Supplementary Materials

Table S1: Location, source, and coordinates of the surface water samples in this study, and the identity of the respective isolates

Sample	Location	Source	Coordinates	Date	Isolates recovered
1	Fonte (Rendufe, Valpaços)	Fountain	41º32'5.7408" N 7º23'56.4" W	14/11/2022	*K. pneumoniae* VS3300
2	Fonte (Rendufe, Valpaços)	Fountain	41º32'15.774" N 7º23'59.1576" W	14/11/2022	
3	Rio Miradeses (Valpaços)	River	41º34'04.9188" N 7º15'35.7084" W	14/11/2022	*Klebsiella* spp. VS3315
4	Ponte Lages (Valpaços)	Stream	41º36'18.9324" N 7º18'29.0196" W	14/11/2022	
5	Rio de Curros (Valpaços)	River	41º30'20.3364"N 7º27'30.0384" W	16/11/2022	*K. pneumoniae* VS3301
6	Rio Ribeira Da Fraga (Valpaços)	River	41º32'13.416" N 7º26'22.4304" W	16/11/2022	*K. pneumoniae* VS3302; *Klebsiella* spp. VS3316, VS3317
7	Poço (Valpaços)	Water Well	41º36'17.46" N 7º19'44.3496" W	21/11/2022	*K. pneumoniae* VS3303
8	Fonte (Rendufe, Valpaços)	Fountain	41º32'5.7408" N 7º23'56.4" W	21/11/2022	
9	Ribeira do Cubo (Valpaços)	Stream	41º32'12.084" N 7º25'9.8832 W	21/11/2022	
10	Prado (Carrazedo de Montenegro, Valpaços)	Fountain	41º33'34.1208" N 7º25'52.8672" W	21/11/2022	
11	Prado (Carrazedo de Montenegro, Valpaços)	Water Tank	41º33'34.164" N 7º25'52.95" W	21/11/2022	
12	Fonte Chafariz (Valpaços)	Fountain	41º36'24.5304" N 7º18'39.1464" W	22/11/2022	

13	Rio Tua (Mirandela)	River	41º29'11.3892" N 7º11'5.07122" W	28/11/2022
14	Rio Tua (Parque de Campismo de Mirandela)	River	41º30'29.8332" N 7º11'52.836" W	28/11/2022
15	Rio Tuela (Vale Juncal, Mirandela)	River	41º31'59.4372" N 7º11'4.884" W	28/11/2022
16	Rio Tuela (Abambres, Mirandela)	River	41º33'18.2448" N 7º10'45.984" W	28/11/2022
17	Rio Tuela (Cabanelas, Mirandela)	River	41º31'26.076" N 7º11'28.3524" W	28/11/2022
18	Poço (Valpaços)	Water Well	41º35'44.07" N 7º18'3.3372" W	28/11/2022
19	Poço (Vilarandelo, Valpaços)	Water Well	41º39'41.5476" N 7º19'44.9292" W	28/11/2022
20	Pereiro (Valpaços)	Water Tank	41º35'59.8812" N 7º24'13.5648" W	05/12/2022
21	Mina (Vilaranda, Valpaços)	Water Mine	41º39'12.3588" N 7º25'5.0448" W	05/12/2022
22	Poço (Argemil, Valpaços)	Water Well	41º35'0.4668" N 7º25'30.0" W	05/12/2022
23	Fonte (Celeirós, Valpaços)	Fountain	41º38'56.5008" N 7º23'58.542" W	05/12/2022
24	Nascente (Santiago da Ribeira de Alhariz, Valpaços)	Spring	41º37'5.7792" N 7º24'15.2568" W	05/12/2022
25	Ribeira (Santiago da Ribeira de Alhariz, Valpaços)	Stream	41º37'5.7648" N 7º24'15.282" W	05/12/2022
26	Fonte (Pereiro, Valpaços)	Fountain	41º36'1.368" N 7º24'14.5188" W	05/12/2022
27	Ribeira (Nozedo, Valpaços)	Stream	41º35'9.7908" N 7º26'10.0392" W	12/12/2022
28	Ribeira da Portela (Carrazedo de Montenegro, Valpaços)	Stream	41º33'34.1208" N 7º25'52.8672" W	12/12/2022

29	Ribeira de Sobrado (Sobrado. Valpaços)	Stream	41º34'20.9028" N 7º27'45.63" W	12/12/2022	
30	Ribeira de Seixedo (Seixedo, Valpaços)	Stream	41º34'30.2592"N 7º28'8.346" W	12/12/2022	
31	Fonte (Padrela, Valpaços)	Fountain	41º34'8.9652" N 7º29'44.7432" W	12/12/2022	
32	Ribeira Quinta D. Adelaide (Valpaços)	Stream	41º35'39.9012" N 7º18'18.9792" W	09/01/2023	*K. pneumoniae* VS3304
33	Ribeira Intermarché (Valpaços)	Stream	41º36'24.48" N 7º18'21.798" W	09/01/2023	*Klebsiella* spp. VS3318
34	Poço (Carrazedo de Montenegro, Valpaços)	Water Well	41º34'11.2296" N 7º25'37.0308" W	09/01/2023	*Klebsiella* spp. VS3319
35	Ponte da Horta (Crasto, Valpaços)	Stream	41º34'20.1468" N 7º19'38.8848" W	16/01/2023	*K. pneumoniae* VS3299
36	Ribeira (Crasto, Valpaços)	Stream	41º33'42.948" N 7º19'36.6348" W	16/01/2023	
37	Ribeira Água Revés (Valpaços)	Stream	41º33'43.6752" N 7º19'27.9768" W	16/01/2023	*Klebsiella* spp. VS3320
38	Poço (Água Revés, Valpaços)	Water Well	41º33'29.8188" N 7º20'49.0272" W	16/01/2023	
39	Poço (Carrazedo de Montenegro, Valpaços)	Water Well	41º33'47.3148" N 7º25'41.4372" W	16/01/2023	
40	Rio Rabaçal (Valpaços)	River	41º37'59.3688" N 7º14'49.8372" W	23/01/2023	*Klebsiella* spp. VS3321, VS3322
41	Barragem do Rabaçal (Rebordelo, Vinhais)	Dam	41º45'11.43" N 7º10'23.4372" W	23/01/2023	*K. pneumoniae* VS3305
42	Nascente (Rebordelo, Vinhais)	Spring	41º45'10.2852" N 7º10'24.8808" W	23/01/2023	*Klebsiella* spp. VS3323
43	Fonte (Sonim, Valpaços)	Fountain	41º44'35.466" N 7º11'22.4808" W	23/01/2023	*Klebsiella* spp. VS3324
44	Rio Calvo (Santa Valha, Valpaços)	River	41º40'42.582" N 7º17'20.1408" W	23/01/2023	*K. pneumoniae* VS3296; *Klebsiella* spp. VS3325

45	Fonte (Sá, Valpaços)	Fountain	41º40'28.3728" N 7º20'49.5492" W	30/01/2023	*K. pneumoniae* VS3306; *Klebsiella* spp. VS3326
46	Rio Torto (Valpaços)	River	41º33'25.6392" N 7º16'59.2068" W	30/01/2023	*K. pneumoniae* VS3307, VS3308, VS3309
47	Fonte (Sá, Valpaços)	Fountain	41º40'30.468" N 7º20'48.372" W	30/01/2023	*K. pneumoniae* VS3210
48	Rio Calvo (Vale de Casas, Valpaços)	River	41º38'34.9692" N 7º16'39.4392" W	01/02/2023	*K. pneumoniae* VS3297
49	Ribeira do Requeiral (Rio de Lobos, Valpaços)	Stream	41º36'27.99" N 7º19'38.6292" W	01/02/2023	*K. pneumoniae* VS3298
50	Ribeira de Vale Machado (Valpaços)	Stream	41º34'0.9912" N 7º15'42.8724" W	23/02/2023	
51	Ribeira Bufão (Mirandela)	Stream	41º34'27.8796" N 7º18'4.3956" W	23/02/2023	*Klebsiella* spp. VS3327
52	Regato Gralheira (Vila Pouca de Aguiar)	Stream	41º26'36.3408" N 7º40'25.23" W	13/03/2023	
53	Ribeira da Mãe de Água (Vila Pouca de Aguiar)	Stream	41º25'13.1628" N 7º41'7.5408" W	13/03/2023	
54	Fonte (Vila Pouca de Aguiar)	Fountain	41º29'21.7968" N 7º38'57.2172" W	13/03/2023	
55	Regato Carrica (Vila Pouca de Aguiar)	Stream	41º26'0.4092" N 7º40'43.9212" W	13/03/2023	
56	Ribeiro Telões (Vila Pouca de Aguiar)	Stream	41º28'32.502" N 7º39'30.0672" W	13/03/2023	
57	Ribeira do Mezio (Vila Pouca de Aguiar)	Stream	41º24'59.562" N 7º41'13.668" W	15/03/2023	
58	Rio de Soutelo (Vila Real)	River	41º22'0.516" N 7º43'34.824" W	15/03/2023	
59	Ribeira da Borralheira (Vila Real)	Stream	41º22'34.608" N 7º43'5.376" W	15/03/2023	
60	Ribeiro Palheiros (São Lourenço, Chaves)	Stream	41º43'16.9428" N 7º25'29.172" W	20/03/2023	

61	Regato de S. Lourenço (Chaves)	Stream	41º43'14.502" N 7º24'33.2748" W	20/03/2023	
62	Regato de Sá (Valpaços)	Stream	41º40'57.9828" N 7º21'35.37" W	20/03/2023	
63	Regadio (Vila Verde da Raia, Chaves)	Irrigation Ditch	41º46'40.6632" N 7º25'27.57" W	20/03/2023	
64	Rio Tâmega (Chaves)	River	41º44'30.048" N 7º27'51.0228" W	20/03/2023	*Klebsiella* spp. VS3328
65	Regato (Sabroso de Aguiar, Vila Pouca de Aguiar)	Stream	41º34'8.022" N 7º35'43.3212" W	27/03/2023	*K. pneumoniae* VS3311
66	Rio Tâmega (Outeiro Jusão, Chaves)	River	41º43'11.8668" N 7º28'43.2948" W	27/03/2023	
67	Regato (Pedras Salgadas, Vila Pouca de Aguiar)	Stream	41º32'39.6348" N 7º36'19.818" W	27/03/2023	
68	Regato Águas Campilho (Vidago, Chaves)	Stream	41º38'29.5332" N 7º34'34.266" W	27/03/2023	*K. pneumoniae* VS3312
69	Rio Avelames (Sabroso de Aguiar, Vila Pouca de Aguiar)	River	41º30'40.4748" N 7º38'26.6712" W	27/03/2023	
70	Ribeira da Maila (Lordelo, Vila Real)	Stream	41º18'58.644" N 7º45'59.13" W	04/04/2023	
71	Regato (Quintelas, Vila Real)	Stream	41º19'9.7464" N 7º47'47.4324" W	04/04/2023	
72	Ribeira de Arnal (Vila Real)	Stream	41º19'11.7588" N 7º47'46.7088" W	04/04/2023	
73	Ribeira do Pontão de Outeiro (Vila Real)	Stream	41º19'10.8336" N 7º44'38.8896" W	04/04/2023	
74	Rio Corgo, parque Corgo (Vila Real)	River	41º18'19.656" N 7º44'7.3608" W	04/04/2023	*K. pneumoniae* VS3313
75	Cascata de Galegos da Serra (Vila Real)	Stream	41º19'32.448" N 7º47'57.5448" W	04/04/2023	
76	Ribeira Galegos (Galegos da Serra, Vila Real)	Stream	41º19'47.8992" N 7º48'2.4012" W	04/04/2023	*K. pneumoniae* VS3314

77	Rio Cabril (Vila Real)	River	41º19'7.23" N 7º44'32.7732" W	04/04/2023

Table S2: Primer pairs used for molecular typing, detection of antimicrobial resistance, and virulence genes in Klebsiella strains.

Target gene	Primers sequences (5'-3´)	Amplicon size (base pairs)	Reference
Antimicrobial resistance			
bla$_{CTX-U}$	F:CGATGTGCAGTACCAGTAA	585	[1]
	R:TTAGTGACCAGAATCAGCGG		
bla$_{CTX-M3}$	F:AATCACTGCGTCAGTTCAC	701	[2]
	R:TTTATCCCCCACAACCCAG		
bla$_{CTX-M9}$	F:GTGACAAAGAGAGTGCAACGG	857	[3]
	R:ATGATTCTCGCCGCTGAAGCC		
bla$_{SHV}$	F: CACTCAAGGATGTATTGTG	885	[4]
	R: TTAGCGTTGCCAGTGCTCG		
bla$_{TEM}$	F:ATTCTTGAAGACGAAAGGGC	1150	[4]
	R:ACGCTCAGTGGAACGAAAAC		
*amp*C	F:AATGGGTTTTCTACGGTCTG	191	[5]
	R:GGGCAGCAAATGTGGAGCAA		
*tet*A	F: GTAATTCTGAGCACTGTCGC	937	[4]
	R:CTGTCCTGGACAACATTGCTT		
*tet*B	F: CTCAGTATTCCAAGCCTTTG	416	[4]
	R: CTAAGCACTTGTCTCCTGTT		

aac(3)-II	F: ACTGTGATGGGATACGCGTC R: CTCCGTCAGCGTTTCAGCTA	237	[4]
aac(3)-IV	F: CTTCAGGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	286	[4]
ant(2)	F:ATGTTACGCAGCAGGGCAGTCG R:CGTCAGATCAATATCATCGTGC	188	[6]
*str*A	F: ATTCTGACTGGTTGCCTGTC R: CGCAGATAGAAGGCAAGG	815	[4]
*str*B	F: TTCTCATTGCGGACAACCT R: TAGATCGCGTTGCTCCTCTT	747	[4]
*aad*A1	F:GCAGCGCAATGACATTCTTG R:ATCCTTCGGCGCGATTTTG	282	[7]
*aad*A5	F:CTTCAGTTCGGTGAGTGGC R:CAATCGTTGCTTTGGCATAT	453	[8]
*sul*1	F:TGGTGACGGTGTTCGGCATTC R: GCGAGGGTTTCCGAGAAGGTG	789	[9]
*sul*2	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	722	[4]
*sul*3	F:CATTCTAGAAAACAGTCGTAGTTCG R:CATCTGCAGCTAACCTAGGGCTTTGGA	990	[4]

bla$_{OXA}$	F:ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	813	[4]
bla$_{OXA-48}$	F:TTGGTGGCATCGATTATCGG R:GAGCACTTCTTTTGTGATGGC	743	[3]
bla$_{VIM}$	F:GATGGTGTTTGGTCGCATA R:CGAATGCGCAGCACCAG	390	[3]
bla$_{IMP}$	F:GTTTATGTTCATACTCG R: GGTTTAAAAAACAACCAC	432	[10]
bla$_{KPC}$	F:GTATCGCCGTCTAGTTCTGC R:GGTCGTGTTTCCCTTTAGCC	638	[11]
*par*C	F:AAACCTGTTCAGCGCCGCATT R:AAACCTGTTCAGCGCCGCATT	395	[12]
mcr-1	F:AGTCCGTTTGTTCTTGTGGC R:AGATCCTTGGTCTCGGCTTG	320	[13]
Virulence			
*pap*C	F:GACGGCTGTACTGCAGGGTGTGGCG R:ATATCCTTTCTGCAGGGATGCAATA	328	[4]
*pap*G-III	F: CATTTATCGTCCTCAACTTAG R: AAGAAGGGATTTTGTAGCGTC	482	[4]
*cnf*1	F:AAGATGGAGTTTCCTATGCAGGAG R:CATTCAGAGTCCTGCCCTCATTATT	498	[4]

aer	F:TACCGGATTGTCATATGCAGACCGT R:AATATCTTCCTCCAGTCCGGAGAAG	602	[4]
bfp	F:AACTACATCTAGTACTCAACAACAG R:ATGTGCTTGAATAACACCATCATCT	575	[14]

Table S3: Principal Components Analysis. I) Source versus Genotype, Eigenvalues for surface waters PCA; II) Source versus Virulence, Eigenvalues for surface waters PCA.

I)

Number	Eigenvalue	Percent	Cum Percent
1	1,219158	10,160	10,160
2	1,121243	9,344	19,503
3	1,085126	9,043	28,546
4	1,064549	8,871	37,417
5	1,047725	8,731	46,148
6	1,031250	8,594	54,742
7	1,031250	8,594	63,336
8	1,031250	8,594	71,930
9	1,031250	8,594	80,523
10	1,031250	8,594	89,117
11	1,000000	8,333	97,450
12	0,305949	2,550	100,000

II)

Number	Eigenvalue	Percent	Cum Percent
1	1,142842	19,047	19,047
2	1,080484	18,008	37,055
3	1,047040	17,451	54,506
4	1,031250	17,187	71,694
5	1,031250	17,187	88,881
6	0,667134	11,119	100,000

1. Zong, Z.; Partridge, S.R.; Thomas, L.; Iredell, J.R. Dominance of BlaCTX-M within an Australian Extended-Spectrum β-Lactamase Gene Pool. *Antimicrob Agents Chemother* **2008**, *52*, 4198–4202, doi:10.1128/AAC.00107-08.
2. Maynard, C.; Bekal, S.; Sanschagrin, F.; Levesque, R.C.; Brousseau, R.; Masson, L.; Larivière, S.; Harel, J. Heterogeneity among Virulence and Antimicrobial Resistance Gene Profiles of Extraintestinal Escherichia Coli Isolates of Animal and Human Origin. *J Clin Microbiol* **2004**, *42*, 5444–5452, doi:10.1128/JCM.42.12.5444-5452.2004.
3. Carvalho, I.; Chenouf, N.S.; Cunha, R.; Martins, C.; Pimenta, P.; Pereira, A.R.; Martínez-álvarez, S.; Ramos, S.; Silva, V.; Igrejas, G.; et al. Antimicrobial Resistance Genes and Diversity of Clones among ESBL- and Acquired AmpC-Producing Escherichia Coli Isolated from Fecal Samples of Healthy and Sick Cats in Portugal. *Antibiotics 2021, Vol. 10, Page 262* **2021**, *10*, 262, doi:10.3390/ANTIBIOTICS10030262.
4. Garcês, A.; Correia, S.; Amorim, F.; Pereira, J.E.; Igrejas, G.; Poeta, P. First Report on Extended-Spectrum Beta-Lactamase (ESBL) Producing Escherichia Coli from European Free-Tailed Bats (Tadarida Teniotis) in Portugal: A One-Health Approach of a Hidden Contamination Problem. *J Hazard Mater* **2019**, *370*, 219–224, doi:10.1016/J.JHAZMAT.2017.12.053.
5. Caro¡ —y, N.; Espaze, E.; Be Èrard ™, I.; Richet, H.; Reynaud, A. Mutations in the AmpC Promoter of Escherichia Coli Isolates Resistant to Oxyiminocephalosporins without Extended Spectrum β-Lactamase Production. *FEMS Microbiol Lett* **1999**, *173*, 459–465, doi:10.1111/J.1574-6968.1999.TB13539.X.
6. Vanhoof, R.; Content, J.; Van Bossuyt, E.; Dewit, L.; Hannecart-pokorni, E. Identification of the AadB Gene Coding for the Aminoglycoside-2″-O-Nucleotidyltraiisferase, ANT(2″), by Means of the Polymerase Chain Reaction. *Journal of Antimicrobial Chemotherapy* **1992**, *29*, 365–374, doi:10.1093/JAC/29.4.365.
7. Sáenz, Y.; Briñas, L.; Domínguez, E.; Ruiz, J.; Zarazaga, M.; Vila, J.; Torres, C. Mechanisms of Resistance in Multiple-Antibiotic-Resistant Escherichia Coli Strains of Human, Animal, and Food Origins. *Antimicrob Agents Chemother* **2004**, *48*, 3996–4001, doi:10.1128/AAC.48.10.3996-4001.2004/ASSET/BAF6C9EB-BDBC-44D8-A8B8-E83040DCD8DC/ASSETS/GRAPHIC/ZAC0100443730001.JPEG.
8. Wei, Q.; Jiang, X.; Yang, Z.; Chen, N.; Chen, X.; Li, G.; Lu, Y. DfrA27, a New Integron-Associated Trimethoprim Resistance Gene from Escherichia Coli., doi:10.1093/jac/dkn474.
9. Mazel, D.; Dychinco, B.; Webb, V.A.; Davies, J. Antibiotic Resistance in the ECOR Collection: Integrons and Identification of a Novel Aad Gene. *Antimicrob Agents Chemother* **2000**, *44*, 1568–1574, doi:10.1128/AAC.44.6.1568-1574.2000/ASSET/0ABD19D4-9409-411A-AFC3-B8BEE4FEEC10/ASSETS/GRAPHIC/AC0600809001.JPEG.
10. Aarestrup, F.M.; Agerso, Y.; Gerner-Smidt, P.; Madsen, M.; Jensen, L.B. Comparison of Antimicrobial Resistance Phenotypes and Resistance Genes in Enterococcus Faecalis and Enterococcus Faecium from Humans in the Community, Broilers, and Pigs in Denmark. *Diagn Microbiol Infect Dis* **2000**, *37*, 127–137, doi:10.1016/S0732-8893(00)00130-9.
11. Neyestanaki, D.K.; Mirsalehian, A.; Rezagholizadeh, F.; Jabalameli, F.; Taherikalani, M.; Emaneini, M. Determination of Extended Spectrum Beta-Lactamases, Metallo-Beta-Lactamases and AmpC-Beta-Lactamases among Carbapenem Resistant Pseudomonas Aeruginosa Isolated from Burn Patients. *Burns* **2014**, *40*, 1556–1561, doi:10.1016/J.BURNS.2014.02.010.
12. Vila, J.; Ruiz, J.; Goñi, P.; Jimenez De Anta, M.T. Detection of Mutations in ParC in Quinolone-Resistant Clinical Isolates of Escherichia Coli. *Antimicrob Agents Chemother* **1996**, *40*, 491–493, doi:10.1128/AAC.40.2.491.

13. Rita Rebelo, A.; Bortolaia, V.; Kjeldgaard, J.S.; Pedersen, S.K.; Leekitcharoenphon, P.; Hansen, I.M.; Guerra, B.; Malorny, B.; Borowiak, M.; Andre Hammerl, J.; et al. Multiplex PCR for Detection of Plasmid-Mediated Colistin Resistance Determinants, Mcr-1, Mcr-2, Mcr-3, Mcr-4 and Mcr-5 for Surveillance Purposes. 1, doi:10.2807/1560-7917.ES.2018.23.6.17-00672.
14. Bardiau, M.; Labrozzo, S.; Mainil, J.G. Study of Polymorphisms in Tir, Eae and TccP2 Genes in Enterohaemorrhagic and Enteropathogenic Escherichia Coli of Serogroup O26. *BMC Microbiol* **2011**, *11*, 1–7, doi:10.1186/1471-2180-11-124/TABLES/4.