

Evolution and Mutations Predisposing to Daptomycin Resistance in Vancomycin-Resistant *Enterococcus faecium* ST736 Strains

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Supplemental information

Table A. Distribution of clonality among *E. faecium* clinical isolates (1995-2013)^a

Year	Total no. of isolates	MSLT sequence type (no. of isolates, %)				
		ST17	ST18	ST412	ST736	Miscellaneous ^b
1994-1995	43	33 (76.7)	3 (7.0)	0	0	7 (16.3)
2009	20	0	2 (10.0)	2 (10.0)	9 (45.0)	7 (35.0)
2010	30	0	1 (3.3)	6 (20.0)	16 (53.3)	7 (23.3)
2011	30	0	2 (6.7)	3 (10.0)	23 (76.7)	2 (6.7)
2012	35	0	6 (17.1)	5 (14.3)	20 (57.1)	4 (11.4)
2013	92	0	24 (26.1)	19 (20.7)	43 (46.7)	6 (6.3)
Total	250	33 (13.2)	38 (15.2)	35 (14.0)	111 (44.1)	33 (13.2)

^a *E. faecium* isolates from 2009 to 2012 are not randomly selected; selective bias may be existed.

^b Miscellaneous ST for 1994-1995 isolates: ST16 (n = 2), ST20 (1), ST186 (1), ST280 (1) and ST535 (2); for 2009 isolates: ST203 (1), ST282 (4), ST750 (1) and one new ST (1); for 2010 isolates: ST282 (1), ST750 (2), ST893 (1), ST894 (1) and ST896 (1) and ST1314; for 2011 isolates: ST656 (1) and ST896 (1); for 2012 isolates: ST32 (1), ST214 (1), ST584 (1) and ST664 (1); for 2013 isolates: ST117 (2), ST448 (1), ST749 (1), ST895 (1), and ST896 (1). See supplemental table S1 for details.

Table B. Clinical, microbiological and mutation of the *liaFSR* and *cls* genes of daptomycin-nonsusceptible *E. faecium* isolates

<i>E. faecium</i> group	Year	Isolate	Source	MLST sequence type (ST)	Vancomycin MIC (µg/mL)	<i>van</i> gene	Daptomycin MIC (µg/mL)	<i>liaR</i> .W73C	<i>liaS</i> .T120A	<i>cls</i> .N131	<i>cls</i> .N13S	<i>cls</i> .N13T	<i>cls</i> .A20D	<i>cls</i> .H215R	<i>cls</i> .R218Q	Average depth of Coverage
ST736 (n=38)	2009	E39	Blood	736	>16	<i>vanA</i>	96	1	1	0	0	1	0	0	0	460.0
	2010	E13	Blood	736	1	-	32	1	1	0	0	0	0	0	0	86.6
	2010	E14	Blood	736	>16	<i>vanA</i>	16	1	1	0	0	0	0	0	0	66.2
	2010	E17	Wound	736	>16	<i>vanA</i>	16	1	1	0	0	1	0	0	0	68.2
	2010	E34	Urine	736	>16	<i>vanA</i>	48	1	1	0	0	1	0	0	0	21.6
	2010	E53	Wound	736	>16	<i>vanA</i>	64	1	1	0	0	1	0	0	0	37.2
	2011	E146	Urine	736	>16	<i>vanA</i>	32	1	1	1	0	0	0	0	0	17.4
	2011	E370	Blood	736	>16	<i>vanA</i>	16	1	1	0	0	0	1	0	0	59.9
	2011	E371	Blood	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	1	0	84.7
	2011	E372	Blood	736	>16	<i>vanA</i>	16	1	1	0	0	0	0	1	0	50.3
	2011	E480	Urine	736	1	-	8	1	1	0	0	0	0	0	1	35.8
	2011	E91	Blood	736	>16	<i>vanA</i>	64	1	1	0	0	1	0	0	0	24.4
	2012	E165	Urine	736	>16	<i>vanA</i>	16	1	1	0	0	0	0	0	0	16.1
	2012	E166	Urine	736	2	-	8	1	1	0	0	0	0	0	0	37.3
	2012	E168	Urine	736	>16	<i>vanA</i>	32	1	1	0	0	0	0	0	0	33.3
	2012	E173	Wound	736	>16	<i>vanA</i>	32	1	1	0	0	0	0	0	0	74.7
	2012	E390	Wound	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	1	0	67.2
	2012	E395	Blood	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	95.0
	2013	E175	Urine	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	15.6
	2013	E203	Blood	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	46.7
	2013	E207	Urine	736	>16	<i>vanA</i>	12	1	1	0	0	0	0	0	0	40.8
	2013	E208	Peritoneal fluid	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	21.3
	2013	E218	Urine	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	87.0
	2013	E222	Urine	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	41.3
	2013	E225	Blood	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	67.6
	2013	E226	Peritoneal fluid	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	48.7
	2013	E231	Wound	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	64.9
	2013	E236	SITE	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	86.6
	2013	E237	Blood	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	62.4
	2013	E242	Wound	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	67.8
	2013	E243	Peritoneal fluid	736	>16	<i>vanA</i>	>256	1	1	0	0	0	0	0	0	51.8
	2013	E249	Blood	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	103.5
	2013	E251	Urine	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	68.9
	2013	E253	Wound	736	>16	<i>vanA</i>	>256	1	1	0	0	0	1	0	0	131.2
	2013	E261	Urine	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	36.1
	2013	E284	Urine	736	>16	<i>vanA</i>	6	1	1	0	0	0	0	0	0	14.0
	2013	E347	Blood	736	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	31.3
	2013	E356	Blood	736	>16	<i>vanA</i>	32	1	1	0	1	0	0	0	0	31.7
Non-ST736 (n=15)	2009	E7	Urine	18	1	-	16	0	0	1	0	0	0	0	0	74.1
	2009	E8	Urine	282	>16	<i>vanA</i>	16	0	0	0	0	0	0	0	0	30.0
	2009	E49	Blood	750	≤2	-	16	0	0	0	0	0	0	0	1	25.9
	2010	E50	Blood	412	>16	<i>vanA</i>	64	0	0	0	0	0	0	0	0	41.3
	2010	E20	Wound	893	2	-	6	1	1	0	0	0	0	0	0	71.7
	2010	E451	Urine	896	>16	<i>vanA</i>	6	0	0	0	0	0	0	0	0	47.2
	2011	E376	Blood	656	>16	<i>vanA</i>	12	1	1	0	0	0	0	1	0	65.2
	2011	E367	Blood	896	>16	<i>vanA</i>	6	0	0	0	0	0	0	0	0	67.0
	2012	E392	Urine	214	1	-	6	0	0	0	0	0	0	0	0	32.0
	2013	E233	Urine	18	>16	<i>vanA</i>	6	0	0	0	0	0	0	0	0	39.8
	2013	E245	Urine	18	>16	<i>vanA</i>	12	0	0	0	0	0	0	0	0	112.7
	2013	E325	Blood	18	>16	<i>vanA</i>	8	1	1	0	0	0	0	0	0	16.8
	2013	E174	Urine	412	>16	<i>vanA</i>	12	0	0	0	0	0	0	0	0	28.4
	2013	E302	Peritoneal fluid	412	>16	<i>vanA</i>	8	0	0	0	0	0	0	0	0	61.2
	2013	E354	Blood	412	>16	<i>vanA</i>	16	1	1	0	0	0	0	0	0	14.3

Table C. Distribution of daptomycin MICs of dominant VREfm clones for each time period of evaluation

Year	MLST ST	No. of isolates	MIC ($\mu\text{g/mL}$)									
			≤ 2	3	4	8	16	32	64	128	≥ 256	
1994-1995	ST17	33	1	1	31							
	ST18	3	2	0	1							
2009-2012	ST18	11	1	1	9							
	ST412	16	5	4	6					1		
	ST736	69	8	8	37	5	5	4	2	1		
2013	ST18	24	4	5	13	2	1					
	ST412	19	5	3	8	1	2					
	ST736	43	5	4	14	16	1	1				2

Figure A

