

## Supplementary Material

**Table S1.** Characterization of 62 ESBL-producing *E. coli* from sheep from Portugal

Animal	Isolate	ESBL enzyme	$\beta$ -lactam resistance	Other resistance	Other resistance genes	Replicon content (Inc)	PG	PFGE pattern
PTOV01	Ov7	SHV-12	AML, CTX, CAZ, EFT, CPT, ATM	TET	<i>tetA</i>	B/O, FIB, I1 $\gamma$ , K, FII	B1	L
PTOV02	Ov2	CTX-M-1	AML, CTX, EFT, FEP, CPT	-	-	L	A	A
PTOV02	Ov3	CTX-M-32	AML, CTX, EFT, CPT	GEN, TET	<i>aac(6')Ib-cr</i> , <i>aac(3')-II</i> , <i>aph(3')-III</i> , <i>tetA</i> , <i>tetB</i>	HI1, FII	A	AE
PTOV02	Ov25	CTX-M-15	AML, AMC, CTX, EFT, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, Y, X2, FII	A	AC
PTOV03	Ov4	CTX-M-98	AML, CTX, EFT, CPT	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul1</i> , <i>sul2</i> , <i>tetA</i>	FIB, FIA, I1 $\gamma$ , FII	A	M
PTOV03	Ov5	CTX-M-15	AML, AMC, CTX, EFT, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV03	Ov6	CTX-M-32	AML, AMC, CTX, EFT, CPT	SXT, TET	<i>sul3</i> , <i>tetB</i>	FIB, L, X2, FII	A	H
PTOV04	Ov8	CTX-M-15	AML, AMC, CTX, EFT, FEP, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV04	Ov9	CTX-M-15	AML, AMC, CTX, CAZ, EFT, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV04	Ov10	CTX-M-15	AML, AMC, CTX, EFT, CPT, ATM	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul1</i> , <i>tetB</i>	FIB, FIA, I1 $\gamma$ , Y, FII	A	Q
PTOV05	Ov11	CTX-M-1	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM, FOX	FUR, COL	<i>aac(6')Ib-cr</i> , <i>mcr-1</i>	I1 $\gamma$ , X1	B1	W
PTOV05	Ov12	CTX-M-15	AML, AMC, CTX, CAZ, EFT, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, X2	A	Z
PTOV05	Ov13	CTX-M-32	AML, CTX, CAZ, EFT, FEP, CPT, ATM	CIP, GEN, TET, FUR	<i>aac(6')Ib-cr</i> , <i>aac(3)-II</i> , <i>sul1</i> , <i>tetB</i>	L	A	AF
PTOV05	Ov64	CTX-M-32	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr</i> , <i>aac(3)-II</i> , <i>tetB</i>	I1 $\alpha$ , L, I1 $\gamma$	A	S
PTOV06	Ov14	CTX-M-14	AML, AMC, CTX, EFT, CPT	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul1</i> , <i>sul2</i> , <i>tetA</i>	I1 $\alpha$ , FIB, P, I1 $\gamma$ , FII	A	N
PTOV06	Ov15	CTX-M-15	AML, AMC, CTX, CAZ, EFT, CPT, ATM	SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV06	Ov16	CTX-M-32	AML, CTX, EFT, CPT, ATM	SXT, TET	<i>sul1</i> , <i>sul3</i> , <i>tetB</i>	L	A	AF
PTOV06	Ov65	CTX-M-32	AML, CTX, EFT, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr</i> , <i>aac(3)-II</i> , <i>tetB</i>	I1 $\alpha$ , L, I1 $\gamma$	A	S
PTOV07	Ov17	CTX-M-15	AML, AMC, CTX, EFT, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV07	Ov18	CTX-M-32	AML, CTX, EFT, FEP, CPT, ATM	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul3</i> , <i>tetA</i>	-	B1	X
PTOV07	Ov19	CTX-M-32	AML, CTX, CAZ, EFT, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr</i> , <i>aac(3)-II</i> , <i>tetB</i>	I1 $\alpha$ , L, I1 $\gamma$	A	D
PTOV07	Ov20	CTX-M-1	AML, AMC, CTX, EFT, CPT	SXT	<i>sul2</i>	I1 $\alpha$ , FIB, X1, FII	B1	G
PTOV07	Ov21	CTX-M-15	AML, AMC, CTX, EFT, CPT	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul3</i> , <i>tetA</i>	L, X3, FII	A	AG
PTOV07	Ov22	CTX-M-15	AML, AMC, CTX, EFT, CPT	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV07	Ov23	CTX-M-15	AML, CTX, EFT, CPT	CIP, SXT	<i>aac(6')Ib-cr</i> , <i>sul2</i>	I1 $\alpha$ , FIB, P, I1 $\gamma$ , FII	A	O
PTOV07	Ov24	CTX-M-15	AML, AMC, CTX, EFT, FEP, CPT, ATM	SXT, TET	<i>sul2</i> , <i>tetA</i>	FIB, L	A	AA
PTOV07	Ov66	CTX-M-32	AML, AMC, CTX, EFT, CPT	GEN, TET	<i>aac(6')Ib-cr</i> , <i>aac(3)-II</i> , <i>aph(3)-III</i> , <i>tetA</i> , <i>tetB</i>	HI1, FII	A	AE
PTOV08	Ov26	CTX-M-32	AML, CTX, EFT, FEP, CPT, ATM	TET	<i>tetA</i>	N, FIB, I1 $\gamma$ , FII	B1	C
PTOV08	Ov27	CTX-M-15	AML, CTX, CAZ, EFT, CPT, ATM	CIP, SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul1</i> , <i>tetB</i>	FIB, FIA, I1 $\gamma$	A	R
PTOV08	Ov28	CTX-M-15	AML, AMC, CTX, EFT, CPT, ATM	TET	<i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV08	Ov29	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	TET	<i>tetB</i>	N, I1 $\gamma$ , FII	A	U
PTOV10	Ov30	CTX-M-32	AML, AMC, CTX, EFT, CPT	SXT, TET, FUR	<i>sul1</i> , <i>tetB</i>	L	A	AF
PTOV11	Ov31	CTX-M-32	AML, AMC, CTX, EFT, CPT, ATM	SXT, TET, CHL	<i>sul1</i> , <i>sul2</i>	L	A	AF
PTOV11	Ov32	CTX-M-32	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM	-	-	N, I1 $\gamma$	D	AB
PTOV11	Ov33	CTX-M-15	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM	SXT, TET	<i>aac(6')Ib-cr</i> , <i>sul2</i> , <i>tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV12	Ov34	CTX-M-1	AML, CTX, EFT, CPT, ATM	TET	<i>tetB</i>	N, I1 $\gamma$	B1	P
PTOV13	Ov35	CTX-M-32	AML, AMC, CTX, EFT, FEP, CPT, ATM	CIP, SXT, TET	<i>sul3</i>	R	B1	T

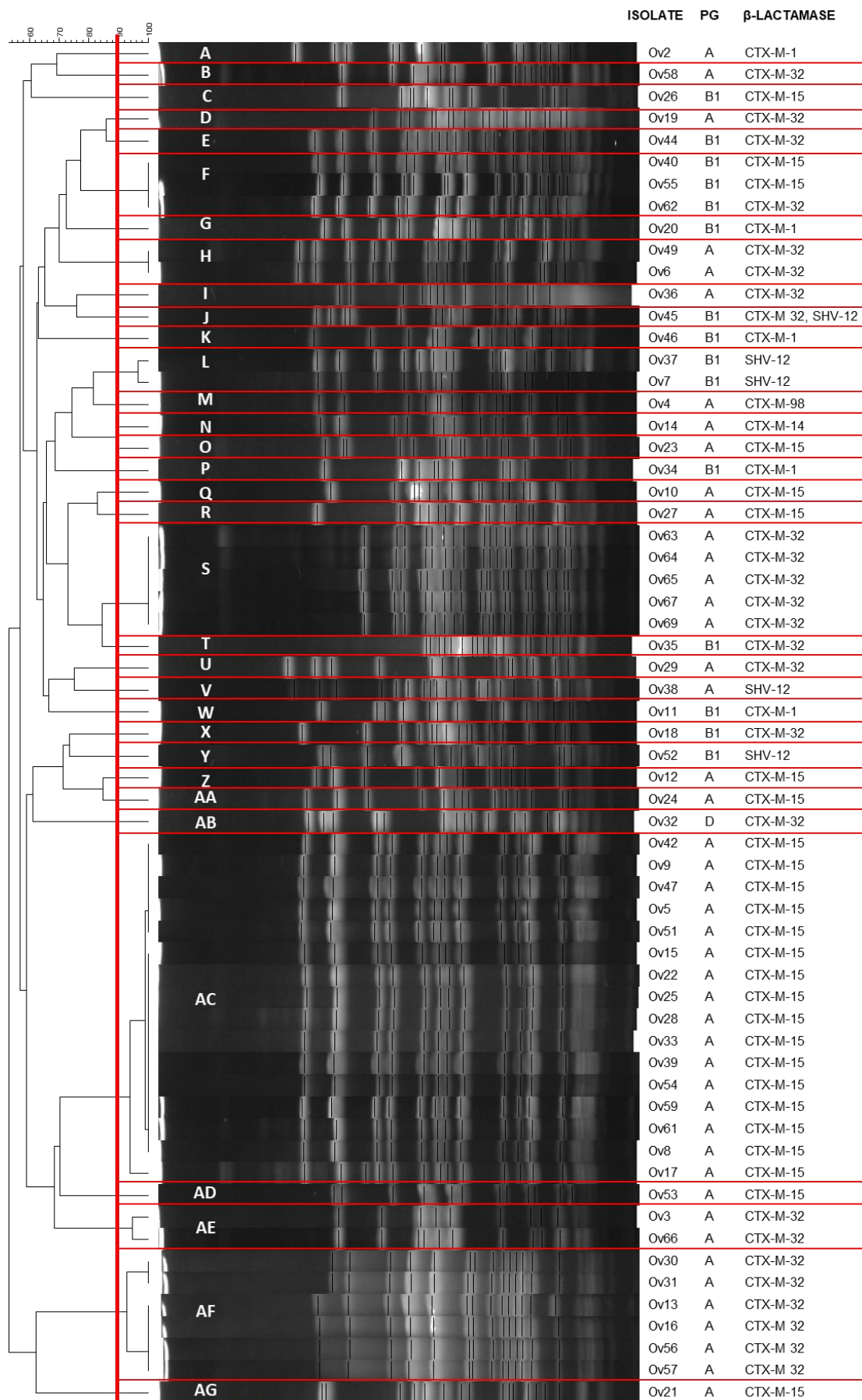
PTOV13	Ov36	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	-	-	N, L	A	I
PTOV13	Ov37	SHV-12	AML, CTX, CAZ, ATM	TET	<i>tetA</i>	N, FIB, II $\gamma$ , K, FII	B1	L
PTOV13	Ov38	SHV-12	AML, CTX, CAZ, ATM	CIP, TET	-	II $\alpha$ , N, FIB, II $\gamma$ , Y, X2, FII	A	V
PTOV13	Ov39	CTX-M-15	AML, AMC, CTX, EFT, FEP, CPT, ATM	SXT	<i>sul2</i>	FIB, L, Y, X2, FII	A	AC
PTOV13	Ov40	CTX-M-15	AML, CTX, CAZ, EFT, FEP, CPT, ATM	SXT, TET	<i>sul2, tetA</i>	II $\gamma$ , Y	B1	F
PTOV15	Ov67	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr, aac(3)-II, tetB</i>	II $\alpha$ , L, II $\gamma$	A	S
PTOV15	Ov42	CTX-M-15	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM	GEN, SXT, TET, FUR	<i>aac(6')Ib-cr, qnrS, aph(3)-III, sul2, tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV15	Ov44	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	GEN	<i>aac(6')Ib-cr, aac(3)-II</i>	II $\alpha$ , II $\gamma$	B1	E
PTOV15	Ov45	CTX-M-32, SHV-12	AML, AMC, CTX, CAZ, ATM	SXT, TET	<i>sul1, sul2, tetA</i>	II $\gamma$ , Y	B1	J
PTOV16	Ov46	CTX-M-1	AML, AMC, CTX, EFT, CPT,	TET	<i>tetA, tetB</i>	II $\alpha$ , FIB, II $\gamma$ , FII	B1	K
PTOV16	Ov47	CTX-M-15	AML, AMC, CTX, CAZ, EFT, CPT, ATM	SXT, TET	<i>sul2, tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV17	Ov49	CTX-M-32	AML, AMC, CTX, EFT, FEP, CPT, ATM	SXT, TET	<i>sul3, tetB</i>	FIB, Y, FII	A	H
PTOV17	Ov51	CTX-M-15	AML, CTX, CAZ, EFT, CPT, ATM	SXT	<i>sul2, sul3</i>	FIB, L, Y, X2, FII	A	AC
PTOV17	Ov52	SHV-12	AML, CTX, CAZ, EFT, ATM	TET, CHL	<i>tetA</i>	II $\alpha$ , II $\gamma$	B1	Y
PTOV17	Ov63	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr, aac(3)-II, tetB</i>	II $\alpha$ , L, II $\gamma$	A	S
PTOV18	Ov53	CTX-M-15	AML, CTX, EFT, CPT	TET	<i>tetA</i>	L	A	AD
PTOV18	Ov54	CTX-M-15	AML, AMC, CTX, EFT, CPT	SXT, TET	<i>sul2, tetA</i>	FIB, L, Y, X2	A	AC
PTOV18	Ov55	CTX-M-15	AML, CTX, EFT, CPT, ATM	SXT, TET	<i>sul2, tetA</i>	II $\gamma$ , Y	B1	F
PTOV18	Ov56	CTX-M-32	AML, CTX, EFT, CPT, ATM	SXT, TET	<i>sul1, sul3, tetB</i>	L	A	AF
PTOV19	Ov57	CTX-M-32	AML, CTX, EFT, CPT	SXT, TET	<i>sul1, sul3, tetB</i>	L	A	AF
PTOV19	Ov58	CTX-M-32	AML, CTX, CAZ, EFT, FEP, CPT, ATM	SXT, TET, CHL	<i>sul1, sul2, sul3, tetB</i>	HI1, FIB, II $\gamma$ , FII	A	B
PTOV19	Ov59	CTX-M-15	AML, CTX, CAZ, EFT, FEP, CPT, ATM	SXT, TET	<i>sul2, tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV20	Ov61	CTX-M-15	AML, AMC, CTX, CAZ, EFT, FEP, CPT, ATM	CIP, SXT, TET, FUR	<i>aac(6')Ib-cr, qnrS, sul2, tetA</i>	FIB, L, Y, X2, FII	A	AC
PTOV20	Ov69	CTX-M-32	AML, AMC, CTX, CAZ, EFT, CPT, ATM	CIP, GEN, TET	<i>aac(6')Ib-cr, aac(3)-II, tetB</i>	II $\alpha$ , L, II $\gamma$	A	S
PTOV21	Ov62	CTX-M-32	AML, CTX, CAZ, EFT, FEP, CPT, ATM	SXT, TET	<i>sul2, tetA</i>	N, L, II $\gamma$ , Y	B1	F

AML – amoxicillin, AMC – amoxicillin-clavulanic acid, CTX – cefotaxime, CAZ – ceftazidime, EFT – ceftiofur, FEP – cefepime, CPT – ceftaroline, ATM – aztreonam, FOX – ceftiofur, CIP – ciprofloxacin, GEN – gentamicin, SXT – sulfamethoxazole-trimethoprim, TET – tetracycline, CHL – chloramphenicol, FUR – nitrofurantoin.  
PG: phylogroup.

**Table S2:** List of primers used in the standard PCRs assays.

Aim	Target	Primer Name	Primer Sequence (5'-3')	Amplicon Size (pb)	Annealing (T)	Reference
Colistin resistance	<i>mcr-1</i>	MCR-1 F	AGTCCGTTTGTTCTGTGGC	320	58°C	[47]
		MCR-1 R	AGATCCTTGGTCTCGGCTTG			
	<i>mcr-2</i>	MCR2-F	CAAGTGTGTTGGTCGAGTT	715	58°C	[47]
		MCR2-R	TCTAGCCCCGACAAGCATACC			
	<i>mcr-3</i>	MCR3-F	AAATAAAAATTGTTCCGCTTATG	929	58°C	[47]
		MCR3-R	AATGGAGATCCCCGTTTTT			
	<i>mcr-4</i>	MCR4-F	TCACTTTCATCACTGCGTTG	1116	58°C	[47]
		MCR4-R	TGGTCCATGACTACCAATG			
	<i>mcr-5</i>	MCR5-F	ATGCGGTTGTCTGCATTATC	1644	58°C	[47]
		MCR5-R	TCATTGTGGTTGCTCTTTCTG			
Beta-lactamases	<i>bla</i> <sub>CTX-M</sub> G1	MP CTX-M G1 F	AAA AAT CAC TGC GCC AGT TC	415	52°C	[48]
		MP CTX-M G1 R	AGC TTA TTC ATC GCC ACG TT			
	<i>bla</i> <sub>CTX-M</sub> G2	MP CTX-M G2 F	CGA CGC TAC CCC TGC TAT T	552	52°C	[48]
		MP CTX-M G2 R	CCA GCG TCA GAT TTT TCA GG			
	<i>bla</i> <sub>CTX-M</sub> G8	MP CTX-M G8 F	CGT TAA GCG GAT GAT GC	666	52°C	[48]
		MP CTX-M G8 R	AAC CCA CGA TGT GGG TAG C			
	<i>bla</i> <sub>CTX-M</sub> G9	MP CTX-M G9 F	CAA AGA GAG TGC AAC GGA TG	205	52°C	[48]
		MP CTX-M G9 R	ATT GGA AAG CGT TCA TCA CC			

	<i>bla</i> <sub>CTX-M G25</sub>	MP CTX-M G25 F MP CTX-M G25 R	GCA CGA TGA CAT TCG GG AAC CCA CGA TGT GGG TAG C	327	52°C	[48]
	<i>bla</i> <sub>OXA</sub>	OXA F OXA R	CATTTCGGTGTGCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800	57°C	[49]
	<i>bla</i> <sub>TEM</sub>	TEM F TEM R	AGCCGCTTGAGCAAATTAAC ATCCCGCAGATAAATCACCAC	713	57°C	[49]
	<i>bla</i> <sub>SHV</sub>	SHV F SHV R	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCTCTGTAAGTG	564	57°C	[49]
Quinolone resistance	<i>qnrA</i>	qnrA F qnrA R	GGATGCCAGTTTCGAGGA TGCCAGGCACAGATCTTG	492	59°C	[52]
	<i>qnrB</i> (1-6)	qnrB (1-6) F qnrB (1-6) R	GGMATHGAAATTCGCCACTG TTTGCYGYCGCCAGTCGAA	580	57°C	[53]
	<i>qnrS</i> (1-2)	qnrS (1-2) R qnrS (1-2) F	GATCTAAACCGTCGAGTTCGG TCGACGTGCTAACTTGCG	466	57°C	[52]
	<i>aac6'</i> <i>Ib-cr</i>	aac-cr- F aac-cr-R	TTGCGATGCTCTATGAGTGG GCGTGTCGCTCGAATGCC	400	57°C	[54]
Aminoglycoside resistance	<i>aac</i> (3)-II	aac(3)-II 1 aac(3)-II 2	GTCGAACAGGTAGCACTGAG TGAAACGCTGACGGAGCCTC	568	63°C	[55]
	<i>aac</i> (3)-IV	aac(3)-IV- F aac(3)-IV- R	GTGTGCTGTGGTCCACAGC AGTTGACCCAGGGCTGTCGC	705	55°C	[56]
	<i>ant</i> (2'')-I	ant(2'')-I F ant(2'')-I R	GGGCGCGTCATGGAGGAGTT TATCGCGACCTGAAAGCGGC		68°C	[57]
	<i>aph</i> (3')-I	aph(3')-I F aph(3')-I R	GCCGATGTGGATTGCGAAAA GCTTGATCCCCAGTAAGTCA		68°C	[58]
	<i>aph</i> (3')-II	aph(3')-II F aph(3')-II R	GCTATTCGGCTATGACTGGGC CCACCATGATATTCGGCAAGC		63°C	[59]
	<i>aph</i> (3')-III	aph(3')-III F aph(3')-III R	AACGCTCTGTCTCGAGGCCGCG GGCAAGATCCTGTGATCGGTC		53°C	[60]
Tetracycline resistance	<i>tetA</i>	tetA 1 tetA 2	GTAATTCTGAGCACTGTTCGC CTGCCTGGACAACATTGCTT	950	57°C	[61]
	<i>tetB</i>	tetB 1 tetB 2	CTCAGTATTCCAAGCCTTTG ACTCCCTGAGCTTGAGGGG	430	53°C	[62]
Sulphonamide resistance	<i>sul1</i>	sul1 F sul1 R	TGAGATCAGACGTATTGCGC TTGAAGGTTTCGACAGCACGT	400	59°C	[63]
	<i>sul2</i>	sul 2 F sul 2 R	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT	270	70°C	[59]
	<i>sul3</i>	sul 3 F sul 3 R	CATCTGCAGCTAACCTAGGGCTTTGGA GAGCAAGATTTTGGAAATCG	990	53°C	[64]
<i>E. coli</i> Phylogenetic Group	<i>chuA</i>	ChuA.1b ChuA.2	ATGGTACCGGACGAACCAAC TGCCGCCAGTACCAAAGACA	288	59°C	[65]
	<i>yjaA</i>	YjaA.1b YjaA.2b	CAAACGTGAAGTGTCAGGAG AATGCGTTCCTCAACCTGTG	211	59°C	[65]
	<i>arpA</i>	AceK.f ArpA1.r	AACGCTATTGCGCAGCTTGC TCTCCCCATACCGTACGCTA	400	59°C	[65]
	TspE4C2	TspE4C2.1b TspE4C2.2b	CACTATTTCGTAAGGTCATCC AGTTTATCGTGC GGTCGC	152	59°C	[65]



PG – Phylogenetic group

**Figure S1.** Clonal relation of 62 ESBL-producing *E. coli* from sheep from Portugal