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Note

Evaluation of DiversiLab®, MLST and PFGE typing for discriminating clinical *Enterococcus faecium* isolates

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ABSTRACT

We evaluated and critically assessed the performance and discriminatory power of a rep-PCR based commercial test DiversiLab® Enterococcus kit (bioMerieux) for typing a set of 65 representative isolates of *Enterococcus faecium*/VRE and compared it to state-of-the-art typing techniques such as PFGE and MLST.

Enterococci are important hospital-acquired pathogens that are frequently resistant to multiple antibiotics, specifically vancomycin. Different surveillance systems report increasing frequencies of vancomycin-resistant enterococci (VRE) in some countries such as Germany in recent years (EARS-NET, n.d.; PEG, n.d.). Generally, almost all VRE belong to the species *Enterococcus faecium*. Microbial typing is essential to detect outbreaks and to trace the emergence and spread of successful VRE strain types. Current VRE typing strategies differ in methodological approach, laboratory requirements, costs and discriminatory power (Werner, 2013). Here we compared the performance of a commercial rep-PCR based typing system for enterococci (DiversiLab® Enterococcus kit, bioMerieux, Nürtingen, Germany) with established techniques such as *Sma*I-macrorestriction in PFGE and MLST. Since the vast majority of VRE belongs to *E. faecium*, only isolates of *E. faecium* were included in this study.

Sixty-five well-defined and pre-characterized clinical strains of *E. faecium* (n = 13 *vanA*-type VRE, n = 50 *vanB*-type VRE) that had been sent to the German National Reference Centre for Staphylococci and Enterococci from outbreaks and individual cases were selected for comparative typing (Supplementary Table S1). The selected isolates originated from invasive infections (bloodstream isolates), non-invasive infections or from asymptomatic colonizations. Isolates belonging to prominent strain types prevalent among German hospitals were also included. Isolates were analysed by *Sma*I-macrorestriction analysis in PFGE (n = 62), MLST typing (n = 65) and DiversiLab (DL) typing (n = 65). PFGE typing was done as described recently (Werner, 2013). MLST typing was done according to the international MLST typing scheme (http://efaecium.mlst.net/). DiversiLab (DL) typing was performed as described in the standard protocol provided by the manufacturer (DiversiLab Enterococcus Kit; bioMerieux, Nürtingen, Germany). The rep-PCR patterns are resolved through a gel-based fluidic microarray in an Agilent Bioanalyser 2100. Genomic DNA was extracted based on a column-based standard protocol (DNeasy Tissue Kit, Qiagen, Germany). Standard settings for cluster definitions were used for PFGE (>82% similarity score (Morrison et al., 1999); BioNumerics 7.1 software) and DL typing (>95% similarity score; DiversiLab typing software, bioMerieux). Statistical tests were calculated using software EpiCompare 1.0 (Ridom, Münster, Germany).

DL typing revealed 18 clusters with a standard similarity setting of 95% identity score (Fig. 1). MLST typing showed 15 different sequence types with 38 ST192 isolates (17 cities), 8 ST203 isolates (5 cities), 7 ST117 (6 cities) and 12 individual STs. ST192 sequence types were detected among epidemiologically related and unrelated isolates (*vanA* and *vanB*; Figs. 1 and 2 and Supplementary Table S1). Sixty-two isolates typed by PFGE revealed 40 different patterns. Consequently, PFGE typing showed the highest discriminatory index, followed by DL typing and MLST (Table 1). Considering the high frequency of ST192 isolates in the strain collection the low discriminatory index for MLST was not surprising. Epidemiologically related isolates, which clustered by MLST and PFGE, revealed also similar DL patterns (see "outbreak"). Only two epidemiologically related and microbiologically indistinguishable outbreak isolates UW7605 and UW7606 with similar PFGE and MLST

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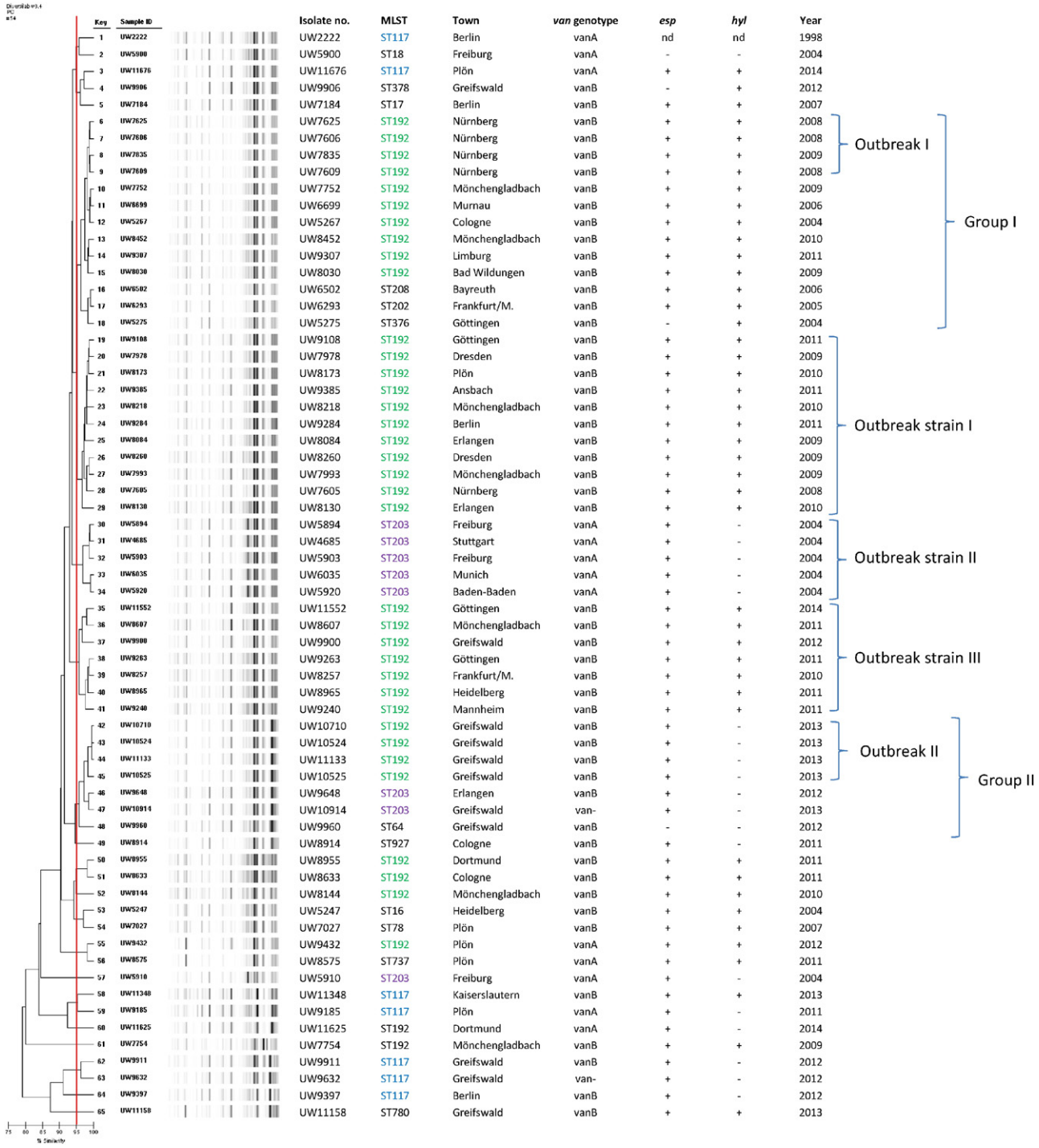

Key	Sample ID	Isolate no.	MLST	Town	*van* genotype	*esp*	*hyl*	Year
1	UW2222	UW2222	ST117	Berlin	vanA	nd	nd	1998
2	UW5900	UW5900	ST18	Freiburg	vanA	-	-	2004
3	UW11676	UW11676	ST117	Plön	vanA	+	+	2014
4	UW9906	UW9906	ST378	Greifswald	vanB	-	+	2012
5	UW7184	UW7184	ST17	Berlin	vanB	+	+	2007
6	UW7625	UW7625	ST192	Nürnberg	vanB	+	+	2008
7	UW7606	UW7606	ST192	Nürnberg	vanB	+	+	2008
8	UW7835	UW7835	ST192	Nürnberg	vanB	+	+	2009
9	UW7609	UW7609	ST192	Nürnberg	vanB	+	+	2008
10	UW7752	UW7752	ST192	Mönchengladbach	vanB	+	+	2009
11	UW6699	UW6699	ST192	Murnau	vanB	+	+	2006
12	UW5267	UW5267	ST192	Cologne	vanB	+	+	2004
13	UW8452	UW8452	ST192	Mönchengladbach	vanB	+	+	2010
14	UW9307	UW9307	ST192	Limburg	vanB	+	+	2011
15	UW8030	UW8030	ST192	Bad Wildungen	vanB	+	+	2009
16	UW6502	UW6502	ST208	Bayreuth	vanB	+	+	2006
17	UW6293	UW6293	ST202	Frankfurt/M.	vanB	+	+	2005
18	UW5275	UW5275	ST376	Göttingen	vanB	-	+	2004
19	UW9108	UW9108	ST192	Göttingen	vanB	+	+	2011
20	UW7978	UW7978	ST192	Dresden	vanB	+	+	2009
21	UW8173	UW8173	ST192	Plön	vanB	+	+	2010
22	UW9385	UW9385	ST192	Ansbach	vanB	+	+	2011
23	UW8218	UW8218	ST192	Mönchengladbach	vanB	+	+	2010
24	UW9284	UW9284	ST192	Berlin	vanB	+	+	2011
25	UW8084	UW8084	ST192	Erlangen	vanB	+	+	2009
26	UW8260	UW8260	ST192	Dresden	vanB	+	+	2009
27	UW7993	UW7993	ST192	Mönchengladbach	vanB	+	+	2009
28	UW7605	UW7605	ST192	Nürnberg	vanB	+	+	2008
29	UW8130	UW8130	ST192	Erlangen	vanB	+	+	2010
30	UW5894	UW5894	ST203	Freiburg	vanA	+	-	2004
31	UW4685	UW4685	ST203	Stuttgart	vanA	+	-	2004
32	UW5903	UW5903	ST203	Freiburg	vanA	+	-	2004
33	UW6035	UW6035	ST203	Munich	vanA	+	-	2004
34	UW5920	UW5920	ST203	Baden-Baden	vanA	+	-	2004
35	UW11552	UW11552	ST192	Göttingen	vanB	+	+	2014
36	UW8607	UW8607	ST192	Mönchengladbach	vanB	+	+	2011
37	UW9900	UW9900	ST192	Greifswald	vanB	+	+	2012
38	UW9263	UW9263	ST192	Göttingen	vanB	+	+	2011
39	UW8257	UW8257	ST192	Frankfurt/M.	vanB	+	+	2010
40	UW8965	UW8965	ST192	Heidelberg	vanB	+	+	2011
41	UW9240	UW9240	ST192	Mannheim	vanB	+	+	2011
42	UW10710	UW10710	ST192	Greifswald	vanB	+	-	2013
43	UW10524	UW10524	ST192	Greifswald	vanB	+	-	2013
44	UW11133	UW11133	ST192	Greifswald	vanB	+	-	2013
45	UW10525	UW10525	ST192	Greifswald	vanB	+	-	2013
46	UW9648	UW9648	ST203	Erlangen	vanB	+	-	2012
47	UW10914	UW10914	ST203	Greifswald	van-	+	-	2013
48	UW9960	UW9960	ST64	Greifswald	vanB	-	-	2012
49	UW8914	UW8914	ST927	Cologne	vanB	+	-	2011
50	UW8955	UW8955	ST192	Dortmund	vanB	+	+	2011
51	UW8633	UW8633	ST192	Cologne	vanB	+	+	2011
52	UW8144	UW8144	ST192	Mönchengladbach	vanB	+	+	2010
53	UW5247	UW5247	ST16	Heidelberg	vanB	+	+	2004
54	UW7027	UW7027	ST78	Plön	vanB	+	+	2007
55	UW9432	UW9432	ST192	Plön	vanA	+	+	2012
56	UW8575	UW8575	ST737	Plön	vanA	+	+	2011
57	UW5910	UW5910	ST203	Freiburg	vanA	+	-	2004
58	UW11348	UW11348	ST117	Kaiserslautern	vanB	+	+	2013
59	UW9185	UW9185	ST117	Plön	vanA	+	-	2011
60	UW11625	UW11625	ST192	Dortmund	vanA	+	-	2014
61	UW7754	UW7754	ST192	Mönchengladbach	vanB	+	+	2009
62	UW9911	UW9911	ST117	Greifswald	vanB	+	-	2012
63	UW9632	UW9632	ST117	Greifswald	van-	+	-	2012
64	UW9397	UW9397	ST117	Berlin	vanB	+	-	2012
65	UW11158	UW11158	ST780	Greifswald	vanB	+	+	2013

Fig. 1. Pseudogel view and comparison of DiversiLab patterns of 65 *E. faecium* isolates (n = 63 VRE). Isolates representing the most prevalent MLST types ST192, ST117 and ST203 are colour-coded. Metadata are given (prevalence of virulence markers *esp* and *hyl* and resistance genes *vanA* and *vanB* are presented; "year" means year of isolation). The red line delineates the similarity score of 95% set by the manufacturer. "Outbreak" defines epidemiologically related isolates from a single hospital; "outbreak strain" defines isolates with an identical MLST type and a related DL pattern; "group" defines isolates with different MLST types but a related DL pattern (see also main text for further details).

types showed different DL types (Fig. 1; "outbreak I" and "outbreak strain I"). Some isolates were grouped into a related DL cluster although they were epidemiologically unrelated and differed microbiologically (different MLST and PFGE types; "Group I" and "Group II", Fig. 1). A group of isolates related by MLST and DL ("outbreak strain I") with similar characteristics (ST192, *vanB*, *esp*- and *hyl*-positive), revealed 10 different PFGE patterns (Fig. 2). Adjusting the DL similarity score to 96% would slightly increase discrimination and performance in comparison to MLST and PFGE results; however, not all groups of epidemiologically unrelated strains with different MLST and PFGE types would be resolved. In this regard, discrimination by DL (and MLST) was limited and insufficient in comparison to PFGE typing. Among epidemiologically unrelated and microbiologically diverse isolates with identical MLST types (e.g., ST192, ST203) different DL types were detected, for example 9 DL types among ST192 isolates. In this respect, DL was more discriminatory and thus more reliable than MLST. Reliability of the PFGE-based