JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2011, p. 367–372 Vol. 49, No. 1
0095-1137/11/$12.00 doi:10.1128/JCM.01120-10
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Genotyping of *Enterococcus faecalis* and *Enterococcus faecium* Isolates by Use of a Set of Eight Single Nucleotide Polymorphisms▽†

I. U. Rathnayake, M. Hargreaves, and F. Huygens*

Cell and Molecular Biosciences, Faculty of Science and Technology, Queensland University of Technology, Australia

Received 2 June 2010/Returned for modification 17 August 2010/Accepted 21 October 2010

A single nucleotide polymorphism (SNP) genotyping method for *Enterococcus faecalis* and *Enterococcus faecium* was developed using the "Minimum SNPs" program. SNP sets were interrogated using allele-specific real-time PCR. SNP typing subdivided clonal complexes 2 and 9 of *E. faecalis* and 17 of *E. faecium*, members of which cause the majority of nosocomial infections globally.

Enterococcus faecium and *Enterococcus faecalis* cause 80 to 90% of human enterococcal infections (9). The genetic subset of *E. faecium* named clonal complex 17 (CC17) seems to be responsible for the worldwide emergence of nosocomial infections by this pathogen (6, 7, 13, 14). CC17 is characterized by quinolone and ampicillin resistance and the presence of a putative pathogenicity island carrying *esp* and *hyl* genes (2, 5, 7, 16). As in the case of *E. faecium*, it is suggested that an adaptation to the hospital environment has occurred in *E. faecalis*. CC2 and CC9 might be designated high-risk CCs of *E. faecalis* because they contain members that are vancomycin and gentamicin resistant, produce β-lactamase, and carry pathogenicity islands (3, 6). The characterization and study of the population structure of *E. faecalis* and *E. faecium* is important to investigate how nosocomial enterococcal populations are evolving toward a predominance of highly specialized enterococcal genetic subpopulations that are capable of surviving, spreading, and infecting patients with increasing frequencies in the hospital environment. Recent efforts have focused on the development of methods for the characterization of enterococci (1, 15–19); however, there is a need to develop and apply new robust, rapid, and cost-effective techniques which are likely to yield more definitive results.

Multilocus sequence typing (MLST) has emerged as a powerful tool for determining the population structure of many bacterial pathogens (4, 8, 13, 14). In the case of enterococci, Homan et al. (4) concluded that MLST is an appropriate technique to establish an unambiguous international database of *E. faecium* genetic lineages; however, MLST is impractical for routine monitoring of *E. faecalis* and *E. faecium* outside major research facilities (10). To overcome this shortcoming, the use of informative single nucleotide polymorphisms (SNPs) has been described as a cost-effective alternative to full MLST characterization (12). Currently, MLST costs around AUD$91.00 per strain, compared to AUD$7.00 per strain for our SNP profiling method, a considerable cost saving. Previous studies have demonstrated that a small number of SNPs derived from the MLST database can be used to define bacterial populations, including *Staphylococcus aureus* (4), *Neisseria meningitidis* (12), and *Campylobacter jejuni* (11). The aim of this study was to develop an SNP-based genotyping method to study the population structure of clinical isolates of *E. faecalis* and *E. faecium* from South East Queensland.

E. faecalis and *E. faecium* isolates sourced from clinical samples were obtained from Pathology Queensland and the QUT culture collection and were confirmed as either *E. faecalis* or *E. faecium* by performing real-time PCR to detect the $ddl_{E.\ faecalis}$ and $ddl_{E.\ faecium}$ genes. The primers used were 5′CAAACTG TTGGCATTCCACAA3′ and 5′TGGATTTCCTTTCCAGTC ACTTC3′ (*E. faecalis* forward and reverse primers, respectively) and 5′GAAGAGCTGCTGCAAAATGCTTTAGC3′ and 5′GCGCGCTTCAATTCCTTGT3′ (*E. faecium* forward and reverse primers, respectively) (F. Huygens, unpublished data). *E. faecalis* ATCC 19433 and *E. faecium* ATCC 27270 strains were used for method development. The Corbett X-tractor Gene automated DNA extraction system was used to extract DNA from all cultured isolates (Corbett Robotics, Australia) using the Core protocol no. 141404 version 02. Informative SNP sets that provide a high Simpson's diversity index (*D*) value (12) were identified for *E. faecalis* and *E. faecium* using the software program "Minimum SNPs," which has been described in detail elsewhere (12). Allele sequences and corresponding sequence types (STs) from the *E. faecalis* (http://efaecalis.mlst.net/) and *E. faecium* (http://efaecium.mlst.net/) MLST databases were used as input data for the Minimum SNPs software. An allele-specific real-time PCR (AS kinetic PCR) methodology was developed to interrogate these high-*D*-value SNPs. The allele-specific primers, designed using Primer Express 2.0 (Applied BioSystems), are listed in Table 1. Each AS kinetic PCR mixture contained 2 μl of DNA and 8 μl of reaction master mix containing 5 μl of 2× SYBR green PCR master mix (Invitrogen, Australia) and 0.125 μl of reverse and forward primers (0.5 μM final concentration). The cycling conditions were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s and a melting stage of 60°C to 90°C (RotorGene 6000; Corbett Robotics, now Qiagen). The kinetic PCR results for the *xpt198*, *aroE355*, *gdh165*, *gyd208*, *gki141*, and *pstS390* SNPs of *E. fae-*

* Corresponding author. Mailing address: Cell and Molecular Biosciences, Faculty of Science and Technology, Queensland University of Technology, 2 George St., Brisbane, Queensland 4001, Australia. Phone: 61 7 3138 9252. Fax: 61 7 3138 1534. E-mail: f.huygens@qut.edu.au.

† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 27 October 2010.

TABLE 1. Primers used for the interrogation of high-*D*-value SNPs in *E. faecalis* and *E. faecium*

Species and SNP	Cumulative *D* value	Primer[a]	Primer sequence (5′–3′)[b]
E. faecalis			
gyd268	0.5034	*gyd*268GF	GACAAAGAAGTTACTGTTGATGAAGT**G**
		*gyd*268AF	GACAAAGAAGTTACTGTTGATGAAGT**A**
		*gyd*268R	CTACGATATCAGAAGAAACGATTTCG
xpt198[c]	0.7536	*xpt*198GR	AAATGAATAAACTGAAGCCGTTAA**G**
		*xpt*198AR	AAATGAATAAACTGAAGCYGTTAA**A**
		*xpt*198F	CTTCGCKCGTAAGGCAAAAAGT
aroE355[c]	0.8766	*aroE*355GR	TGGGATTATAAATAGCATCATACAC**G**
		*aroE*355AR	TGGGATTATAAATAGCATCATACAC**A**
		*aroE*355F	CCACATGCRCATAGTAGTCCTATAGAAAA
gdh165	0.9386	*gdh*165AF	CAGCCTATCGTGATGAACC**A**
		*gdh*165GF	CAGCCTATCGTGATGAACC**G**
		*gdh*165R	CGCCAGACCAACGGAAAT
gyd208	0.9682	*gyd*208AF	GCTCAACGTGTTCCTGTAGC**A**
		*gyd*208TF	GCTCAACGTGTTCCTGTAGC**T**
		*gyd*208R	CCATTACTGCATTYACTTCATCAAC
gki141[c]	0.9824	*gki*141TR	TTCCCCCGCGCC**T**
		*gki*141CR	TTCCCCCGCGCC**C**
		*gki*141F	TTCCGTTTGCHTTAGATAATGATG
pstS87[c]	0.9886	*psts*87GR	GACCACTGGTCCCATACC**G**
		*psts*87AR	GACCACTGGTCCCATACC**A**
		*psts*87F	CGTGGATGCCTCTAAATTAGTYGA
pstS390[c]	0.992	*pstS*390GR	CATCAATGCTTAAGGCAAC**G**
		*pstS*390AR	CATCAATGCTTAAGGCAAC**A**
		*pstS*390F	CAGTTCGTAAAATTGTTGAACAAACA
E. faecium			
pstS452[c]	0.3888	*pstS*452CR	GTGTACATATGTTCATATGACCAGATT**C**
		*pstS*452TR	GTGTACATATGTTCATAKGACCAGATT**T**
		*pstS*452F	TCGACGGTGTAGAACCAAAAGA
atpA485[c]	0.6456	*atpA*485CR	CAGCATATGGTGCGATATAAAG**C**
		*atpA*485TR	CAGCATATGGTGCGATATAAAG**T**
		*atpA*485F	ACATTGAAAAAAATATGGCGCAAT
gyd160[c]	0.7359	*gyd*160GR	CCGTCTAATTTACCGTTCAATTC**G**
		*gyd*160TR	CCGTCTAATTTACCGTTCAATTC**T**
		*gyd*160AR	CCGTCTAATTTACCRTTCAATTC**A**
		*gyd*160F	GCAAACATCGTWCCTAACTCAACW
purK115	0.8276	*purK*115TF	AGAAAAAATCTTTTTTGGAAACGAA**T**
		*purK*115CF	RGAAAAAATCTTTTTTGGAAACGAA**C**
		*purK*115R	GATCCCGTCAATCGCATCTT
pstS87	0.8782	*pstS*87CF	GTGGATCATAAAGTAGCAGTGGT**C**
		*pstS*87TF	GTGGATCATAAAGTAGCAGTRGT**T**
		*pstS*87R	GTAAAGATATCAATCAATTCCTGTTTKG
atpA314	0.9122	*atpA*314CF	CCGTAAAACAGGGAAAACTTC**C**
		*atpA*314TF	CCGTAAAACAGGGAAAACTTC**T**
		*atpA*314R	GATCATATCTTGRCCTTTTTGGTTRA
atpA188[c]	0.9373	*atpA*188GR	GTTAACAGATTTACGTTGCATAAC**G**
		*atpA*188AR	GTTAACAGATTTACGTTGCATAAC**A**
		*atpA*188F	AATYGACGGACTAGGTGAAATCG
purK217[c]	0.9509	*purK*217AR	CCCTTGCCATCATAGCC**A**
		*purK*217GR	CCYTTGCCATCATARCC**G**
		*purK*217F	GATCGTCAGTCCGACRGATATC

[a] F, forward primer; R, reverse primer.
[b] The allele-specific primers are indicated with a nucleotide base in boldface at the 3′ end of the sequence. Key to symbols: H = A+T+C, K = G+T, R = A+G, W = A+T, Y = C+T.
[c] The SNP is in the reverse primer.

calis and the *purK115*, *atpA314*, and *purK217* SNPs of *E. faecium* gave sufficiently large differences in cycle threshold (ΔC_T) values to provide a clear distinction between the matched and mismatched reactions. The primers for *gyd268* and *pstS87* of *E. faecalis* and *pstS452*, *atpA485*, *gyd160*, *pstS87*, and *atpA188* of *E. faecium* were redesigned with a subterminal mismatched nucleotide at the 3′ end of the primer to improve the allele specificity by increasing the ΔC_T between the matched and the mismatched primers while having little or no effect on C_T values for the matched primers. The likely reason for this effect is that the mismatch lowers the melting point of the target-primer duplex, thus reducing the probability that the primer site will be occupied at any given time point during the annealing step.

Isolate-specific SNP profiles were generated, consisting of the polymorphism present at each of the SNP positions. SNP

TABLE 2. SNP profiles of *E. faecalis* isolates

No. of isolates with profile or strain[a]	Polymorphism at SNP:								SNP profile	ST(s) in MLST
	1, *gyd268*	2, *xpt198*	3, *aroE355*	4, *gdh165*	5, *gyd208*	6, *gki141*	7, *pstS87*	8, *pstS390*		
1	A	C	C	A	A	G	T	C	ACCAAGTC	ST41, ST146, ST216, ST219, ST239
2	A	C	C	A	T	G	T	T	ACCATGTT	ST44, ST189
1	A	C	C	G	A	A	T	C	ACCGAATC	ST62, ST85
1	A	C	C	G	T	G	T	T	ACCGTGTT	ST113
1	A	C	T	A	A	G	T	T	ACTAAGTT	New[b]
1	A	C	T	A	T	G	C	C	ACTATGCC	ST79, ST82
2	A	C	T	G	A	A	C	C	ACTGAACC	ST138
2	A	C	T	G	T	A	T	C	ACTGTATC	ST40, ST114, ST148, ST198
1	A	T	C	A	A	A	C	C	ATCAAACC	ST5, ST21, ST46, ST50, ST70
1	A	T	C	A	A	A	C	C	ATCAAACC	ST145, ST152, ST157
7	A	T	T	A	A	G	C	T	ATTAAGCT	ST6, ST139, ST181, ST183, ST241
1	A	T	T	A	T	G	C	C	ATTATGCC	ST170
1	A	T	T	G	A	G	C	T	ATTGAGCT	New
1	G	C	C	A	A	A	C	C	GCCAAACC	ST186, ST192
2	G	C	C	A	T	A	T	T	GCCATATT	ST19, ST20, ST120
1	G	C	C	A	T	G	C	C	GCCATGCC	New
1	G	C	C	G	A	A	T	C	GCCGAATC	ST30, ST56, ST217
1	G	C	C	G	T	A	T	T	GCCGTATT	New
17	G	C	T	G	A	A	C	C	GCTGAACC	ST16, ST66, ST67
4	G	C	T	G	A	A	T	C	GCTGAATC	ST26, ST60, ST209, ST214
1	G	T	C	G	T	G	T	T	GTCGTGTT	ST36, ST118, ST180
1	G	T	T	G	A	A	C	C	GTTGAACC	ST95, ST179
1	G	T	T	G	A	G	T	C	GTTGAGTC	ST64, ST101, ST161, ST205
TX2486	A	T	T	G	A	A	C	T	ATTGAACT	ST2
TX2708	A	T	T	A	A	G	C	T	ATTAAGCT	ST6
TX0630	A	T	C	A	T	A	C	T	ATCATACT	ST9
ΔC_T value (mean ± SD[c]) of:										
A	6.97 ± 0.37	NA[b]	NA	2.02 ± 0.20	7.42 ± 0.54	8.88 ± 0.37	NA	NA		
C	NA	4.36 ± 0.27	3.32 ± 0.47	NA	NA	NA	9.77 ± 0.78	2.49 ± 0.38		
G	3.51 ± 0.38	NA	NA	3.69 ± 0.44	NA	7.73 ± 0.29	NA	NA		
T	NA	2.61 ± 0.18	1.48 ± 0.30	NA	9.43 ± 0.63	NA	9.74 ± 0.92	2.2 ± 0.31		

[a] Strains were obtained from University of Texas and are fully MLST characterized.
[b] New, STs not found in MLST database; NA, not applicable.
[c] From pooled results for each polymorphism.

profiles were determined for 55 *E. faecalis* isolates and 30 *E. faecium* isolates (Tables 2 and 3). The SNP profiles were assigned to either STs or CCs. Amounts of between 18 and 30 ng of template DNA from randomly chosen isolates of *E. faecalis* and *E. faecium* were sequenced to validate the SNPs as described previously (17). The SNP profiles for 160 *E. faecalis* STs and 414 *E. faecium* STs (listed on the MLST database) were determined *in silico*. The nucleotides present at the SNP positions were manually determined for all the STs to determine the *in silico* SNP profile. The web-based eBURST (Based Upon Related Sequence Types) algorithm was used to aid the visualization of the relationship between high-*D*-value SNP profiles and MLST sequence types generated for *E. faecalis* and *E. faecium* isolates.

The relationship between the SNP profile of each isolate and the MLST-defined population structure was determined for both *E. faecalis* and *E. faecium* isolates, using the MLST database and the "working backwards" mode of the Minimum SNPs program. Twenty-one and 19 SNP profiles were identified for *E. faecalis* and *E. faecium* isolates, respectively. A number of SNP profiles were new, and these isolates are likely to be new STs that warrant further characterization. The most dominant SNP profile for *E. faecalis* clinical isolates was GCTGAACC (corresponding to STs 16, 66, and 67), which is shared by 17 isolates in our collection. SNP profile GGCTCCCC (corresponding to STs 267 and 349) is the dominant profile for *E. faecium*, which is shared by six isolates in our collection.

One hundred sixty STs (350 isolates) of *E. faecalis* and 414 STs (1,319 isolates) of *E. faecium* were subjected to *in silico* analysis of the high-*D*-value SNPs. The 160 *E. faecalis* STs were resolved into 86 SNP profiles. The 414 *E. faecium* STs were subdivided into 55 SNP profiles. The SNP profiles of all STs listed in the MLST database are shown in the supplemental material.

eBURST analysis of all STs was correlated with the SNP profiles of *E. faecalis* and *E. faecium* (Fig. 1). The STs of the major *E. faecalis* clonal complex CC21 were found to share the same SNP profile, ATCAAACC. The most prevalent ST in MLST, ST 16, has the GCTGAACC SNP profile. Previous studies of the *E. faecalis* population structure have found that CC2 contains STs 6, 2, and 51 and CC9 contains STs 9, 17, 18, 42, and 52 and that these CCs were associated almost exclusively with hospital-derived isolates (6). In contrast, our study found that none of the clinical isolates belonged to either CC2 or CC9. To date, members of CC2 and CC9 have not been documented in Australia. *In silico* SNP analysis of the MLST

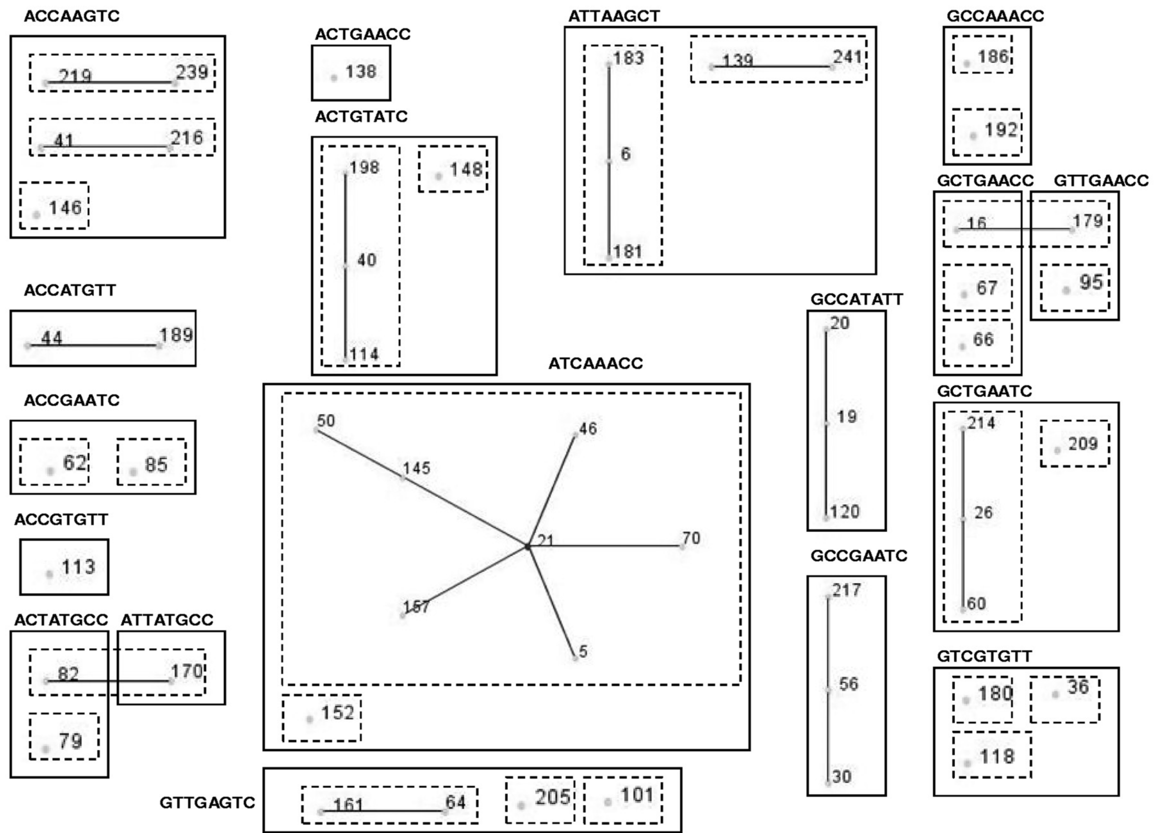
TABLE 3. SNP profiles of *E. faecium* isolates

No. of isolates with profile	Polymorphism at SNP:								SNP profile	ST(s) in MLST
	1, *pstS452*	2, *atpA485*	3, *gyd160*	4, *purK115*	5, *pstS87*	6, *atpA314*	7, *atpA188*	8, *purk217*		
1	A	A	C	C	C	T	T	C	AACCCTTC	New[a]
1	A	G	A	C	C	C	T	C	AGACCCTC	New
2	A	G	A	T	C	T	C	C	AGATCTCC	New
1	A	G	A	T	C	T	T	C	AGATCTTC	New
1	A	G	C	C	T	T	T	C	AGCCTTTC	New
3	A	G	C	T	C	T	C	C	AGCTCTCC	ST260, ST262, ST273, ST322
1	A	G	T	C	T	T	T	C	AGTCTTTC	ST60, ST61, ST74, ST75, ST76, ST85, ST94, ST96, ST152, ST178, ST213, ST218, ST225, ST289, ST329, ST334, ST346, ST352, ST356, ST361
2	G	A	C	T	C	T	T	C	GACTCTTC	ST227, ST230, ST313, ST316
5	G	A	T	T	C	T	T	C	GATTCTTC	ST78, ST145, ST201, ST203, ST204, ST249, ST283, ST287, ST288, ST304, ST323, ST339, ST341, ST365, ST393, ST414
1	G	G	C	C	C	C	C	C	GGCCCCCC	New
2	G	G	C	C	C	T	C	C	GGCCCTCC	ST162
6	G	G	C	T	C	C	C	C	GGCTCCCC	ST267, ST349
2	G	G	C	T	C	T	C	C	GGCTCTCC	ST18, ST125, ST132, ST173, ST186, ST275, ST276, ST282, ST302, ST305, ST319, ST336, ST340, ST344, ST351, ST368, ST380, ST388, ST391, ST409
2	G	G	T	T	C	C	C	C	GGTTCCCC	ST16, ST17, ST31, ST63, ST65, ST103, ST168, ST174, ST180, ST187, ST206, ST208, ST209, ST233, ST234, ST252, ST280, ST290, ST294, ST295, ST300, ST306, ST307, ST308, ST360, ST371, ST389, ST390, ST415
ΔC_T value (mean ± SD[b]) of:										
A	7 ± 0.57	13.07 ± 0.65	14.14	NA[c]	NA	NA	NA	NA		
C	NA	NA	19.5 ± 0.35	7.78 ± 0.07	5.49 ± 0.33	4.24 ± 0.59	15.84 ± 0.53	3.04 ± 0.14		
G	10.78 ± 0.44	14.14 ± 0.25	NA	NA	NA	NA	NA	NA		
T	NA	NA	18.69 ± 0.18	7.72 ± 0.35	12.68 ± 0.17	5.02 ± 0.57	9.51 ± 1.1	1.58 ± 0.29		

[a] New, STs not found in MLST database.
[b] From pooled results for each polymorphism.
[c] NA, not applicable.

STs, together with the *in vitro* SNP profiling of ST 2, ST 6, and ST 9 strains (obtained from the University of Texas) (Table 2), revealed that CC9 and CC2 can be subdivided by using the SNP method. For these CCs, SNP typing is able to further discriminate between STs in the same clonal complex, indicating that it is ideally suited to further discriminate very closely related STs. The 91 *E. faecium* STs were grouped into seven SNP profiles. Based on MLST typing, a distinct high-risk enterococcal clonal complex, CC17, can be differentiated. This CC is associated with the majority of hospital outbreaks and clinical infections on five continents (6, 13). Recently, genetic population studies have shown that the majority of vancomycin-resistant *E. faecium* strains associated with nosocomial infections worldwide are part of the same CC17. The eight high-

E. faecalis



E. faecium

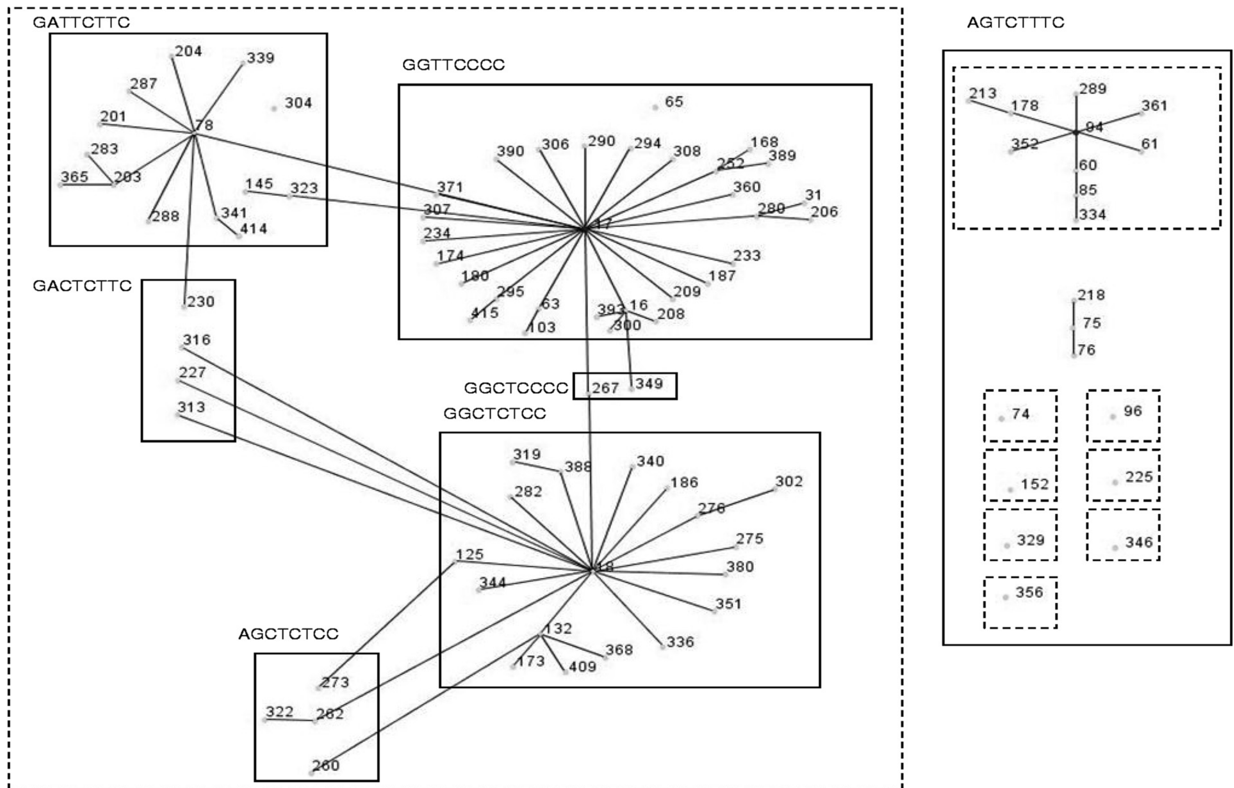


FIG. 1. An eBURST population snapshot of 51 *E. faecalis* STs grouped into 18 SNP profiles and 27 *E. faecium* STs grouped into 7 SNP profiles. The dotted-line boxes represent clonal complexes as defined by the *E. faecalis* and *E. faecium* MLST databases; the solid-line boxes represent STs grouped according to their corresponding 8-nucleotide high-*D*-value SNP profiles. Single local variants are connected by solid lines.

D-value SNPs were able to further differentiate this major CC17 into 6 SNP profiles. The SNP profile with the most STs (29 in total) had the GGTTCCCC profile. This subdivision of CC17 can be useful in investigating the association of these STs with specific disease profiles, something that MLST is unable to perform, as all these STs are grouped into the same clonal complex by MLST.

The Simpson's index of diversity (*D* value) was calculated for both *E. faecalis* and *E. faecium* to determine the comparative discriminatory powers of MLST and SNP typing. An important finding was that there was little difference in resolving power between MLST and SNP typing either for *E. faecalis* isolates (MLST $D = 0.97$ and SNP $D = 0.96$) or for *E. faecium* isolates (MLST $D = 0.96$ and SNP $D = 0.91$). This finding clearly demonstrates that the high discriminatory power of the SNP genotyping method is as good as that of MLST.

In conclusion, we have developed a novel and widely applicable approach for the typing of *E. faecalis* and *E. faecium* isolates that has a high discriminatory power and can be applied to the investigation of nosocomial enterococcal outbreaks. SNP typing subdivided isolates of clonal complexes 2 and 9 of *E. faecalis* and 17 of *E. faecium*, members of which are known to be the major causative agents of nosocomial infections globally. This method represents an efficient means of classifying *E. faecalis* and *E. faecium* isolates into groups that are concordant with the population structure of these organisms. These SNPs can be used on their own or combined with other rapidly evolving markers, such as virulence genes and antibiotic resistance genes, to yield highly informative genotyping methods.

We acknowledge Narelle George (Pathology Queensland) and Sue Gill (Queensland University of Technology) for kindly providing the *E. faecalis* and *E. faecium* isolates. We also acknowledge Barbara Murray and Karen Jacques-Palaz (Health Science Center at Houston, University of Texas) for supplying *E. faecalis* ST 2, 6, and 9 strains.

Irani U. Rathnayake is in receipt of an International Postgraduate Research Scholarship (IPRS), and the study is supported by the Institute of Sustainable Resources, QUT.

REFERENCES

1. **Coque, T. M., P. Seetulsingh, K. V. Singh, and B. E. Murray.** 1998. Application of molecular techniques to the study of nosocomial infections caused by enterococci, p. 469–493. *In* N. Woodford and A. Johnson (ed.), Methods in molecular medicine, vol. 15. Molecular bacteriology: protocols and clinical applications. Humana Press, Totowa, NJ.
2. **Coque, T. M., R. Willems, R. Canton, R. Del Campo, and F. Baquero.** 2002. High occurrence of *esp* among ampicillin-resistant and vancomycin-susceptible *Enterococcus faecium* clones from hospitalized patients. J. Antimicrob. Chemother. **50:**1035–1038.
3. **Hiraga, N., T. Muratani, S. Naito, and T. Matsumoto.** 2008. Genetic analysis of faropenem-resistant *Enterococcus faecalis* in urinary isolates. J. Antibiot. **61:**213–221.
4. **Homan, W. L., D. Tribe, S. Poznanski, M. Li, G. Hogg, E. Spalburg, J. D. Van Embden, and R. J. Willems.** 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. J. Clin. Microbiol. **40:**1963–1971.
5. **Huygens, F., J. Inman-Bamber, G. R. Nimmo, W. Munckhof, J. Schooneveldt, B. Harrison, J. A. McMahon, and P. M. Giffard.** 2006. *Staphylococcus aureus* genotyping using novel real-time PCR formats. J. Clin. Microbiol. **44:**3712–3719.
6. **Leavis, H. L., M. J. Bonten, and R. J. Willems.** 2006. Identification of high-risk enterococcal clonal complexes: global dispersion and antibiotic resistance. Curr. Opin. Microbiol. **9:**454–460.
7. **Leavis, H. L., R. J. Willems, J. Top, E. Spalburg, E. M. Mascini, A. C. Fluit, A. Hoepelman, A. J. de Neeling, and M. J. Bonten.** 2003. Epidemic and nonepidemic multidrug-resistant *Enterococcus faecium*. Emerg. Infect. Dis. **9:**1108–1115.
8. **Maiden, M. C. J., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140–3145.
9. **Ogier, J.-C., and P. Serror.** 2008. Safety assessment of dairy microorganisms: the *Enterococcus* genus. Int. J. Food Microbiol. **126:**291–301.
10. **Persing, D. H., F. C. Tenover, J. Versalovic, Y.-W. Tang, E. R. Unger, D. A. Relman, and T. J. White (ed.).** 2004. Molecular microbiology: diagnostic principles and practice. ASM Press, Washington, DC.
11. **Price, E. P., V. Thiruvenkataswamy, L. Mickan, L. Unicomb, R. E. Rios, F. Huygens, and P. M. Giffard.** 2006. Genotyping of *Campylobacter jejuni* using seven single-nucleotide polymorphisms in combination with *flaA* short variable region sequencing. J. Med. Microbiol. **55:**1061–1070.
12. **Robertson, G. A., V. Thiruvenkataswamy, H. Shilling, E. P. Price, F. Huygens, F. A. Henskens, and P. M. Giffard.** 2004. Identification and interrogation of highly informative single nucleotide polymorphism sets defined by bacterial multilocus sequence typing databases. J. Med. Microbiol. **53:**35–45.
13. **Top, J., R. Willems, and M. Bonten.** 2008. Emergence of CC17 *Enterococcus faecium*: from commensal to hospital-adapted pathogen. FEMS Immunol. Med. Microbiol. **52:**297–308.
14. **Valdezate, S., C. Labayru, A. Navarro, M. A. Mantecon, M. Orteqa, T. M. Coque, M. Garcia, and J. A. Saez-Nieto.** 2009. Large clonal outbreak of multidrug-resistant CC17 ST17 Enterococcus faecium containing Tn5382 in a Spanish hospital. J. Antimicrob. Chemother. **63:**17–20.
15. **Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau.** 1995. AFLP: a new concept for DNA fingerprinting. Nucleic Acids Research. **23:**4407–4414.
16. **Willems, R. J., J. Top, M. van Santen, D. A. Robinson, T. M. Coque, F. Baquero, H. Grundmann, and M. J. Bonten.** 2005. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. Emerg. Infect. Dis. **11:**821–828.
17. **Woodford, N., L. Tysall, C. Auckland, M. W. Stockdale, A. J. Lawson, R. A. Walker, and D. M. Livermore.** 2002. Detection of oxazolidinone-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains by real-time PCR and PCR-restriction fragment length polymorphism analysis. J. Clin. Microbiol. **40:**4298–4300.
18. **Zdragas, A., P. Partheniou, C. Kotzamanidis, L. Psoni, O. Koutita, E. Moraitou, N. Tzanetakis, and M. Yiangou.** 2008. Molecular characterisation of low-level vancomycin-resistant enterococci found in coastal water of Thermaikos Gulf, Northern Greece. Water Res. **42:**1274–1280.
19. **Zhu, X., B. Zheng, S. Wang, R. J. L. Willems, F. Xue, X. Cao, Y. Li, S. Bo, and J. Liu.** 2009. Molecular characterisation of outbreak-related strains of vancomycin-resistant *E. faecium* from an intensive care unit in Beijing, China. J. Hosp. Infect. **72:**147–154.