Supplementary Material

Table S1. Primers of antibiotic resistance used for PCR in the study.

Determining Resistance to	Target Gene	Sequences (5'- 3')	Annealing Temperature (℃)	Amplicon Size (bp)	References
Glycopeptide	*vanA*	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	54	732	Dutka-Malen et al. (1995)
	vanB	F: ATGGGAAGCCGATAGTC R: GATTTCGTTCCTCGACC	54	635	Dutka-Malen et al. (1995)
Penicillin	*pbp4*	F: CAACGAAAGCCTGATGAAATGG R: AATCGCCTTTTTGAGGATCGG	55	1272	Ono et al. (2005)
	pbp5	F: AACAAAATGACAAACGGG R: TATCCTTGGTTATCAGGG	54	779	Obeng et al. (2013)
Aminoglycoside	*aac(6')-Ie-aph(2'')-Ia* (Gentamicin)	F: CAGAGCCTTGGGAAGATGAAG R: CCTCGTGTAATTCATGTTCTGGC	56	348	Vakulenko et al. (2003)
	aph(2'')-Ib (Gentamicin)	F: CTTGGACGCTGAGATATATGAGCAC R: GTTTGTAGCAATTCAGAAACACCCTT	56	867	
	aph(2'')-Ic (Gentamicin)	F: CCACAATGATAATGACTCAGTTCCC R: CCACAGCTTCCGATAGCAAGAG	56	444	
	aph(2'')-Id (Gentamicin)	F: GTGGTTTTTACAGGAATGCCATC R: CCCTCTTCATACCAATCCATATAACC	56	641	
	aph(3')-IIIa (Kanamycin)	F: GGCTAAAATGAGAATATCACCGG R: CTTTAAAAAATCATACAGCTCGCG	56	523	
	ant(4')-Ia (Neomycin, kanamycin, tobramycin and amikacin)	F: CAAACTGCTAAATCGGTAGAAGCC R: GGAAAGTTGACCAGACATTACGAACT	56	294	
	ant(6)-Ia (Streptomycin)	F: CGGGAGAATGGGAGACTTTG R: CTGTGGCTCCACAATCTGAT	55	563	Kobayashi et al. (2001)
	aac(6')-Ii (Gentamicin and other aminoglycosides)	F: TGGCCGGAAGAATATGGAGA R: GCATTTGGTAAGACACCTACG	55	410	
Tetracycline	*tet*(M)	F: GTGGACAAAGGTACAACGAG R CGGTAAAGTTCGTCACACAC	61	406	Malhotra-Kumar et al. (2005)
	tet(L)	F: TGGTGGAATGATAGCCCATT R: CAGGAATGACAGCACGCTAA	61	229	
	tet(O)	F: AACTTAGGCATTCTGGCTCAC R: TCCCACTGTTCCATATCGTCA	61	515	
	tet(K)	F: GATCAATTGTAGCTTTAGGTGAAGG R: TTTTGTTGATTTACCAGGTACCATT	61	155	

Macrolide	*erm*(A)	F: CCCGAAAAATACGCAAAATTTCAT R: CCCTGTTTACCCATTTATAAACG	61	590	Malhotra-Kumar et al. (2005)
	erm(B)	F: TGGTATTCCAAATGCGTAATG R: CTGTGGTATGGCGGGTAAGT	61	745	Malhotra-Kumar et al. (2005)
	mef(A/E)	F: CAATATGGGCAGGGCAAG R: AAGCTGTTCCAATGCTACGC	61	317	Malhotra-Kumar et al. (2005)
	msr(A/B)	F: GCAAATGGTGTAGGTAAGACAACT R: ATCATGTGATGTAAACAAAAT	55	399	Sutcliffe et al. (1996)
	msr(C)	F: TATAACAAACCTGCAAGTTC R: CTTCAATTAGTCGATCCATA	52	410	McDermott et al. (2005)
Lincosamide	*lnuB*	F: CCTACCTATTGTTTGTGGAA R: ATAACGTTACTCTCCTATTC	50	925	Bozdogan et al. (1999)
Streptogramin	*vatE*	F: ACTATACCTGACGCAAATGC R: GGTTCAAATCTTGGTCCG	52	511	Soltani et al. (2000)
	vatD	F: GCTCAATAGGACCAGGTGTA R: TCCAGCTAACATGTATGGCG	55	271	Soltani et al. (2000)
Chloramphenicol	*cat*	F: TAAGGTTATTGGGATAAGTTA R: GCATGRTAACCATCACAWAC	54	340	Hummel et al. (2007)
Int-Tn	(Tn*916*/Tn*1545*)	F: GCGTGATTGTATCTCACT R: GACGCTCCTGTTGCTTCT	50	1028	Doherty et al. (2000)

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Table S2. Target genes and primers used in this study.

Genes	Encoded Virulence Factor	Primer Sequence 5′–3′	Annealing Temperature	Amplicon length (bp)	Reference
asa1	aggregation substance	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	56	375	Vankerckhoven et al. (2004)
esp	enterococcal surface protein	F: AGATTTCATCTTTGATTCTTGG R: AATTGATTCTTTAGCATCTGG	56	510	Vankerckhoven et al. (2004)
efaA$_{fs}$	*E. faecalis* specific endocarditis antigen	F: GACAGACCCTCACGAATA R: AGTTCATCATGCTGTAGTA	51	705	Eaton & Gasson (2001)
efaA$_{fm}$	*E.faecium* specific surface antigen	F: AACAGATCCGCATGAATA R: CATTTCATCATCTGATAGTA	48	735	Eaton & Gasson (2001)
gelE	gelatinase	F: ACCCCGTATCATTGGTTT R: ACGCATTGCTTTTCCATC	48	419	Lopes et al. (2006)
hyl	hyaluronidase	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	56	276	Vankerckhoven et al. (2004)
cylA	cytolysin activator	F: ACTCGGGGATTGATAGGC R: GCTGCTAAAGCTGCGCTT	56	688	Vankerckhoven et al. (2004)
sgrA	serine–glutamate repeat containing protein A *fms2* or *orf2351*	F: AATGAACGGGCAAATGAG R: CTTTTGTTCCTTAGTTGGTATGA	53	671	Fritas et al. (2018)
pstD	enzyme IID subunit of a putative phosphotransferase system	F: TATCAACGCGATCAAAACGA R: CGTTCGCATACAGCTTTTCA	53	241	Fritas et al. (2018)
orf1481	sugar-binding protein encoded by a genomic island (8.5 kb)	F: GTTTATCAACATGCTAGCCCA R: GCCAATGAGTTAGATGTAGCC	55	437	Fritas et al. (2018)
IS16	*element IS*	F: CATGTTCCACGAACCAGAG R: TCAAAAAGTGGGCTTGGC	55	547	Fritas et al. (2018)

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Table S3. Origin, species, phenotypic and genotypic profile of each *Enterococcus* spp. isolates tested.

Strain	Source	Species	Resistance pattern	MIC Ampi	HLSR	HLGR	Phenotypic resistance profiles	Genotypic resistance profiles	Biofilm production	Virulence profile
E244	Stools	*E. faecalis*	MDR	32	-	+	RD C W N CN S CIP ENR E QD DA OX AMP TE TIG	*pbp4 tet(M) erm(B) aph(3')-IIIa cat Int-Tn*	MBP	*asa1 efaAfs cylA esp*
E54	Stools	*E. faecalis*	MDR	/	+	+	N CN S E QD DA OX TE	*pbp4 tet(M) lnuB erm(B) aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*gelE asa1 efaAfs cylA esp*
E53	Stools	*E. faecalis*	MDR	/	+	+	C F W N CN S E QD DA OX TE	*pbp4 tet(M) lnuB erm(B) aph(3')-IIIa ant(6')-Ia cat Int-Tn*	SBP	*asa1 efaAfs cylA esp*
E220	Stools	*E. faecalis*	MDR	/	+	+	RD C LZD W N CN S KF CIP ENR E QD DA OX TE TIG	*pbp4 tet(M) lnuB erm(B) aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*asa1 efaAfs cylA esp*
E234	Stools	*E. faecalis*	XDR	/	+	+	RD C LZD W N CN S KF CIP TEC E QD DA OX TE TIG	*tet(M) tet(L) erm(B) aph(3')-IIIa cat*	WBP	*gelE efaAfs*
E121	Stools	*E. faecalis*	MDR	/	+	+	C LZD W N CN S CIP ENR E QD DA OX TE	*pbp4 tet(M) tet(L) lnuB erm(B) aph(3')-IIIa ant(6')-Ia cat*	SBP	*gelE efaAfs*
E165	Stools	*E. faecalis*	MDR	/	-	+	C LZD F W N CN S CIP ENR QD DA TE TIG	*tet(M) erm(B) Int-Tn*	SBP	*asa1 efaAfs cylA esp*
E188	Stools	*E. faecalis*	MDR	/	+	-	RD C LZD W N CN S ENR E QD DA OX TE	*pbp4 tet(M) aph(3')-IIIa Int-Tn*	MBP	*asa1 efaAfs*
E232	Stools	*E. faecalis*	MDR	/	+	+	RD C LZD W N CN S ENR E QD DA OX TE TIG	*pbp4 tet(M) lnuB aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*gelE asa1 efaAfs cylA esp*
E270	Stools	*E. faecalis*	MDR	/	+	-	LZD N CN S QD DA OX TE	*pbp4 tet(M) aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*gelE asa1 efaAfs cylA esp*
E218	Stools	*E. faecalis*	XDR	/	+	-	RD C LZD W N CN S KF CIP ENR E QD DA OX TE TIG	*pbp4 tet(M) lnuB aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*asa1 efaAfs cylA esp*
E119	Stools	*E. faecalis*	MDR	/	+	+	RD C LZD W N CN S CIP ENR E QD DA OX TE TIG	*pbp4 tet(M) tet(L) lnuB erm(B) aph(3')-IIIa ant(6')-Ia cat*	MBP	*gelE efaAfs*
E233	Stools	*E. faecalis*	XDR	128	+	-	RD LZD W N CN S KF CIP QD DA OX AMP TE TIG	*pbp4 tet(M) aph(3')-IIIa ant(6')-Ia*	SBP	*gelE asa1 efaAfs cylA esp*
E52	Stools	*E. faecalis*	MDR	/	+	+	N S QD DA OX TE	*pbp4 tet(M) Int-Tn*	SBP	*gelE efaAfs esp*
1079B	UTI	*E. faecalis*	MDR	/	-	-	RD N CN S KF CIP ENR QD DA OX	*pbp4*	MBP	*gelE efaAfs*
654	UTI	*E. faecalis*	MDR	32	-	-	W S QD DA OX TE	*pbp4 tet(M) Int-Tn*	SBP	*gelE asa1 efaAfs cylA*
1091	UTI	*E. faecalis*	MDR	>256	-	-	RD N CN S KF CIP ENR QD DA OX AMP TE	*tet(M)*	MBP	*gelE efaAfs*
793	UTI	*E. faecalis*	MDR	64	+	-	RD C N S CIP ENR E QD DA OX TE	*pbp4 tet(M) tet(L) erm(B) aph(3')-IIIa cat Int-Tn*	WBP	*gelE asa1 efaAfs*
1079	UTI	*E. faecalis*	MDR	64	-	-	RD N CN S KF CIP ENR QD DA OX AMP	*pbp4*	SBP	*gelE efaAfs*

46	UTI	*E. faecalis*	MDR	8	+	-	C W N CN S KF QD DA OX TE	*pbp4 tet(M) cat Int-Tn*	SBP	*asa1 efaAfs cylA*
835C	UTI	*E. faecalis*	MDR	>256	-	-	N CN S KF CIP ENR E QD DA OX TE	*pbp4 tet(M) tet(L) erm(B) Int-Tn*	SBP	*gelE asa1 efaAfs*
568	UTI	*E. faecalis*	MDR	16	+	-	C W N CN S KF CIP ENR E QD DA OX TE	*pbp4 tet(M) tet(L) lnuB erm(B) aph(3')-IIIa ant(6')-Ia cat Int-Tn*	SBP	*asa1 efaAfs cylA esp*
260	UTI	*E. faecalis*	MDR	>256	+	-	N CN S KF CIP ENR QD DA OX	*pbp4*	MBP	*gelE efaAfs*
323	UTI	*E. faecalis*	MDR	/	-	-	W N CN S E QD DA OX TE	*pbp4 tet(M) Int-Tn*	SBP	*asa1 efaAfs cylA esp*
324	UTI	*E. faecalis*	MDR	/	-	-	W N CN S KF QD DA OX	*pbp4*	WBP	*gelE asa1 efaAfs*
320	UTI	*E. faecalis*	MDR	32	-	-	RD N CN S CIP ENR E QD DA OX	*pbp4 erm(B)*	MBP	*gelE asa1 efaAfs*
359	UTI	*E. faecalis*	MDR	32	-	-	RD W N CN S KF E QD DA OX TE	*pbp4 tet(M) Int-Tn*	SBP	*asa1 efaAfs cylA esp*
357B	UTI	*E. faecalis*	MDR	32	-	-	N CN S KF CIP ENR E QD DA OX AMC AMP TE	*pbp4 erm(B)*	MBP	*gelE asa1 efaAfs*
415	UTI	*E. faecalis*	MDR	16	-	-	W N CN S KF E QD OX AMC AMP TE	*pbp4 tet(M) Int-Tn*	SBP	*asa1 efaAfs cylA esp*
447	UTI	*E. faecalis*	MDR	>256	-	-	F W S KF CIP ENR E QD DA OX AMC AMP TE	*pbp4 tet(M) Int-Tn*	WBP	*asa1 efaAfs cylA esp*
E227	Stools	*E. faecium*	MDR	128	-	-	RD LZD F W N CN S E DA OX AMP	*pbp5 tet(M) lnuB erm(B)) mrs(A/B) mrs(C) aph(3')-IIIa ant(6')-Ia aac(6')-Ii Int-Tn*	WBP	*asa1 efaAfm cylA esp*
E260	Stools	*E.faecium*	MDR	>256	-	-	F W N S KF CIP ENR QD OX AMP TE	*tet(M) mrs(A/B) mrs(C) aph(3')-IIIa ant(6')-Ia aac(6')-Ii Int-Tn*	WBP	*gelE asa1 efaAfm cylA esp*
E169	Stools	*E. faecium*	MDR	64	-	-	RD C LZD F W N CN S KF CIP ENR QD OX TE TIG	*tet(M) mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm IS16*
E153	Stools	*E.faecium*	MDR	64	-	-	RD C LZD F W N S KF CIP ENR E OX AMP TE TIG	*tet(M) tet(L) mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm IS16*
E251	Stools	*E.faecium*	MDR	256	-	-	LZD F W N S KF CIP ENR E DA OX AMP	*pbp5 mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm sgrA*
E236	Stools	*E.faecium*	MDR	>256	-	-	RD W S KF ENR OX AMP	*mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*gelE efaAfm*
E239	Stools	*E.faecium*	XDR	128	-	-	C LZD F W N CN S KF CIP QD OX AMP TE	*tet(M) mrs(A/B) mrs(C) aac(6')-Ii Int-Tn*	WBP	*efaAfm IS16*
E160	Stools	*E.faecium*	XDR	16	-	-	RD C LZD F W N CN S KF CIP ENR QD OX AMP TE	*tet(M) mrs(A/B) mrs(C) aac(6')-Ii Int-Tn*	WBP	*efaAfm*
E257	Stools	*E.faecium*	MDR	128	-	-	LZD N KF CIP ENR QD DA OX AMP TIG	*mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm IS16*
E177	Stools	*E.faecium*	XDR	32	-	-	LZD F W N CN S KF CIP ENR TEC OX AMP TE TIG	*tet(M) mrs(A/B) mrs(C) aac(6')-Ii Int-Tn*	WBP	*efaAfm IS16*

E99	Stools	*E.faecium*	MDR	>256	+	-	F W N CN S KF E DA OX AMP TE TIG	*tet(M) tet(L) lnuB erm(B) mrs(A/B) mrs(C) aac(6')-Ie-aph(2")-Ia ant(6')-Ia aac(6')-Ii*	WBP	*efaAfm IS16*
E254	Stools	*E.faecium*	MDR	>256	+	-	C LZD W N CN S KF CIP ENR E DA OX AMC AMP TE TIG	*tet(L) lnuB erm(B) mrs(A/B) mrs(C) mef(A/E) aph(3')-IIIa ant(6')-Ia aac(6')-Ii*	WBP	*efaAfm sgrA pstD orf1481 IS16*
E241	Stools	*E.faecium*	/	256	-	-	RD N S KF DA OX AMP	*pbp5 mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm*
E238	Stools	*E.faecium*	MDR	>256	-	-	C LZD F W N S KF OX AMP TE TIG	*mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm*
E154	Stools	*E.faecium*	MDR	64	-	-	RD LZD F N S CIP ENR E DA OX AMP TE	*mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm*
24A	UTI	*E. faecium*	MDR	>256	-	+	C N CN S KF CIP ENR E QD DA OX AMC AMP TE	*tet(M) tet(L) mrs(A/B) mrs(C) ant(6')-Ia aac(6')-Ii cat Int-Tn*	MBP	*asa1 efaAfm cylA sgrA pstD orf1481 IS16*
1118	UTI	*E.faecium*	MDR	>256	+	+	W N CN S KF CIP ENR E DA OX AMC AMP TE	*erm(B) mrs(A/B) mrs(C) aac(6')-Ie-aph(2")-Ia aph(3')-IIIa ant(6')-Ia aac(6')-Ii*	WBP	*efaAfm sgrA pstD orf1481 IS16*
399	UTI	*E.faecium*	MDR	/	-	-	S KF CIP ENR QD DA OX	*mrs(A/B) mrs(C) aac(6')-Ii*	WBP	*efaAfm orf1481*
618	UTI	*E. gallinarum*	MDR	>256	+	+	RD CIP ENR QD DA OX TE	*tet(M) erm(B) aph(3')-IIIa cat Int-Tn*	MBP	*gelE asa1*
556B	UTI	*E. gallinarum*	MDR	<8	-	-	N S CIP ENR E QD DA OX TE	*tet(M) Int-Tn*	MBP	*asa1*
1034	UTI	*E. gallinarum*	MDR	64	+	-	N S KF CIP ENR E QD DA OX AMP TE	*tet(M) aph(3')-IIIa ant(6')-Ia Int-Tn*	SBP	*esp*

Legend: Stools = isolate from healthy dog stools; UTI = isolate from sick dog urines; AMPI = Minimum Inhibitory Concentration (MIC) for ampicillin expressed in μg/ml; HLSR = High Level Streptomycin Resistance; HLGR = High Level Gentamicin Resistance; RD = rifampicin; C = chloramphenicol; LZD = linezolid; F = nitrofurantoin; W = trimethoprim; N = neomycin; CN = gentamicin; S = streptomycin; KF = cephalothin; CIP = ciprofloxacin; ENR = enrofloxacin; TEC = teicoplanin; E = erythromycin; QD = quinupristin–dalfopristin; DA = clindamycin; OX = oxacillin; AMC = amoxicillin–clavulanic acid; AMP = ampicillin; TE = tetracycline; TIG = tigecycline; WBP = Weak Biofilm Producer; MBP = Moderate Biofilm Producer; SBP = Strong Biofilm Producer.