

**Characterization of a tigecycline-, linezolid- and vancomycin-resistant clinical *Enterococcus faecium* isolate, carrying *vanA* and *vanB* genes**

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Supplementary Table 1. Genomic relationships of the 4995/20 isolate with other *E. faecium* isolates reported to the GenBank database (accessed on the 9<sup>th</sup> August 2023). Data on 4995/20 isolate included for comparative purposes.

Isolate	ST	SNP cluster	Country of isolation	Source	<i>vanA</i>	<i>vanB</i>	<i>tet(M)</i>	<i>tet(L)</i>	<i>dfrG</i>	<i>poxtA</i>	<i>fexB</i>	Assembly/ Short Reads Archive
4995/20	18	PDS000100071.5	Poland	human (hematoma)	+	+	+	+	-	+	+	GCA_020221735.2
SJ267	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021338535.1
RC-19-040	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021330095.1
SJ178	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021327155.1
JH019	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021331535.1
JH017	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021326645.1
BSI-SJ82	18	PDS000100071.5	Ireland	human	+	-	+	-	+	-	-	GCA_021333455.1
P01-D01-M4	18	PDS000100071.5	Uganda	human (blood)	-	-	+	+	+	-	-	SRS18227107
P01-B02-M10	18	PDS000100071.5	Uganda	human (blood)	-	-	+	+	+	-	-	SRS18227101
P01-F01-M6	18	PDS000100071.5	Uganda	human (blood)	-	-	+	+	+	-	-	SRS18227096
EFM0003	18	PDS000100071.5	n/a	n/a	+	-	+	-	+	-	-	ERS4370981
EFM0461	18	PDS000100071.5	n/a	n/a	+	-	+	-	+	-	-	ERS5061401
EFM0469	18	PDS000100071.5	n/a	n/a	+	-	+	-	+	-	-	ERS5061407

n/a, data not available.

Supplementary Table 2. Occurrence of the *fexB-poxA*-PCT among bacterial isolates reported to GenBank (the nt-prok database, accessed the 9<sup>th</sup> of May 2023). Data on 4995/20 isolate included for comparative purposes.

Species	Isolate	Localization	Plasmid size (kb)	del 904 nt <sup>a</sup> in PCT	Other variants in PCT <sup>a</sup>	Country	Host	Accession number
<i>E. faecium</i>	4995/20	p4995_2	42.4	+	none	Poland	human (hematoma)	CP084180.1
<i>E. faecium</i>	18-465	p18-465_1	24.4	+	ins 95 nt	France	human (faeces)	CP065753.1
	M1	pEfM1	30.9	+	ins 95 nt	Italy	river	ON009374.1
	R186-2	pR186-2-B	46.5	+	ins 95 nt	Switzerland	cattle	CP116522.1
	1818	p1818_c	23.9	+	ins 95 nt	Switzerland	human (faeces)	CP091209.1
	211b	p211b-b	28.2	+	ins 95 nt	Switzerland	human (faeces)	CP091212.1
	AT02	pAT02-b	52.5	+	ins 95 nt	Switzerland	pet food	CP097063.1
	F179	pF197_3	26.7	+	del 14 bp; ins 95 nt	Switzerland	water	CP072887.1
	K69-1a	pK69-1a-C	28.6	-	ins 95 nt	Switzerland	cattle	CP116526.1
	K191-3	pK191-3-B	24.8	-	ins 95 nt	Switzerland	cattle	CP116534.1
	K195-6b	pK195-6b-B	44.0	-	ins 95 nt	Switzerland	cattle	CP116516.1
	1525	p1525-b	21.4	-	ins 75 nt	Switzerland	human (faeces)	CP091219.1
	AT45b	pAT45b-b	24.8	-	ins 95 nt	Switzerland	pet food	CP097024.1
	K188-5	pK188-5-B	24.6	-	del 107 nt	Switzerland	cattle	CP116530.1
	116	p116-1	21.6	+	ins 95 nt	China	animal	CP047328.1
<i>E. faecalis</i>	LS05-2	chromosome	n/a	+	ins 95 nt	Switzerland	pet food	CP097004.1
	1521	chromosome	n/a	+	ins 95 nt	Switzerland	human (faeces)	CP091237.1
<i>E. hirae</i>	GE2	pEh-GE2	24.8	+	ins 95 nt	Italy	marine zooplankton	MT683616.1
	697	p697-a	53.0	-	ins 72 nt	Switzerland	human (faeces)	CP091202.1
<i>S. aureus</i>	AOUC-0915	chromosome	n/a	+	del 104 nt; ins 95 nt	Italy	human	MH746818.1

<sup>a</sup>see Supplementary Fig. 4 for details; n/a, not applicable; n/k, not known.

Supplementary Table 3. Occurrence of the *tet(M)*-*tet(L)*-PCT among bacterial isolates reported to GenBank (the nt-prok database, accessed the 11<sup>th</sup> of May 2023). Data on 4995/20 isolate included for comparative purposes.

Species	Isolate	Plasmid name	Plasmid size (kb)	PCT variants <sup>a</sup>	Country	Source	Accession number
<i>E. faecium</i>	4995/20	p4995_2	42.4	none	Poland	human (hematoma)	CP084180.1
<i>E. faecium</i> *	EF3	pEfm-EF3	27.7	none	Italy	marine sediment	MT683615.1
	M1	pEfm-M1	30.9	ins 27 nt	Italy	river	ON009374.1
	AT45b	pAT45b-d	18.5	ins 27 nt	Switzerland	pet food	CP097026.1
	S2-6b	pS2-6b-b	30.8	ins 27 nt	Switzerland	pig	CP088193.1
	264a	p264a-c	27.4	ins 27 nt	Switzerland	human (faeces)	CP091216.1
	642	p642-b	21.8	ins 27 nt	Switzerland	human (faeces)	CP091223.1
	K70-1a	pK70-1a-B	39.5	ins 27 nt	Switzerland	cattle	CP116545.1
	27	pC27-2	62.4	ins 27 nt	China	pig	MH784602.1
	25	pC25-1	67.8	ins 27 nt	China	pig	MH784601.1
	SDGJP3	pSDGJP3	51.7	ins 27 nt	China	pig	CP038171.1
<i>E. faecalis</i>							
	T90-1	pT90-1	54.3	ins 27 nt	China	pig	CP068250.1
	T90-3	pT90-3	71.1	ins 27 nt	China	pig	CP069131.1
	T90-4	pT90-4	35.6	ins 27 nt	China	pig	CP069155.1
	T90-6	pT90-6	149.5	ins 27 nt	China	pig	CP069129.1
<i>E. hirae</i>	TF52	pTF52-21k	21.8	del 9 nt	China	pig	CP109810.1
	TF34	pTF34-21k	21.8	ins 27 nt	China	pig	CP109806.1
	DY27	pDY27-poxTA	53.5	ins 27 nt	France	pig	MW207669.1
	DY13	pDY13-poxTA	25.2	ins 27 nt	China	pig	MW207667.1
	fas4	pfas4-1	57.3	ins 27 nt	China	pig	MK798157.1
	S46-3	pS46-3-a	35.2	ins 27 nt	Switzerland	pig	CP088188.1
	DSM 20160	p3ZJ02	26.0	none	China	pig	CP122307.1
<i>E. durans</i>	S8-3	pS8-3-a	21.8	ins 27 nt	Switzerland	pig	CP088204.1
	R109-1	pR109-1-A	27.9	ins 27 nt	Switzerland	cattle	CP116580.1
<i>E. gallinarum</i>	FS4	pEgFS4-2	38.4	ins 27 nt	Italy	pig	MZ291453.1

<sup>a</sup>see Supplementary Fig. 5 for details; \*for *E. faecium* 10 top hits are shown.

Supplementary Table 4. Occurrence of the p4995\_4-like plasmids among bacterial isolates reported to GenBank (the nt-prok database, accessed the 15<sup>th</sup> of August 2023). Data on 4995/20 isolate included for comparative purposes.

Species	Isolate	Plasmid name	Plasmid size (kb)	Country	Source	Accession number
<i>E. faecium</i>	4995/20	p4995_4	11.6	Poland	human (hematoma)	CP084184.1
<i>E. faecium</i> *	V1836	pHVH-V1836-4	11.6	Denmark	human (rectum)	CP044268.1
	15-307-1	p15-307-1_06	11.8	France	human (rectum)	CP044322.1
	UK040	pUK040_3	21.2 <sup>a</sup>	Germany	human	CP091580.1
	VB3378	pVB3378_p7	11.6	India	human (blood)	CP072714.1
	VB3338	pVB3338_P7	11.6	India	human (blood)	CP062272.1
	BP3378	pBP3378_P7	11.6	India	human (blood)	CP059800.1
	BP657	pBP657_P4	11.8	India	human (blood)	CP059820.1
	A7214	pA7214_P4	11.8	India	human (blood)	CP059751.1
	A4694	pA4694_P5	11.8	India	human (blood)	CP059744.1
	ME3	plasmid_unnamed2	51.6 <sup>b</sup>	India	river water	CP043867.1
	AA290	pAA290_4 DNA	11.8	Japan	human	AP026637.1
	A11051	pA11051_P4	9.9	India	human (blood)	CP059768.1
	VB6521	pVB6521_p4	9.9	India	human (blood)	CP072724.1
	VB6171	pVB6171_p4	9.9	India	human (blood)	CP072735.1
	VB13828	p4VB13828	9.9	India	human (blood)	CP071962.1
	A6521	pA6521_4	9.9	India	human (blood)	CP061821.1
	VRE3382	plasmid_unnamed2	19.9 <sup>a</sup>	Australia	human (rectum)	CP065530.1
	E7160	plasmid: 6	9.9	Slovenia	human (faeces)	LR536667.1
	BMT-1-3-1	pIN-3-1_03	11.4	Germany	human (rectum)	CP063596.1

\*no hits to other species were found; <sup>a</sup>plasmid dimer; <sup>b</sup>p4995\_4-like plasmid included in a larger plasmid.

Supplementary Table 5. Occurrence of the *rep*<sub>p4995\_5</sub> gene among bacterial isolates reported to GenBank (the nt-prok database, accessed the 15<sup>th</sup> of May 2023). Data on 4995/20 isolate included for comparative purposes.

Species	Isolate	Plasmid name	Plasmid size (kb)	Country	Source	Accession number
<i>E. faecium</i>	4995/20	p4995_5	4.5	Poland	human (hematoma)	CP084185.1
<i>E. faecium</i> *	VRE3363	plasmid 6	4.5	Australia	human	CP064349.1
	SC1762-D	p9	4.5	China	human	CP085915.1
	SC1762	p9	4.5	China	human	CP085903.1
	V1836	pHVH-V1836-7	4.5	Denmark	human (rectum)	CP044271.1
	V24	pV24-9	4.5	Denmark	human (rectum)	CP036160.1
	V2937	pHVH-V2937-5	4.5	Denmark	human (rectum)	CP044279.1
	18-276	p18-276_4	4.5	France	human (rectum)	CP065758.1
	18-204	p18-204_6	4.5	France	human (rectum)	CP066211.1
	VREfm1	pPEC012	4.5	Hungary	human (wound)	KY595964.1
	A11051	pA11051_P6	4.5	India	human (blood)	CP059770.1
	A15023	pA15023_P5	4.5	India	human (blood)	CP059828.1
	A6521	pA6521_5	4.5	India	human (blood)	CP061822.1
	BA17124	pBA17124_P6	4.5	India	human (blood)	CP059790.1
	BP5067	pBP5067_P5	4.5	India	human (blood)	CP059811.1
	VB13828	p7VB13828	4.5	India	human (blood)	CP071965.1
	VB6171	pVB6171_p5	4.5	India	human (blood)	CP072736.1
	VB6521	pVB6521_p5	4.5	India	human (blood)	CP072725.1
	VB976	pVB976_p5	4.5	India	human (blood)	CP072591.1
	E8172	plasmid: 8	4.5	The Netherlands	human	LR135379.1
	E7948	plasmid: 6	4.5	The Netherlands	human	LR135362.1
	Dallas 51_4	p51_4_4kb	4.5	US	human (rectum)	CP066408.1
	Dallas 16_1	p16_1_4kb	4.5	US	human (rectum)	CP066388.1
	BMT-1-3-1	pIN-3-1_04	5.1	Germany	human	CP063597.1
	E7114	plasmid: 8	6.4	Latvia	human	LR135450.1
	UK040	pUK040_4	8.5	Germany	human	CP091581.1
	EF0656	pEF0656-4	9.4	China	human (urine)	CP114621.1
	E7025	plasmid: 6	9.6	Latvia	human	LR135231.1
	15-307-1	p15-307-1_05	24.5	France	human (rectum)	CP044321.1
	ME3	unnamed2	51.5	India	river water	CP043867.1
	CEP232	pCEP232	n/k	France	human (rectum)	MT901633.1

\*no hits to other species were found; n/k, not known (partial sequence available).

Supplementary Table 6. Occurrence of the *rep*<sub>p4995\_5</sub> gene among bacterial isolates reported to GenBank (the nt-prok database, accessed the 15<sup>th</sup> of May 2023). Data on 4995/20 isolate included for comparative purposes.

Species	Isolate	Plasmid name	Plasmid size (kb)	Country	Source	Accession number
<i>E. faecium</i>	4995/20	p4995_5	2.1	Poland	human (hematoma)	CP084186.1
<i>E. faecium</i>	HJP554	pHJP554-4	2.1	China	human	CP083477.1
	NY11066	pNY11066-5	2.1	China	human	CP093952.1
	NY11067	pNY11067-3	2.1	China	human (urine)	CP093956.1
	SC1762	p10	2.1	China	human	CP085904.1
	SC1762-D	p10	2.1	China	human	CP085916.1
	VRE1	pVRE1-4	2.1	China	human (urine)	CP040744.1
	V2937	pHVH-V2937-8	2.1	Denmark	human (rectum)	CP044282.1
	15-307-1	p15-307-1_11	2.1	France	human (rectum)	CP044327.1
	A10290	pA10290_P7	2.1	India	human (blood)	CP059762.1
	A4694	pA4694_P7	2.1	India	human (blood)	CP059746.1
	BA17124	pBA17124_P8	2.1	India	human (blood)	CP059792.1
	BP3378	pBP3378_p10	2.1	India	human (blood)	CP059803.1
	BP5067	pBP5067_P9	2.1	India	human (blood)	CP059815.1
	BP657	pBP657_P6	2.1	India	human (blood)	CP059822.1
	VB12993	pVB12993_p6	2.1	India	human (blood)	CP072585.1
	VB13828	p10VB13828	2.1	India	human (blood)	CP071968.1
	VB3338	pVB3338_p10	2.1	India	human (blood)	CP062275.1
	VB3378	pVB3378_p10	2.1	India	human (blood)	CP072717.1
	VB976	pVB976_p7	2.1	India	human (blood)	CP072593.1
	AA290	pAA290_7	2.1	Japan	human	AP026640.1
	AA94	pAA94_3	2.1	Japan	human	AP026616.1
	E8691	plasmid: 8	2.1	The Netherlands	dog	LR135442.1
	E4402	plasmid: 5	2.1	Denmark	dog	LR135178.1
	E7025	plasmid: 9	2.1	Latvia	human	LR135234.1
	E7933	plasmid: 10	2.1	The Netherlands	human	LR135393.1
	E8440	plasmid: 9	2.1	The Netherlands	human	LR135427.1
	Dallas 51_4	p51_4_2kb	2.1	US	human (rectum)	CP066398.1
	HOU503	unnamed1	2.1	US	human	CP040707.1
	E7429	plasmid: 6	8.1	The Netherlands	human	LR135302.1
	Ef_aus00233	plasmid: 5	22.1	Australia	human	LT598667.1
	VRE3355	unnamed2	24.2	Australia	human (rectum)	CP071929.1
	E6055	plasmid: 4	41.1	Portugal	human (faeces)	LR135200.1
	AA708	pELF1	138.9	Japan	human (faeces)	LC495616.1
	ef332	plas4	203.6	China	human	CP058895.1
<i>E. faecalis</i>	1207/14	p1207_6	2.1	Poland	human (faeces)	CP075610.1

n/k, not known.