlortetracycline	11.7	C ₂₂ H ₂₃ ClN ₂ O ₈	[M+H] ⁺	479.1216	30	444.1	154.1
49	Neospiramycin	11.9	C ₃₆ H ₆₂ N ₂ O ₁₁	[M+2H] ⁺⁺	350.2250	15	174.1	160.1
IS	Spiramycin-I-d3	12.6	C ₄₃ H ₇₁ D ₃ N ₂ O ₁₄	[M+2H] ⁺⁺	423.7737	-	-	-
50	Spiramycin	12.6	C ₄₃ H ₇₄ N ₂ O ₁₄	[M+2H] ⁺⁺	422.2643	13	422.3	540.3
51	Chlortetracycline	12.7	C ₂₂ H ₂₃ ClN ₂ O ₈	[M+H] ⁺	479.1216	25	154.1	444.1
IS	Sulfadimethoxine	13.2	C ₁₂ H ₁₄ N ₄ O ₄ S	[M+H] ⁺	311.0809	25	156.0	108.1
52	Sulfaquinoxaline	13.6	C ₁₄ H ₁₂ N ₄ O ₂ S	[M+H] ⁺	301.0754	25	156.0	108.1
IS	Methacycline	13.8	C ₂₂ H ₂₂ N ₂ O ₈	[M+H] ⁺	443.1449	-	-	-
53	Oxolinic Acid	13.8	C ₁₃ H ₁₁ NO ₅	[M+H] ⁺	262.0710	40	160.0	234.1
IS	Ceftiofur-d3	13.8	C ₁₉ H ₁₄ D ₃ N ₅ O ₇ S ₃	[M+H] ⁺	527.0598	-	-	-
54	Ceftiofur	13.8	C ₁₉ H ₁₇ N ₅ O ₇ S ₃	[M+H] ⁺	524.0363	27	126.0	197.0
55	Gamithromycin	13.9	C ₄₀ H ₇₆ N ₂ O ₁₂	[M+H] ⁺⁺	389.2772	10	389.3	619.5
56	Tilmicosin	14.0	C ₄₆ H ₈₀ N ₂ O ₁₃	[M+2H] ⁺⁺	435.2903	20	695.5	435.3
57	Pirlimycin	14.4	C ₁₇ H ₃₁ ClN ₂ O ₅ S	[M+H] ⁺	411.1715	20	363.2	112.1
58	Doxycycline	14.4	C ₂₂ H ₂₄ N ₂ O ₈	[M+H] ⁺	445.1605	30	321.1	428.1
59	Nalidixic acid	15.5	C ₁₂ H ₁₂ N ₂ O ₃	[M+H] ⁺	233.0921	30	233.1	205.1
IS	Penicillin G-d7	15.9	C ₁₆ H ₁₁ D ₇ N ₂ O ₄ S	[M+Na] ⁺	364.1319	-	-	-
60	Penicillin G	15.9	C ₁₆ H ₁₈ N ₂ O ₄ S	[M+Na] ⁺	357.0880	20	198.1	182.0
61	Tiamulin	15.9	C ₂₈ H ₄₇ NO ₄ S	[M+H] ⁺	494.3299	13	192.0	494.3
62	Flumequine	16.0	C ₁₄ H ₁₂ FNO ₃	[M+H] ⁺	262.0874	35	238.1	262.1
63	Tylosin A	16.1	C ₄₆ H ₇₇ NO ₁₇	[M-C ₈ H ₁₅ O ₅ +3H] ⁺⁺	363.7248	22	174.1	363.2
64	Erythromycin A	16.4	C ₃₇ H ₆₇ NO ₁₃	[M+H] ⁺	734.4685	15	576.4	734.5
65	Tylosin-3-acetate	16.5	C ₄₈ H ₇₉ NO ₁₈	[M-C ₈ H ₁₅ O ₅ +3H] ⁺⁺	384.7301	17	109.1	174.1
66	Oxacillin	17.0	C ₁₉ H ₁₉ N ₃ O ₅ S	[M+Na] ⁺	424.0938	10	265.1	424.1
67	Penicillin V	17.0	C ₁₆ H ₁₈ N ₂ O ₅ S	[M+Na] ⁺	373.0829	12	373.1	214.0
68	Cloxacillin	17.3	C ₁₉ H ₁₈ ClN ₃ O ₅ S	[M+Na] ⁺	458.0548	10	299.0	458.1
69	Valnemulin	17.9	C ₃₁ H ₅₂ N ₂ O ₅ S	[M+H] ⁺	565.3670	11	263.1	565.4
70	Dicloxacillin	17.9	C ₁₉ H ₁₇ Cl ₂ N ₃ O ₅ S	[M+Na] ⁺	492.0158	13	492.0	333.0
71	Nafcillin	18.0	C ₂₁ H ₂₂ N ₂ O ₅ S	[M+H] ⁺	415.1322	12	199.1	256.1
72	Tylvalosin	18.2	C ₅₃ H ₈₇ NO ₁₉	[M+CH ₃ OH+Na] ⁺⁺	548.8050	17	109.1	174.1
73	Rifaximin	19.3	C ₄₃ H ₅₁ N ₃ O ₁₁	[M+H] ⁺	786.3596	20	754.4	786.4

¹ Ions used only for qualitative purposes (ion ratio calculated in Product Ion scan spectrum).

References

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