ESBL resistance genes in fecal *E. coli* of calves fed waste milk with antimicrobial residues

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SUPPLEMENTARY FILE

In the following tables, results of the faecal bacteriological results and of the questionnaire are displayed for better understanding of the results in the manuscript.

Farms were located in mainland Portugal, in the areas followed by three veterinary practices that collaborated in the study. The 31 farms that contributed with *E. coli* isolates to the study, are located in 14 different city councils, from the north through to the south of Portugal.

Table S1 contains the primers that were used for the amplification of ESBL coding genes.

Table S2 displays results of both phenotypic and genotypic evaluation of ESBL production by *E. coli* isolates from calves. Presence of at least one positive isolate per farm was considered sufficient to render farm status as positive for a certain ESBL type.

Tables S3 and S4 display individual farm responses to the questionnaire that focused on putative risk factors for ESBL production by *E. coli* isolated from calves' faeces.

The question regarding the number of intramammary formulations used on each farm in the previous year, was made with reference to a full list of the medicines available for that purpose in the market (n=18) at the moment the questionnaire was delivered.

Table *S1.* Primers that were used for the amplification of ESBL coding genes, their target gene, sequence and size, and respective references.

Target gene	Primer	Primer sequence	Segment size	Reference
*bla*CTX-M1	CTX-M gp1F	5'-AAA AAT CAC TGC GCC AGT TC-3'	415 bp	(Youngquist *et al.*, 2016)
	CTX-M gp1R	5'-AGC TTA TTC ATC GCC ACG TT-3'		
*bla*CTX-M2	CTX-M gp2F	5'-CGA CGC TAC CCC TGC TAT T-3'	552 bp	
	CTX-M gp2R	5'-CCA GCG TCA GAT TTT TCA GG-3'		
*bla*CTX-M8	CTX-M gp8F	5'-TCG CGT TAA GCG GAT GAT GC-3'	666 bp	
	CTX-M gp8/25R	5'-AAC CCA CGA TGT GGG TAG-3'		
*bla*CTX-M9	CTX-M gp9F	5'-CAA AGA GAG TGC AAC GGA TG-3'	205 bp	
	CTX-M gp9R	5'-ATT GGA AAG CGT TCA TCA CC-3'		
*bla*CMY-2	CMY-2 F	5'-ATG ATG AAA AAA TCG TTA TGC GC-3'	1145 pb	(Beneragama *et al.*, 2013)
	CMY-2R	5'-TTA TTG CAG CTT TTC AAG AAT GCG CCA-3'		

Table S2. Results of phenotypic and genotypic evaluation of ESBL production by *E. coli* isolates from calves in each farm.

Farm	Number of lactating cows	Phenotypic ESBL production	*E. coli* isolates producing ESBL types					Total ESBL production types
			CTX-M 1	CTX-M 2	CTX-M 8	CTX-M 9	CMY-2	
1	91	Negative	0	2	0	0	0	1
2	38	Negative	0	0	0	0	0	0
3	40	Negative	0	1	0	0	0	1
4	28	Negative	0	0	0	0	0	0
5	30	Positive	1	0	0	0	0	1
6	105	Positive	1	1	0	1	1	3
7	38	Positive	1	0	0	0	0	1
8	49	Negative	0	1	0	0	0	1
9	40	Positive	4	4	0	0	0	2
10	148	Negative	0	2	0	0	0	1
11	57	Negative	0	2	0	0	0	1
12	63	Negative	0	0	0	0	0	0
13	80	Positive	1	0	0	0	1	2
14	22	Negative	0	0	0	0	0	0
15	60	Positive	2	0	0	0	0	1
16	16	Positive	2	1	0	0	0	2
17	60	Negative	0	1	0	0	0	1
18	36	Negative	0	0	0	0	0	0
19	200	Positive	4	0	0	0	0	1
20	205	Positive	5	0	0	0	0	1
21	715	Positive	4	0	0	0	0	1
22	194	Positive	0	0	0	5	0	1
23	579	Positive	0	0	0	1	0	1
24	387	Negative	0	0	0	0	0	0
25	215	Negative	0	0	0	0	0	0
26	63	Negative	0	0	0	0	0	0
27	423	Positive	4	0	0	1	0	2
28	414	Positive	2	0	0	0	0	1
29	45	Negative	0	0	0	0	0	0
30	174	Negative	0	0	0	0	0	0
31	70	Positive	0	0	0	0	0	0

Table S3. Individual farm responses to the questionnaire focused on putative risk factors for ESBL production regarding the milk being fed.

Farm	Feeds waste milk	Waste milk from mastitis	Waste milk from withdrawal period	Waste milk from high cell count	Processing of waste milk	Adds antibiotic to milk
1	Yes	Yes	Yes	Yes	None	Yes
2	Yes	Yes	Yes	No	None	No
3	No	No	No	No	None	No
4	Yes	Yes	Yes	No	None	No
5	Yes	Yes	Yes	No	None	No
6	Yes	Yes	Yes	Yes	None	No
7	Yes	Yes	Yes	Yes	None	No
8	Yes	Yes	Yes	Yes	None	No
9	Yes	Yes	Yes	Yes	None	No
10	Yes	Yes	Yes	Yes	None	No
11	Yes	Yes	Yes	Yes	None	No
12	Yes	Yes	Yes	Yes	None	Yes
13	Yes	Yes	Yes	Yes	None	No
14	Yes	Yes	Yes	No	None	No
15	Yes	Yes	Yes	Yes	None	No
16	Yes	Yes	Yes	Yes	None	No
17	Yes	Yes	Yes	Yes	None	No
18	Yes	Yes	Yes	Yes	None	No
19	No	No	No	No	Pasteurizes	Yes
20	Yes	Yes	Yes	No	None	No
21	Yes	Yes	Yes	No	Pasteurizes	No
22	Yes	Yes	Yes	Yes	None	Yes
23	Yes	Yes	Yes	No	None	No
24	Yes	Yes	Yes	No	None	Yes
25	Yes	Yes	Yes	Yes	None	No
26	Yes	Yes	Yes	No	None	No
27	Yes	Yes	Yes	No	None	Yes
28	Yes	Yes	Yes	No	None	Yes
29	No	No	No	No	Pasteurizes	Yes
30	Yes	Yes	Yes	Yes	None	No
31	Yes	Yes	Yes	Yes	None	No

Table S4. Individual farm responses to the questionnaire focused on putative risk factors for ESBL production regarding biosecurity and treatment options.

Farm	Treats diarrhea with oral antibiotic	Treats diarrhea with systemic antibiotic	Treats diarrhea with oral and systemic antibiotic	Buys-in animals	When were the last animals bought	Origin of bought in animals	No. of intramammary formulations used in previous year
1	No	Yes	No	Yes	2 to 3 years	Trader	1
2	Yes	No	No	Yes	Previous year	Trader	2
3	No	Yes	No	Yes	Previous year	Single farm	1
4	No	Yes	No	Yes	Over 3 years ago	Single farm	2
5	Yes	Yes	Yes	No	Not applicable	Not applicable	4
6	Yes	Yes	Yes	Yes	Previous year	Single farm	6
7	Yes	Yes	Yes	No	Not applicable	Not applicable	1
8	No	No	No	Yes	2 to 3 years	Trader	2
9	Yes	Yes	Yes	No	Not applicable	Not applicable	7
10	No	Yes	No	Yes	1 to 2 years ago	Single farm	2
11	Yes	Yes	Yes	Yes	Previous year	Trader	3
12	No	Yes	No	No	Not applicable	Not applicable	3
13	No	Yes	No	Yes	1 to 2 years ago	Trader	2
14	No	Yes	No	No	Not applicable	Not applicable	3
15	Yes	Yes	Yes	Yes	Previous year	Trader	6
16	No	Yes	No	No	Not applicable	Not applicable	1
17	Yes	Yes	Yes	No	Not applicable	Not applicable	4
18	Yes	Yes	Yes	No	Not applicable	Not applicable	1
19	Yes	Yes	Yes	No	Not applicable	Not applicable	3
20	No	Yes	No	No	Not applicable	Not applicable	3
21	Yes	No	No	Yes	Not applicable	Trader	2
22	Yes	No	No	Yes	Previous year	Single farm	3
23	Yes	No	No	Yes	Previous year	Trader	3
24	No	Yes	No	No	Not applicable	Not applicable	2
25	Yes	Yes	Yes	No	Not applicable	Not applicable	1
26	Yes	Yes	Yes	Yes	Previous year	Trader	2
27	No	Yes	No	No	Not applicable	Not applicable	3
28	Yes	Yes	Yes	No	Not applicable	Not applicable	4
29	No	Yes	No	No	Not applicable	Not applicable	7
30	No	Yes	No	No	Not applicable	Not applicable	2
31	No	Yes	No	Yes	Over 3 years ago	Trader	3