antibiotics

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Article

Prevalence of Antibiotic Resistance Genes in Multidrug-Resistant *Enterobacteriaceae* on Portuguese Livestock Manure

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Supplementary Materials

Table S1. Primers used for the identification of chloramphenicol (*cat*), trimethoprim (*dfr*), quinolones (*qnr, aac*(6′)-Ib, *oqx, qep*), sulphonamides (*sul*) and tetracyclines (*tet*) resistance genes and for integron class.

Target gene/group	Primers sequences (5′-3′) Fw/Rv	Amplicon size (bp)	Primers (μM)	Reference
***cat* I**	GGTGATATGGGATAGTGTT/CCATCACATACTGCATGATG	349	1.0	[1]
***cat* II**	GATTGACCTGAATACCTGGAA/CCATCACATACTGCATGATG	567	1.0	[1]
***cat* III**	CCATACTCATCCGATATTGA/CCATCACATACTGCATGATG	275	1.0	[1]
***cat* IV**	CCGGTAAAGCGAAATTGTAT/CCATCACATACTGCATGATG	451	1.0	[1]
***dfr* Ia**	GTGAAACTATCACTAATGG/ACCCTTTTGCCAGATTTG	471	1.0	[2]
***dfr* Ib**	TTGGGAAGGACAACGCACTT/ACCATTTCGGCCAGATCAAC	382	1.0	[3]
***dfr* Ic**	GGTGAGCARAAGATYTTTCGC/TGGGAAGAAGGCGTCACCCTC	309	1.0	[2]
***dfr* IIa**	GCBAAAGGDGARCAGCT/TTTMCCAYATTTGATAGC	394	1.0	[2]
***dfr* IIb**	AAAATTTCATTGATTTCTGCA/TTAGCCTTTTTTCCAAATCT	471	1.0	[2]
***dfr* IIc**	TTTATTGTGGTAAGCAATAC/GTATACATCTGCATCAAAAC	201	1.0	[2]
***dfr* IIIa**	ACCTGCCGATCTGCGTCAT/TCGCAGGCATAGCTGTTCTT	387	1.0	[3]
***dfr* IIIb**	ACCAGAGCATTCGGTAATCA/TTGGATCACCTACCCATAGA	445	1.0	[3]
***dfr* IIIc**	CACAGTCTATCGCCTTAATC/ATAGACCACAAAGCTAAACG	233	1.0	[2]
***dfr* IVa**	GTTTCCGAGAATGGAGTAAT/GGTACGTGTAATCAATATTTG	429	1.0	[3]
***dfr* IVb**	TCACCAAGAAGTCAGAGATT/TAAAACCAGATTCGACTTTC	311	1.0	[2]
***dfr* IVc**	AGAATTCCCTTCTCTTTGAT/ATGCCAACAGTTGAGATTAT	218	1.0	[2]
***dfr* Va**	GATCACGTRCGCAAGAARTC/GACTCGACVGCRTASCCTTC	95	1.0	[2]
***dfr* Vb**	TGAACCAGAAGATTTAAAACAC/AATGGTCGGGACCTCAGAT	384	1.0	[2]
***dfr* Vc**	AGTCGCTGTGGATTCTAAGT/CAATGTGAAAATTGTTCTGG	455	1.0	[2]
***dfr* Vd**	ATGATTTGCTTTGGCACTTA/CCACCAATAATGAAGCATGT	250	1.0	[2]
***sul*1**	CGGCGTGGGCTACCTGAACG/GCCGATCGCGTGAAGTTCCG	433	0.4	[4]
***sul*2**	GCGCTCAAGGCAGATGGCATT/GCGTTTGATACCGGCTCCCGT	293	0.4	[4]
***sul*3**	GAGCAAGATTTTGGAATCG/CATCTGCAGCTAACCTAGGGCTTTGGA	790	0.4	[5]
***tet*(A)**	GCTACATCCTGCTTGCCTTC/CATAGATCGCCGTGAAGAGG	210	1.0	[6]
***tet*(B)**	TTGGTTAGGGGCAAGTTTTG/GTAATGGGCCAATAACACCG	659	0.25	[6]
***tet*(C)**	CTTGAGAGCCTTCAACCCAG/ATGGTCGTCATCTACCTGCC	418	0.25	[6]
***tet*(D)**	AAACCATTACGGCATTCTGC/GACCGGATACACCATCCATC	787	2.0	[6]
***tet*(E)**	AAACCACATCCTCCATACGC/AAATAGGCCACAACCGTCAG	278	1.0	[6]
***tet*(G)**	CAGCTTTCGGATTCTTACGG/GATTGGTGAGGCTCGTTAGC	468	1.0	[6]
***tet*(K)**	TCGATAGGAACAGCAGTA/CAGCAGATCCTACTCCTT	844	1.25	[6]
***tet*(L)**	TCGTTAGCGTGCTGTCATTC/GTATCCCACCAATGTAGCCG	267	1.0	[6]

Target gene/group	Primers sequences (5′-3′) Fw/Rv	Amplicon size (bp)	Primers (μM)	Reference
***tet*(M)**	GTGGACAAAGGTACAACGAG/CGGTAAAGTTCGTCACACAC	406	0.5	[6]
***tet*(O)**	AACTTAGGCATTCTGGCTCAC/TCCCACTGTTCCATATCGTCA	515	1.25	[6]
***tet*(S)**	CATAGACAAGCCGTTGACC/ATGTTTTTGGAACGCCAGAG	667	0.5	[6]
***tet*A(P)**	CTTGGATTGCGGAAGAAGAG/ATATGCCCATTTAACCACGC	676	1.25	[6]
***tet*(Q)**	TTATACTTCCTCCGGCATCG/ATCGGTTCGAGAATGTCCAC	904	1.25	[6]
***tet*(X)**	CAATAATTGGTGGTGGACCC/TTCTTACCTTGGACATCCCG	468	1.25	[6]
***qnr* A**	AGAGGATTTCTCACGCCAGG/TGCCAGGCACAGATCTTGAC	580	0.25	[7]
***qnr* B**	GGMATHGAAATTCGCCACTG/TTTGCYGYYCGCCAGTCGAA	264	0.25	[7]
***qnr* C**	GGGTTGTACATTTATTGAATCG/CACCTACCCATTTATTTTCA	307	0.25	[8]
***qnr* D**	CGAGATCAATTTACGGGGAATA/AACAAGCTGAAGCGCCTG	465	0.25	[9]
***qnr* S**	GCAAGTTCATTGAACAGGGT/TCTAAACCGTCGAGTTCGGCG	428	0.25	[7]
***aac*(6′)-Ib**	TTGCGATGCTCTATGAGTGGCTA/CTCGAATGCCTGGCGTGTTT	482	0.25	[10]
***oqx* A**	CTCGGCGCGATGATGCT/CCACTCTTCACGGGAGACGA	392	0.25	[8]
***oqx* B**	TCCTGATCTCCATTAACGCCCA/ACCGGAACCCATCTCGATGC	131	0.25	[8]
***qep* A**	CCAGCTCGGCAACTTGATAC/ATGCTCGCCTTCCAGAAAA	570	0.25	[11]
***intI*1**	GGGTCAAGGATCTGGATTTCG/ACATGCGTGTAAATCATCGTCG	465	0.3	[12]
***intI*2**	CACGGATATGCGACAAAAAGGT/GTAGCAAACGAGTGACGAAATG	788	0.3	[12]
***intI*3**	GCCTCCGGCAGCGACTTTCAG/ACGGATCTGCCAAACCTGACT	979	0.3	[12]

Table S2. Multiplex PCR conditions for target genes.

Target gene/group		Cycling conditions						Final concentrations		
		1st step[a]	Cycles	2nd step[b]	3rd step[c]	4th step[d]	5th step[e]	$MgCl_2$ (mM)	dNTP (μM)	*Taq* pol. (U)
cat	**I, II, III, IV**	95/3′	34x	95/60′′	55/60′′	72/90′′	72/5′	3.0	300	1.5
intI	***1, 2, 3***	94/5′	30x	94/30′′	62/30′′	72/60′′	72/8′	5.0	360	1.0
sul	**1, 2**	94/5′	30x	94/15′′	69/30′′	72/60′′	72/7′	2.0	200	0.5
sul	**3**				51/30′′					
tet	**A, E, G, K, L, M, O, S**	94/5′	35x	94/60′′	55/60′′	72/90′′	72/7′	3.0	300	2.5
tet	**B, C, D, A(P), Q, X**							4.0		
dfr	**I**	94/2′	30x	94/30′′	46/30′′	72/30′′	72/1′	2.5	200	1.0
dfr	**II**				44/30′′					
dfr	**III, IV**				52/30′′					
dfr	**V**				45/30′′					
qnr	**A, B**	95/5′	35x	95/45′′	60/45′′	72/60′′	72/10′	2.5	300	1.0
aac	**(6′)-Ib-cr**									
qnr	**C, D**				52/45′′				200	
qep	**A**									
qnr	**S**				54/45′′				300	
oqx	**A**									
oqx	**B**				62/45′′					

a) 1st denaturation, b) 2nd denaturation, c) annealing, d) extension, e) final extension, all expressed with temperature in °C /time in minutes, ′ or seconds, ′′.

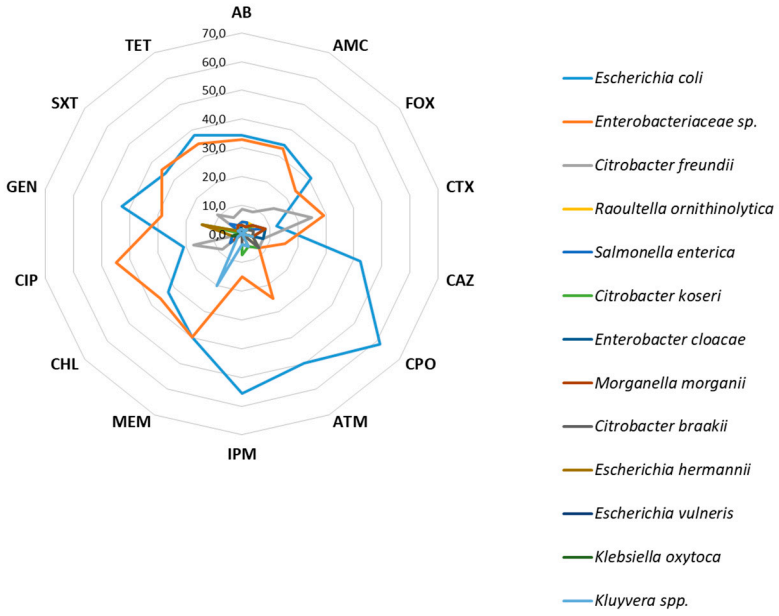

Figure S1. Relative frequency of antibiotics resistance by species.

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