

TECHNICAL ADVANCE

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IS element IS16 as a molecular screening tool to identify hospital-associated strains of *Enterococcus faecium*

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Abstract

Background: Hospital strains of *Enterococcus faecium* could be characterized and typed by various molecular methods (MLST, AFLP, MLVA) and allocated to a distinct clonal complex known as MLST CC17. However, these techniques are laborious, time-consuming and cost-intensive. Our aim was to identify hospital *E. faecium* strains and differentiate them from colonizing and animal variants by a simple, inexpensive and reliable PCR-based screening assay. We describe here performance and predictive value of a single PCR detecting the insertion element, IS16, to identify hospital *E. faecium* isolates within a collection of 260 strains of hospital, animal and human commensal origins.

Methods: Specific primers were selected amplifying a 547-bp fragment of IS16. Presence of IS16 was determined by PCR screenings among the 260 *E. faecium* isolates. Distribution of IS16 was compared with a prevalence of commonly used markers for hospital strains, *esp* and *hyl_{Efm}*. All isolates were typed by MLST and partly by PFGE. Location of IS16 was analysed by Southern hybridization of plasmid and chromosomal DNA.

Results: IS16 was exclusively distributed only among 155 invasive strains belonging to the clonal complex of hospital-associated strains ("CC17"; 28 MLST types) and various vancomycin resistance genotypes (*vanA/B*/negative). The five invasive IS16-negative strains did not belong to the clonal complex of hospital-associated strains (CC17). IS16 was absent in all but three isolates from 100 livestock, food-associated and human commensal strains ("non-CC17"; 64 MLST types). The three IS16-positive human commensal isolates revealed MLST types belonging to the clonal complex of hospital-associated strains (CC17). The values predicting a hospital-associated strain ("CC17") deduced from presence and absence of IS16 was 100% and thus superior to screening for the presence of *esp* (66%) and/or *hyl_{Efm}* (46%). Southern hybridizations revealed chromosomal as well as plasmid localization of IS16.

Conclusions: This simple screening assay for insertion element IS16 is capable of differentiating hospital-associated from human commensal, livestock- and food-associated *E. faecium* strains and thus allows predicting the epidemic strengths or supposed pathogenic potential of a given *E. faecium* isolate identified within the nosocomial setting.

Background

Vancomycin- and multi-resistant *Enterococcus* strains, especially strains of *E. faecium*, raise major concerns in intensive care medicine due to limited treatment options [1]. *E. faecium* is ecologically widely distributed and expected to play a central role as a reservoir and "turntable" for antibiotic resistance determinants in the bacterial world, and especially among Gram-positive

bacteria [2]. A number of molecular typing and characterization techniques such as AFLP, MLVA and MLST and comparative genomic hybridizations allowed differentiating strains of *E. faecium* and allocating them into various clonal complexes as based on core as well as accessory genome content [3,4]. Isolates belonging to hospital-associated clonal types (for instance, MLST CC17) could be identified and a supposed enhanced spreading potential among the nosocomial setting for isolates of this specific subgroup is predictable [4]. Reports from recent years described increasing annual rates of *E. faecium* bacteraemia in European countries

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[5,6]. When investigated in greater detail, the increase was due to increasing numbers of hospital-associated clonal types of *E. faecium* (MLST CC17) whereas numbers of other clonal types remained constant over time again emphasizing the aforementioned increased potential for nosocomial spread [5]. The molecular techniques to identify hospital-associated *E. faecium* strains are laborious, time-consuming and cost-intensive and are thus not applicable for routine diagnostics. Results of recent bacterial, epidemiological, microarray-based and genomic studies suggest that a number of markers are enriched among hospital strains of *E. faecium* including collagen adhesion factors *acm* and *scm* [7,8], other matrix-binding proteins and pili [9,10], a supposed hyaluronidase, *hyl_{Efm}* [11,12], biofilm-associated markers including the enterococcal surface protein gene, *esp* [13,14], and various genomic islands encoding proteins of unknown functions [15,16]. Having acquired a number of these aforementioned determinants, these strains may have an increased pathogenic potential [4,10,17]. However, distribution of these markers among hospital strains is not exclusive and thus the predictive value is limited in terms of specificity and sensitivity. Certain markers possess pseudogenes and partly deleted variants complicating establishing a simple PCR based screening test [8,17]. Acquired ampicillin resistance appeared as a phenotypic trait of hospital strains, at least in Europe; however, this feature is also prevalent among non-hospital strains in some parts of the world, such as Asia and North America [18-20]. Microarray based genomic comparisons revealed a specific mobile element, insertion element *IS16*, exclusively prevalent among hospital *E. faecium* strains from an international collection of isolates [3]. In the present study, we established a PCR-based *IS16* screening test and evaluated its robustness, sensitivity, specificity and predictive value for 260 pre-characterized isolates of *E. faecium* from different human and animal sources. *IS16* was localized in a subset of *E. faecium* isolates (n = 45) representing 29 MLST types by Southern hybridization. Available *E. faecium* genome data were examined for the presence of *IS16* homologous sequences and a genetic association of *IS16* with genomic markers is discussed.

Methods

Strain collection

Our laboratory serves as a Focal Laboratory for Enterococci in Germany. In general, enterococcal isolates sent to us are pre-selected for being vancomycin- and multi-resistant or with the suspicion of clonal spread (local, regional, and country-wide outbreak analysis). Our collection of *E. faecium* test strains included 160 invasive isolates (blood cultures) from 60 different German diagnostic or hospital laboratories isolated between 1995

and 2007. Blood culture strains were pre-selected by geography and hospital origin, year of isolation, and different *van* genotypes (*van*-A/B). The blood culture isolates were compared against 68 human commensal (outpatient) isolates from four different prevalence studies performed in different parts of Germany (1996, 1997, 1999, 2003) [24,25] and 32 isolates from animal (chicken, pigs) and meat samples (broiler, pork) (1994, 1995, 1999) [21-23]. In all cases duplicate isolates were excluded based on clinical/epidemiological data (same patient/hospital or animal sample/flock) and of molecular typing results (for instance, based on *Sma*I-macrorestriction using PFGE for human commensal isolates [24,25]; unpublished results). Antibiotic susceptibilities were determined by microbouillon dilution in MH bouillon. Ampicillin resistance is defined by an MIC of >8 mg/L and vancomycin resistance by >4 mg/L (<http://www.eucast.org>). Summarized data are presented in Table 1 and detailed information is given in Additional files 1, 2, 3. Ethical approval was not needed for this study; all strains were pre-selected and were received anonymously from German microbiological or hospital laboratories.

DNA isolation

Genomic DNA was prepared using a DNA extraction kit (DNeasy Tissue Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. An initial cell wall lysis step was added dissolving the cell pellet in TES buffer [10 mM Tris, 0.5 mM ethylene diamine tetra-acetic acid (EDTA), 10% sucrose (pH 8.0)] plus 10 mg/mL lysozyme (Roche Applied Science, Mannheim, Germany) followed by incubation at 37°C for 30 min. Plasmid DNA was isolated using a common phenol/chloroform-based alkaline lysis strategy [26].

PCR

PCR was performed with illustra™ PuRe Taq Ready-To-Go™ PCR Beads (GE Healthcare, Freiburg, Germany) according to the manufacturer's instructions. Approximately 0.5 µL of isolated genomic DNA (ca. 10 ng) and primers (200 nM each) were added. Amplification of fragments representing the *esp*, *hyl_{Efm}* and *vanA/B* genes was performed in a multiplex PCR as described elsewhere [27]. The following strains and plasmids were used as positive control samples: plasmid pRUM (*IS16*), plasmid pLG1 (*hyl_{Efm}*); plasmid pIP816 (*vanA*; *E. faecium* BM4147), *E. faecium* U0317 (*esp*), and *E. faecalis* V583 (*vanB*). *E. faecalis* OG1RF served as a negative control sample for all PCR assays. PCR primers and conditions for screening *IS16* elements were as follows: *IS16*-F: 5'-CATGTTCCACGAACCAGAG and *IS16*-R: 5'-TCAAAAAGTGGGCTTGGC, annealing temperature was 53°C, the predicted size of the product was 547 bp

Table 1 Prevalence of epidemic markers among *E. faecium* isolates from different origins (see also Figure 1 and Additional Files 1, 2, 3)

Origin	No. of isolates	No. of MLST types	AmpR	PCR IS16 +	PCR <i>esp</i> +	PCR <i>hyl_{Efm}</i> +	PCR <i>vanA+/B</i> +
Blood cultures	160	32	98.1% (n = 157)	96.9% (n = 155)	63.8% (n = 102)	45.6% (n = 73)	58.1% (n = 93)
<i>Hosp.-associated E. faecium</i> (CC17) ¹	155	28	100% (n = 156)	100% (n = 156)	65.8% (n = 102)	46.5% (n = 72)	58.7% (n = 91)
Human commensal	68	46	2.9% (n = 2)	4.4% (n = 3) ²	0%	0%	14.7% (n = 10)
Animal commensal and meat sources	32	22	6.2% (n = 2)	0%	0%	0%	40.6% (n = 13)

BC MLST CC17 (second line) shown in italics (n = 155) is a subgroup of all blood culture *E. faecium* isolates presented in the first line representing all isolates allocated to the clonal complex of hospital-associated strains (CC17).

AmpR, ampicillin resistance; ¹ including two isolates of ST65 representing a singleton but known to be found only among clinical strains; ² all three belonged to the clonal complex of hospital-associated strain types (MLST CC17; 2x ST18, 1x ST413; see also Additional file 4).

(reference *E. faecium* plasmid pRUM; GenBank acc. no. AF507977). Primers were established using software Acelrys DS Gene 1.5.

PFGE typing

Genomic DNA for PFGE analysis was isolated and treated as described recently [27]. The agarose gel concentration was 1%, the CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA) was used for PFGE. The ramped pulsed times were as follows: 1 - 11 sec for 15 h and 11 - 30 sec for 14 h at 14°C. *Sma*I-digested *Staphylococcus aureus* NCTC 8325 was used as a molecular mass standard on all PFGE gels. Chromosomal DNA of *E. faecium* strains was digested with *I-Ceu*-I for 16 h at 37°C. Plasmids were linearized by S1 nuclease (TaKaRa, Germany) treatment for 15 min (37°C) and subsequent resolution of the linearized plasmid DNA in PFGE. The ramped pulsed times for *I-Ceu*-I gels were 5 - 30 sec for 22 h and for S1 PFGE 5 - 35 sec for 22 h at 14°C [28].

Southern hybridizations

Southern hybridization experiments were done as described elsewhere using a PCR-generated digoxigenin-labelled IS16 probe (DIG High Prime; Roche Applied Science, D), hybridization chemicals and equipment from commercial kits and according to recommendations of the manufacturer (Roche, Germany). Immunological detection was done as recommended using a chemiluminescent probe (CDP-Star™, Roche, D) and several readouts were taken at 10, 30, 60 and 120 min in a chemi-imager from Bio-Rad (Chemidoc XRS, Bio-Rad Labs., Hercules, US).

MLST and DNA sequencing

PCRs amplifying the seven loci used for MLST were done according to the reference (<http://efaecium.mlst.net/>). Sequencing reactions were performed according

to the manufacturer's recommendations for cycle sequencing of PCR products (Applied Biosystems, Germany). Sequence files were read, evaluated, aligned and compared to the reference set of alleles using sequencing software Lasergene 8.0 from DNA-STAR (SeqMan 8.0; EditSeq 8.0), TraceEditPro v. 1.1.1 from Ridom (<http://www.ridom.de>), and via the official MLST webpage (<http://efaecium.mlst.net/>). Phylogenetic trees were generated using software programmes eBURST [29] for the set of determined MLST types and global optimised eBURST [30] for the entire set of all available 524 MLST types (<http://efaecium.mlst.net/>).

Statistics

Statistical analyses were performed with software package EpiCompare 1.0 (Ridom).

Results

Identification of IS16 primer sequences

Based on available sequences (pRUM [31] or composite (*vanB*) transposons [32]) we established PCR primers for an IS16-specific internal fragment (547 bp). Specificity and sensitivity was evaluated against available bacterial databases (BlastN, GenBank). The PCR was established using DNA from reference samples known to be positive for IS16 (plasmid pRUM) and from negative control samples known to lack IS16 (*E. faecalis* OG1RF). PCR results were as expected and amplified products from reference samples were sequenced for confirming specificity of the amplified PCR product (not shown).

Screening for markers in the two sets of strains

Genomic DNA was screened by PCR using the established PCR primers for IS16, *esp* and *hyl_{Efm}*. IS16 was highly prevalent among invasive *E. faecium* (n = 155/160; Table 1) and mainly absent (n = 3/100) from human commensal or animal and meat sources.

The three IS16-positive commensal isolates were from humans. Other markers supposedly enriched among clinical or hospital-associated *E. faecium* isolates were also assessed: the *esp* gene was prevalent among 64% of all blood culture isolates and 46% of all invasive isolates were *hyl_{Efm}*-positive (Table 1). None of the 100 non-clinical strains possessed *esp* or *hyl_{Efm}*. Ampicillin resistance was prevalent among almost all clinical strains (98%). Two human commensal and two animal isolates were also ampicillin-resistant.

MLST typing

All 260 *E. faecium* isolates were MLST typed. Discriminatory power among clinical isolates (n = 160 isolates; 32 MLST types DI = 0.889 95% CI [0.864 - 0.913]) was much less than among human and animal commensal isolates (n = 100 isolates, 64 MLST types; DI = 0.988; 95% CI [0.983 - 0.994]). This suggests that prevalent hospital-associated clonal types differ less than their colonizing counterparts in animals and humans. In fact, 126 of the 160 invasive *E. faecium* isolates belonged to only eight MLST types known to represent major

hospital-associated clonal types (ST16, ST17, ST18, ST78, ST117, ST192, ST202, ST203) whereas the other 24 MLST types occurred only occasionally. Based on their MLST types and subsequent eBURST and goeBURST analyses, isolates could be sorted into different clonal complexes which were in agreement with their corresponding ecological background (compare also to [4]). Figure 1 shows eBURST trees generated with all MLST types determined in this study. A phylogenetic tree of all currently detached MLST types (n = 524; 21.04.2010; http://efaecium.mlst.net/) using goeBURST is shown as Additional File 4. Based on these two calculations, almost all (n = 155/160) invasive isolates belonged to the clonal complex of hospital-associated strains (CC17 + ST65 [singleton]). The five IS16-negative clinical isolates represent colonizing strain types not associated with clonal types of hospital strains ("non-CC17"; ST9 [n = 2], ST104; ST271; ST374). The majority of commensal isolates represented many individual MLST types but belonging to the major clonal complexes of animal and human colonizing strain types

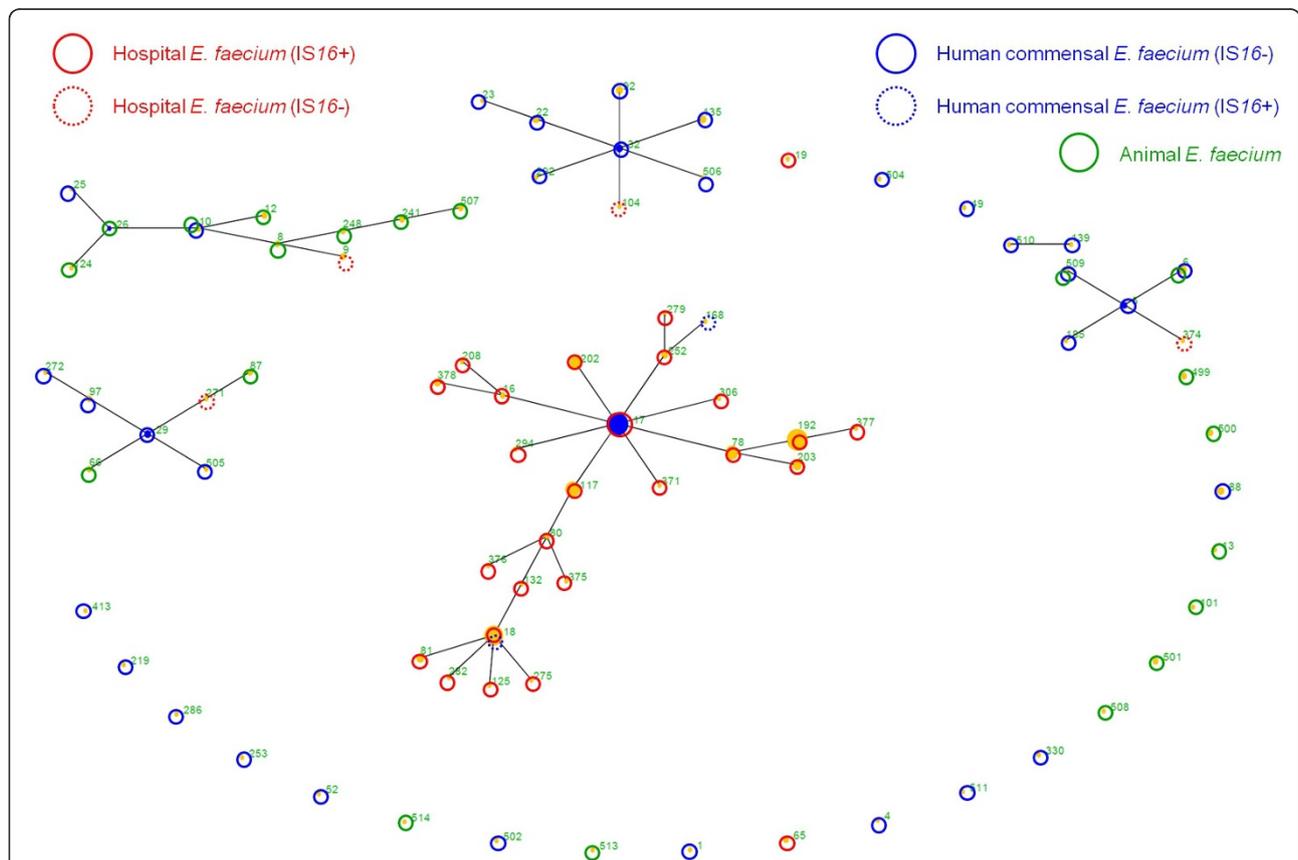


Figure 1 eBURST cluster of MLST types generated from this set of 260 clinical, commensal and livestock-associated *E. faecium* strains. (The newly identified MLST types are not presented as well as the following MLST types which appear as singletons in this set: ST39, ST74, ST94, ST289, ST345, ST503, ST413; ST512; compare also to Additional file 4). The origin of the isolates is given in colour codes. ST19 appears here as a singleton but belongs to CC17 when resolved among the entire set of MLST types (Additional file 4).

(CC5, CC22/36, CC1). The three IS16-positive commensal *E. faecium* isolates revealed MLST types (ST18, ST413) belonging to the clonal complex of hospital-associated strains (CC17).

Southern hybridizations of IS16 probes

The localization of IS16 among 45 representative *E. faecium* isolates from various origins and altogether 29 different MLST types was investigated. I-Ceu-I digested chromosomal DNA resolved in PFGE and plasmid DNA analysed in S1 PFGE and common agarose gels were transferred onto nylon membranes and hybridized against a labelled IS16 probe. Results are shown in Table 2. If available, several representatives of identical MLST types differing in their *van* gene content were considered for analysis (see for instance ST17 *vanA/B*-negative isolates). In general, the IS16 probe hybridized to (up to three) chromosomal bands suggesting several copies of the same element present in the genome (a single hybridizing chromosomal band could still result from several IS16 copies on it). Four isolates (ST17, ST81, ST202, ST275) showed IS16 only hybridizing to plasmid bands. Twenty-two strains (n = 22/45) revealed a signal for both chromosomal as well as plasmid bands. Hybridization patterns were independent of the *van* gene content (Table 2).

Screening *E. faecium* genomes for IS16

Several *E. faecium* genomes are available as genomic fragments for preliminary analysis (collection I: seven *E. faecium* genomes; [33]; collection II: eleven *E. faecium* isolates from the Human Microbiome Project; (<http://nihroadmap.nih.gov/hmp/>; <http://www.ncbi.nlm.nih.gov/genomes/gebblast.cgi?gi=6511#SearchSet>). A BLAST search for IS16-related sequences in both *E. faecium* collections revealed several hits. However, as far as some detailed information was available, the neighbouring DNA fragments all showed different genetic links. IS16 was prevalent among four out of seven genomes in collection I [33]. IS16 was linked to (a) several putative fragments of a conjugative transposon (strain E1162, ST17), (b) ORF involved in sugar metabolism (strain E1636; ST106), (c) a genomic fragment containing other transposases of the IS256 family (strain U0317, ST78) and (d) the supposed pathogenicity island containing the *esp* gene (strain E1679, ST104). None of the other two strains also containing the *esp* PAI possessed IS16 within its structure. In the deposited genomic fragments from the Human Microbiome Project, IS16 was infrequently identified, for instance, in *E. faecium* 1,230,933 linked to a metal-dependent hydrolase or in *E. faecium* TC6 linked to an extracellular solute-binding protein, family 1. In *E. faecium* DO a 5' link to a C4-dicarboxylate anaerobic carrier gene and a 3' link to a

Table 2 Localization of IS16 to I-Ceu-I digested chromosomal DNA resolved in PFGE and plasmid DNA as based on Southern hybridizations

Strain no.	Origin	MLST type	<i>van</i> genotype	I-Ceu-I PFGE	Plasmid
UW 6714	BC	202	-	.*	+
UW 1245	BC	202	<i>vanA</i>	-	+
UW 6293	BC	202	<i>vanB</i>	.*	-#
UW 6295	BC	202	<i>vanB</i>	.*	-#
UW 4962	BC	203	<i>vanA</i>	+	-#
UW 6989	BC	203	-	+	-#
UW 6502	BC	208	<i>vanB</i>	+	+
UW 5918	BC	252	<i>vanA</i>	+	+
UW 4675	BC	252	<i>vanB</i>	+	-
UW 6617	BC	275	<i>vanA</i>	-	+
UW 4960	BC	279	<i>vanB</i>	+	-
UW 6033	BC	282	<i>vanA</i>	+	+
UW 4512	BC	294	-	+	+
UW 6297	BC	306	-	+	+
UW 6935	BC	371	-	+	+
UW 3142	BC	375	<i>vanA</i>	+	+
UW 5275	BC	376	<i>vanB</i>	+	+
UW 6983	BC	377	-	+	+
UW 6112	BC	378	-	+	+
UW 4208	BC	New	-	+	+
UW 6642	BC	New	<i>vanA</i>	+	+
AK-EM 40	HC	18	-	+	+
AK-EM 53	HC	413	-	+	+
K226.7	HC	18	-	+	-
UW 2055	BC	16	<i>vanA</i>	.*	+
UW 3695	BC	17	-	+	+
UW 3544	BC	17	<i>vanA</i>	+	-#
UW 5352	BC	17	<i>vanB</i>	-	+
UW 6990	BC	18	-	+	+
UW 1952	BC	18	<i>vanA</i>	+	+
UW 2900	BC	18	<i>vanB</i>	+	-#
UW 1218	BC	19	<i>vanA</i>	+	-#
UW 3056	BC	65	-	+	+
UW 6880	BC	78	-	+	+
UW 4671	BC	78	<i>vanA</i>	+	-
UW 2457	BC	80	<i>vanA</i>	+	-
UW 6985	BC	81	-	-	+
UW 2771	BC	81	<i>vanA</i>	+	-
UW 6882	BC	117	-	+	-
UW 1983	BC	117	<i>vanA</i>	+	-
UW 6805	BC	125	<i>vanA</i>	+	+
UW 2824	BC	132	<i>vanA</i>	+	-#
UW 1716	BC	192	-	+	-#
UW 5852	BC	192	<i>vanA</i>	+	+
UW 5267	BC	192	<i>vanB</i>	+	-#

If available, different representatives of identical MLST types were considered (see for instance ST17 *vanA/B*-negative isolates).

PFGE, pulsed-field gel electrophoresis; MLST, Multi-locus sequence typing; BC, blood culture; HC, human commensal; *, Hybridization signal with PFGE slot; #, negative results in repeated experiments.

chloramphenicol acetyltransferase resistance gene followed by a replicase and relaxase genes indicated a supposed plasmid origin of this contig (not shown in details). Available sequence data allow extracting the MLST type related allele information out of the sequenced genomes and a corresponding analysis was done on the seven (collection I) and eleven (collection II) accessible genomes. Of the 11 isolates positive for *IS16* eight strains possessed hospital-associated MLST types (ST16, ST17, ST18, ST78, ST203, ST-NEW) and three did not belong to clonal complexes of hospital-associated types (ST25, ST104, ST114). Intriguingly, of the latter three, one was a clinical isolate also positive for *esp*, a marker commonly associated only with hospital-associated strains.

Epidemiological analysis of clinical strains

We introduced PCR-based screening for *IS16* for all *E. faecium* isolates sent to our Focal Laboratory for Enterococci in Germany in late 2007. In 2008 we received 314 *E. faecium* isolates (from >57 hospitals located in 13 of the 16 German federal states; all VRE) and in 2009 334 *E. faecium* isolates (from >49 hospitals located in 11 German federal states, 90% VRE) from clinical sources for further analyses. Altogether 99.4% (n = 312/314) in 2008 and 97.3% (n = 325/334) in 2009 were *IS16*-positive demonstrating wide prevalence of multi-resistant (VRE), hospital-associated strain types in hospitals in Germany.

Discussion

Early recognition of epidemic *E. faecium* strains is critical for a timely introduction of strategies to prevent and control their further spread. Hospital-associated clonal types of *E. faecium* often disseminate unrecognized among the nosocomial setting before and until first VRE cases suddenly appear [5,6]. But standardised methods for rapid diagnostics of these hospital-associated *E. faecium* strains are missing and modern, DNA sequence-based typing techniques are laborious, time-consuming and comparably expensive. Acquired ampicillin resistance [5,6,34] and high-level ciprofloxacin resistance [35,36] appear as phenotypic traits of hospital-associated strains. However, ampicillin resistance in animal or commensal enterococci is also prevalent to a certain extent [37], at least in many North American and Asian countries [38] and the predictive value of acquired ciprofloxacin resistance is limited since it accumulates over time and differently in various clonal types of hospital strains [36]. Several genetic markers, such as *esp*, *hyl_{Efm}* or the *purK1* allele (used as part of the MLST scheme) are not ubiquitous traits of all hospital-associated *E. faecium* strains and failure to detect them does not reliably indicate a strain with a limited spreading or pathogenic potential [27,34,36,39-41]. It was

also shown recently that *hyl_{Efm}* is plasmid-located [11,42], transferable in vitro into several non-hospital *E. faecium* strains and thus cannot be suggested to serve as a specific marker to predict a clonal background of a given strain [43]. A reliable and rapid molecular test to differentiate commensal from hospital-acquired strains is desirable; results from comparative genomic hybridizations and genome sequencing projects show promising candidate markers including the described insertion element *IS16* [3].

According to our data, *IS16* is highly specific for predicting hospital-associated strain types (Table 1; Additional files 1, 2, 3). Altogether 155 of the 160 blood culture isolates from 1995 to 2007 from >50 contributing laboratories or hospitals and representing 28 MLST types were all *IS16* positive. The comparably high number of (a) participating hospitals/laboratories contributing strains, (b) blood culture strains representing many different MLST types and different *van* genotypes, and (c) isolated from over 13 years compensates for a geographical limitation of a strain collection that only included isolates from Germany. The presence of *IS16* was independent of resistance gene clusters known to possess this element; only 19 isolates were *vanB*-positive (12%) and the remaining isolates were vancomycin susceptible (n = 67; 42%) or of the *vanA*-type (n = 74; 46%). Since it is known from previous plasmid analysis that *IS16* could be associated with specific plasmid types (e.g., pRUM), we identified the location of *IS16* in a representative set of *E. faecium* isolates (*vanA/B*-negative; 29 MLST types; Table 2). Southern hybridizations revealed chromosomal, plasmid and both localizations for *IS16* suggesting a somehow restricted mobile gene pool within hospital-associated clonal types. All but three investigated human commensal and all animal strains possessed MLST types different from hospital-associated strains; all but the three aforementioned strains were *IS16* negative. The three human commensal isolates possessing *IS16* revealed MLST types ST18 and ST413, allocating them to hospital-associated strains and again emphasising the excellent predictive value of this element for identifying hospital-associated strain types. PCRs for *esp* and *hyl_{Efm}* were negative for these three strains and one was ampicillin-resistant. Absence of *IS16* among non-clinical *E. faecium* strains also suggests a very limited host range of *IS16*-bearing plasmids (e.g., pRUM-like) only among hospital-associated strains. Prevalence of hospital-associated *E. faecium* isolates among the general human population is low and more detailed information was only available in one of the three cases. This outpatient from a community surveillance study received beta-lactam treatment during the time of sampling which could be the reason for identifying an ampicillin- and multiresistant, epidemic strain here (association with a hospital stay was not documented).

An epidemiological analysis of 648 *E. faecium* clinical isolates sent to our Focal Laboratory for Enterococci in 2008/2009 from German hospital patients revealed that 98% (n = 637) were IS16-positive, demonstrating country-wide prevalence of hospital-associated strain types among German hospitals in recent years. This finding was supported by wide prevalence of acquired high-level ciprofloxacin resistance among the same strain collection serving as an additional marker for hospital-associated strains [36]. Admittedly, most of the strains from 2008/2009 were vancomycin-resistant showing in retrospect that hospital-associated strains of *E. faecium* and not commensal strain types accumulated in the nosocomial setting and acquired *vanA*- or *vanB*-type resistance determinants.

The observation that an IS element, IS16, is among the most specific markers to define hospital-associated strains [3] appears curious regarding the promiscuous nature of these elements to transpose between several genomic backgrounds within a cell and the possible horizontal mobilization in cis or trans by activation via conjugative elements and subsequent transfer into new hosts. It has been argued that acquisition of specific elements, especially IS16, has contributed to the ecological success of hospital-associated *E. faecium* strains allowing higher levels of genetic variability including easier acquisition of mobile genetic elements that may support lifestyle in new or changing environments or ecological niches like the hospital environment [3,44].

Conclusions

Our data show that an IS16-based screening assay appears as the best molecular screening tool to differentiate colonizing *E. faecium* isolates from hospital-associated *E. faecium* strains that show an enhanced spreading potential in the nosocomial setting. The predictive value of an IS16 result is superior to the predictive strengths of established tests targeting molecular markers like *esp* or *hyl_{Efm}* or phenotypic traits like acquired ampicillin or high-level ciprofloxacin resistance. Progress in comparative genome-based analysis will most probably reveal additional markers to identify hospital associated *E. faecium* isolates with an enhanced spreading potential in the nosocomial setting [15,33].

Additional material

Additional file 1: Detailed information on the 160 clinical *E. faecium* isolates. The blood culture strains were part of this study and of Werner et al., [36]. "0" indicates absence, "1" indicates presence of the corresponding marker (*esp*, *hyl_{Efm}*, IS16, *vanA/B/O*). Legend: AmpR, resistance to ampicillin; year, year of isolation.

Additional file 2: Detailed information on the 68 human commensal *E. faecium* isolates. The human commensal strains were part of several community prevalence studies performed in different parts of Germany and at different time periods (see text for details). "0" indicates absence,

"1" indicates presence of the corresponding marker (*esp*, *hyl_{Efm}*, IS16, *vanA/B/O*). Legend: AmpR, resistance to ampicillin; year, year of isolation.

Additional file 3: Detailed information on the 32 animal *E. faecium* isolates. The animal strains were part of several prevalence studies performed in different parts of Germany and at different time periods (see text for details). "0" indicates absence, "1" indicates presence of the corresponding marker (*esp*, *hyl_{Efm}*, IS16, *vanA/B/O*). Legend: AmpR, resistance to ampicillin; year, year of isolation.

Additional file 4: goeBURST analysis of all MLST types of the *E. faecium* database (n = 524 STs; 21.4.2010; http://efaecium.mlst.net/).

Only the major, superior complex without singletons and smaller complexes is shown. A number of singletons from Figure 1 belong to clonal complexes such as ST19 which is part of the large cluster and related to CC17. Other complexes separated in Figure 1 (CC32, CC29) are linked here to form a main, superior cluster. ST65 remains a singleton and is the only IS16-positive ST not phylogenetically linked to the clonal complex of hospital-associated strains (CC17).

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Authors' contributions

IK manages and supervises the central enterococcal database and records all typing and characterization data from routine enterococcal diagnostics. UG performed standard PCRs, characterization and identification experiments. CF performed additional typing experiments such as PFGE, MLST and Southern hybridizations. WVS provided seven unpublished genome data and analyzed them regarding genetic linkages between IS16 and neighbouring regions. GW and WW supervised the study; GW selected the primer sequences, evaluated and calculated all the PCR, MLST, PFGE and hybridisation results. GW, IK and WW wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Arias CA, Murray BE: Antibiotic-resistant bugs in the 21st century - a clinical super-challenge. *N Engl J Med* 2009, **360**:439-443.
2. Tenover FC: Development and spread of bacterial resistance to antimicrobial agents: an overview. *Clin Infect Dis* 2001, **33**(Suppl 3): S108-S115.
3. Leavis HL, Willems RJ, van Wamel WJ, Schuren FH, Caspers MP, Bonten MJ: Insertion sequence-driven diversification creates a globally dispersed emerging multiresistant subspecies of *E. faecium*. *PLoS Pathog* 2007, **3**:e7-75.
4. Willems RJ, van SW: Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. *Future Microbiol* 2009, **4**:1125-1135.
5. Lester CH, Sandvang D, Olsen SS, Schonheyder HC, Jarlov JO, Bangsborg J, Hansen DS, Jensen TG, Frimodt-Moller N, Hammerum AM: Emergence of ampicillin-resistant *Enterococcus faecium* in Danish hospitals. *J Antimicrob Chemother* 2008, **62**:1203-1206.
6. Top J, Willems R, van d V, Asbroek M, Bonten M: Emergence of clonal complex 17 *Enterococcus faecium* in The Netherlands. *J Clin Microbiol* 2008, **46**:214-219.
7. Nallapareddy SR, Singh KV, Okhuysen PC, Murray BE: A functional collagen adhesin gene, *acm*, in clinical isolates of *Enterococcus faecium* correlates

- with the recent success of this emerging nosocomial pathogen. *Infect Immun* 2008, **76**:4110-4119.
8. Sillanpaa J, Prakash VP, Nallapareddy SR, Murray BE: Distribution of genes encoding MSCRAMMs and pili in clinical and natural populations of *Enterococcus faecium*. *J Clin Microbiol* 2009, **47**:896-901.
 9. Hendrickx APA, Van Wamel WJB, Posthuma G, Bonten MJM, Willems RJL: Five genes encoding surface exposed LPXTG proteins are enriched in hospital-adapted *Enterococcus faecium* Clonal Complex-17 isolates. *J Bacteriol* 2007, **189**:8321-8332.
 10. Hendrickx AP, Willems RJ, Bonten MJ, van SW: LPxTG surface proteins of enterococci. *Trends Microbiol* 2009, **17**:423-430.
 11. Freitas AR, Tedim AP, Novais C, Ruiz-Garbajosa P, Werner G, Laverde-Gomez JA, Canton R, Peixe L, Baquero F, Coque TM: Global spread of colonization-virulence *hyl*_{Efm} gene in megaplasms of CC17 *Enterococcus faecium* polyclonal sub-cluster. *Antimicrob Agents Chemother* 2010, **54**:2660-2665.
 12. Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, Klare I, Nallapareddy SR, Huang W, Murray BE: A potential virulence gene, *hyl*_{Efm}, predominates in *Enterococcus faecium* of clinical origin. *J Infect Dis* 2003, **187**:508-512.
 13. Heikens E, Bonten MJM, Willems RJL: Enterococcal surface protein Esp is important for biofilm formation of *Enterococcus faecium* E1162. *J Bacteriol* 2007, **189**:8233-8240.
 14. Willems RJ, Homan W, Top J, van Santen-Verheul M, Tribe D, Manziors X, Gaillard C, Vandenbroucke-Grauls CM, Mascini EM, van KE, et al: Variant *esp* gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. *Lancet* 2001, **357**:853-855.
 15. Heikens E, van Schaik W, Leavis HL, Bonten MJM, Willems RJL: Identification of a novel genomic island specific to hospital-acquired clonal complex 17 *Enterococcus faecium* isolates. *Appl Environ Microbiol* 2008, **74**:7094-7097.
 16. Lester CH, Olsen SS, Schonheyder HC, Hansen DS, Tvede M, Holm A, Arpi M, Friis-Moller A, Jensen KT, Kemp M, et al: Typing of vancomycin-resistant enterococci obtained from patients at Danish hospitals and detection of a genomic island specific to CC17 *Enterococcus faecium*. *Int J Antimicrob Agents* 2009, **35**:312-314.
 17. Nallapareddy SR, Weinstock GM, Murray BE: Clinical isolates of *Enterococcus faecium* exhibit strain-specific collagen binding mediated by *Acm*, a new member of the MSCRAMM family. *Mol Microbiol* 2003, **47**:1733-1747.
 18. Donabedian S, Thal LA, Bozgar P, Zervos T, Hershberger E, Zervos M: Antimicrobial resistance in swine and chickens fed virginiamycin for growth promotion. *J Microbiol Methods* 2003, **55**:739-743.
 19. McDonald LC, Rossiter S, Mackinson C, Wang YY, Johnson S, Sullivan M, Sokolow R, DeBess E, Gilbert L, Benson JA, et al: Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. *N Engl J Med* 2001, **345**:1155-1160.
 20. Jung WK, Lim JY, Kwon NH, Kim JM, Hong SK, Koo HC, Kim SH, Park YH: Vancomycin-resistant enterococci from animal sources in Korea. *Int J Food Microbiol* 2007, **113**:102-107.
 21. Klare I, Heier H, Claus H, Bohme G, Marin S, Seltmann G, Hakenbeck R, Antanassova V, Witte W: *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. *Microb Drug Resist* 1995, **1**:265-272.
 22. Klare I, Heier H, Claus H, Reissbrodt R, Witte W: *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. *FEMS Microbiol Lett* 1995, **125**:165-171.
 23. Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W: Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microb Drug Resist* 1999, **5**:45-52.
 24. Lietzau S, Hoewner M, von BH, Marre R, Brenner H: Antibiotic resistant fecal isolates of Enterococci among unselected patients outside the clinical sector: an epidemiological study from Southern Germany. *Pharmacoepidemiol Drug Saf* 2006, **15**:275-277.
 25. Witte W, Werner G, Tietze E, Reissbrodt R, Klare I: Routes of transmission of antibiotic-resistant bacteria and their resistance determinants to humans via various environmental sources like crop food or bathing freshwater. *Final report projekt UFOPLAN*, 20162211, 2001 - 2004. 1-95. 1-3-2005.
 26. Werner G, Klare I, Witte W: Large conjugative *vanA* plasmids in vancomycin-resistant *Enterococcus faecium*. *J Clin Microbiol* 1999, **37**:2383-2384.
 27. Klare I, Konstabel C, Mueller-Bertling S, Werner G, Strommenger B, Kettlitz C, Borgmann S, Schulte B, Jonas D, Serr A, et al: Spread of ampicillin/vancomycin-resistant *Enterococcus faecium* of the epidemic-virulent clonal complex-17 carrying the genes *esp* and *hyl* in German hospitals. *Eur J Clin Microbiol Infect Dis* 2005, **24**:815-825.
 28. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L: Clonal expansion within clonal complex 2 and spread of vancomycin-resistant plasmids among different genetic lineages of *Enterococcus faecalis* from Portugal. *J Antimicrob Chemother* 2009, **63**:1104-1111.
 29. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG: eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004, **186**:1518-1530.
 30. Francisco A, Bugalho M, Ramirez M, Carrico J: Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. *BMC Bioinformatics* 2009, **10**:152.
 31. Grady R, Hayes F: Axe-Txe, a broad-spectrum proteic toxin-antitoxin system specified by a multidrug-resistant, clinical isolate of *Enterococcus faecium*. *Mol Microbiol* 2003, **47**:1419-1432.
 32. Dahl KH, Rokenes TP, Lundblad EW, Sundsfjord A: Nonconjugative transposition of the *vanB*-containing Tn5382-like element in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2003, **47**:786-789.
 33. van Schaik W, Top J, Riley DR, Boekhorst J, Vrijenhoek JEPV, Schapendonk CME, Hendrickx APA, Nijman IJ, Bonten MJM, Tettelin H, et al: Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island. *BMC Genomics* 2010, **11**:239. *BMC Genomics* 2010.
 34. Galloway-Pena JR, Nallapareddy SR, Arias CA, Eliopoulos GM, Murray BE: Analysis of clonality and antibiotic resistance among early clinical isolates of *Enterococcus faecium* in the United States. *J Infect Dis* 2009, **15**:1566-1573.
 35. Leavis HL, Willems RJL, Top J, Bonten MJM: High-Level ciprofloxacin resistance from point mutations in *gyrA* and *parC* confined to global hospital-adapted clonal lineage CC17 of *Enterococcus faecium*. *J Clin Microbiol* 2006, **44**:1059-1064.
 36. Werner G, Fleige C, Ewert B, Laverde-Gomez JA, Klare I, Witte W: High-level ciprofloxacin resistance among hospital-adapted *Enterococcus faecium* (CC17). *Int J Antimicrob Agents* 2010, **35**:119-125.
 37. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH, et al: Emergence and spread of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. *Appl Environ Microbiol* 2009, **75**:4904-4908.
 40. Willems RJ, Bonten MJ: Glycopeptide-resistant enterococci: deciphering virulence, resistance and epidemicity. *Curr Opin Inf Dis* 2007, **20**:384-390.
 41. Freitas AR, Novais C, Ruiz-Garbajosa P, Coque TM, Peixe L: Dispersion of multidrug-resistant *Enterococcus faecium* isolates belonging to major clonal complexes in different Portuguese settings. *Appl Environ Microbiol* 2009, **75**:4904-4908.
 42. Arias CA, Panesso D, Singh KV, Rice LB, Murray BE: Co-Transfer of antibiotic resistance genes and a *hyl*(Efm)-containing virulence plasmid in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2009, **53**:4240-4246.
 43. Laverde-Gomez JA, van Schaik W, Freitas AR, Coque TM, Weaver K, Francia MV, Witte W, Werner G: A multiresistance megaplasmid bearing a *hyl*_{Efm} genomic island in hospital *Enterococcus faecium* isolates. *Int J Med Microbiol* 2011, **301**:165-175.
 44. Burrus V, Waldor MK: Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology* 2004, **155**:376-386.
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