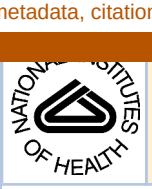

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Analysis of Clonality and Antibiotic Resistance among Early Clinical Isolates of *Enterococcus faecium* in the United States

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Abstract

Background—The *Enterococcus faecium* genogroup, referred to as clonal complex 17 (CC17), seems to possess multiple determinants that increase its ability to survive and cause disease in nosocomial environments.

Methods—Using 53 clinical and geographically diverse US *E. faecium* isolates dating from 1971 to 1994 we determined the multi-locus sequence type, the presence of 16 putative virulence genes (hyl_{Efm}, esp_{Efm} and *fms* genes), resistance to ampicillin (AMP^R), vancomycin (VAN^R) and high-levels of gentamicin and streptomycin.

Results—Overall, 16 different sequence types (STs), mostly CC17 isolates, were identified in 9 different regions of the US. The earliest CC17 isolates were part of an outbreak in 1982 in Richmond, VA. Characteristics of CC17 isolates included increases in AMP^R, the presence of hyl_{Efm} and esp_{Efm}, emergence of VAN^R and the presence of at least 13/14 *fms* genes. Eight out of forty-one of the early AMP^R isolates, however, were not within CC17.

Conclusions—While not all early US AMP^R isolates were clonally related, *E. faecium* CC17 isolates have been circulating in the US since at least 1982 and appear to have progressively acquired additional virulence and antibiotic resistance determinants, perhaps explaining the recent success of this species in the hospital environment.

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Keywords

E. faecium; Clonal Complex 17; multi-locus sequence typing; ampicillin resistance; vancomycin resistance; hyl_{Efm}; esp_{Efm}; *fms*

INTRODUCTION

Enterococci are commensals of the digestive tract and are now among the leading causes of nosocomial infections in the United States [1]. Among enterococcal species, *Enterococcus faecalis* and *Enterococcus faecium* are the most frequently encountered [2]. Over the past 2 decades, outbreaks of nosocomial E. faecium infection have become particularly problematic, increasing to 3.5% of all healthcare-associated infections [3]. The most recent data from the National Healthcare Safety Network at the Centers for Disease Control and Prevention reported that E. faecium accounted for approximately one-third of all nosocomial enterococcal related infections when organisms were identified to the species level[3].

Hospital outbreaks in five continents and the worldwide emergence of multi-resistant *E. faecium* have been attributed to a particular genetic lineage designated clonal complex 17 (CC17) [4]. However, a recent publication from Turner et al. [5] suggests that clustering of multi-locus sequence typing-based allelic profiles using eBURST is unreliable for the ancestral prediction of species with relatively high recombination rates. Recent efforts by the Willems and Carrico groups (personal communication) indicate that this is the case for *E. faecium* by using novel algorithms such as fBURST (presently being developed by the group of João Carriço at the Institute for Microbiology, Lisbon University, Portugal), which split CC17 into smaller clusters. Nevertheless, certain STs (ST16, ST17, ST18, and ST19) appear to be prominent among the hospital- associated genogroup that has been designated clonal complex 17 (CC17), which we refer to as the CC17 genogroup in this study. CC17 genogroup isolates are characterized by ampicillin resistance, frequent resistance to vancomycin, and higher rates than non-CC17 genotypes of virulence determinants such as hyl_{Efm} (encoding a hyaluronidase-like protein) [6] and a putative pathogenicity island which includes esp_{Efm} (**e**nterococcal **s**urface **p**rotein in *E. faecium*) [7,8]. The recently identified *fms* genes (*E. **f**aeciu**m** **s**urface proteins), also enriched in CC17, are factors encoding MSCRAMM (microbial surface components recognizing adhesive matrix molecules)-like proteins harboring the typical domains which include a secretion signal sequence, unique regions with Ig-like folds, and a C-terminal cell-wall anchoring domain with an LPXTG-like motif [9,10]. Such surface exposed proteins could contribute to virulence by providing a selective advantage in the hospital setting by conferring, for example, attachment to host tissues and/or environmental surfaces and possibly by increasing the colonizing ability of strains harboring them [9–11]. In addition, *fms9* (designated $ebpC_{fm}$ in [10]) and *fms21* (designated *pilA* in [12]) have been shown to encode proteins necessary for pilus formation. The ebp_{fm} encoded proteins are highly similar to the *ebp* proteins encoding pili in *E. faecalis*, which have been previously associated with pathogenesis in experimental endocarditis [13].

Extensive research has been done on the emergence of ampicillin and vancomycin resistance and the origins of the CC17 genogroup, mainly in Europe [4,14,15]. Although outbreaks of ampicillin-resistant *E. faecium* were first reported in the early 1980's in the U.S. [16–18], clonality and population genetics of early U.S. isolates exhibiting ampicillin or vancomycin resistance have not been characterized. In this study, we determined the clonality of early ampicillin and vancomycin resistant *E. faecium* in a US based set of isolates collected between 1971 and 1994. Using these isolates, we analyzed the presence of putative virulence genes in relation to their clonality and the emergence of ampicillin and vancomycin resistance.

MATERIALS AND METHODS

Bacterial isolates and species-specific PCR

A total of 53 *E. faecium* isolates were selected from our collection of over 450 *E. faecium* clinical (approximately 75%) and non-clinical isolates (including community, fecal, and animal isolates) collected between 1970 to the present from diverse origins and geographic locations with over 15 countries represented. To confirm the identity of the isolates, species specific amplification of the D-alanyl-D-alanine ligase gene (*ddl*) was used [19] in addition to initial biochemical tests. The isolates were selected based on a set of pre-determined criteria which included: i) the earliest US *E. faecium* isolates in our collection; ii) isolates recovered from clinical samples with the majority representing nosocomial outbreaks [16–18,20–22]; and iii) diversity in geographical origin with nine different areas in the US represented (Table 1).

Genomic DNA isolation, multi-locus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE)

Genomic DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Inc., Valencia, Calif.) from 5 ml overnight cultures in brain heart infusion broth (Difco Laboratories, Detroit, Mich.). PCR and MLST was performed as described previously [23]. Fragments of 7 housekeeping genes (*atpA*, *ddl*, *gdh*, *purk*, *gyd*, *pstS*, and *adk*) were sequenced, allelic profiles were obtained, and a ST was designated for each unique allelic profile based on the MLST website (http://efaecium.mlst.net). For select isolates that had an identical pulsotype to those of typed strains recovered from the same area or in the same outbreak, only *purK* was sequenced and an inferred sequence type was given. Pulsed-field gel electrophoresis was performed as described previously [26].

Susceptibility testing

Ampicillin and vancomycin susceptibilities and high-level resistance to gentamicin and streptomycin were determined using standard agar dilution methods [24]. In accordance Clinical and Laboratory Standards Institute (CLSI) guidelines, minimal inhibitory concentrations (MICs) of ≥16 μg/ml for ampicillin and ≥ 32 μg/ml for vancomycin were considered resistant. Isolates with growth on plates with concentrations of 500 μg/ml of gentamicin and 2000 μg/ml of streptomycin were considered to have high-level resistance.

Colony hybridization and *van* gene PCR

Preparation of colony lysates on nylon membranes and hybridization under high-stringency conditions were performed as described previously [25]. DNA probes for hyl_{Efm}, esp_{Efm}, and the 14 *fms* genes were made using primers previously published [6,11] and found in Supplementary Table 1. Of note, all isolates of this study had the *acm* gene (*fms8*) encoding a collagen-binding adhesin consistent with our previous finding of its presence in the vast majority of clinical isolates [26]. These probes were radiolabelled using the RadPrime DNA labeling system (Invitrogen, Carlsbad, Calif.). Vancomycin resistant isolates were tested for the presence of *vanA* and *vanB* genes using the oligodeoxynucleotides and conditions previously described [19].

Statistical analysis

The difference between CC17 and non-CC17 isolates in their distributions of the *fms* genes, hyl_{Efm}, esp_{Efm}, ampicillin resistance, and vancomycin resistance were compared using two-tailed Fisher's exact test. *P*-values of <0.05 were considered statistically significant.

RESULTS

While not a prospective collection, the isolates used in this study represent most of the published *E. faecium* outbreaks up to 1994 and early reports of ampicillin resistant and vancomycin resistant strains in the US. In chronological order, the earliest isolates available to us were from the 1970s and were recovered at the Massachusetts General Hospital in Boston during a 22-year study at this institution [22]. The 1970's isolates were both ampicillin and vancomycin susceptible and MLST analysis revealed that they did not belong to the CC17 genogroup. Instead they were ST296, ST25, or *purK* allele 8 (NEDH 4901 and NEDH 4586 have an identical pulsotype; data not shown). Colony hybridization analysis showed that these isolates did not contain the putative virulence determinants hyl_{Efm} or esp_{Efm} and harbored only eight to nine of the fourteen *fms* genes (Supplementary Table 2). High-level resistance to streptomycin was displayed in two of these isolates.

Two published studies reported ampicillin resistant *E. faecium* infections between the years of 1981–1987. We obtained seven isolates from one study that occurred at the Rancho Los Amigos Medical Center in Downey, California between January 1981 and September 1987 [18,27]. This was one of the first reports of an increased presence of ampicillin resistance (MICs 16–32 μg/ml). We found high-level resistance to streptomycin in some of the isolates but none had high-level resistance to gentamicin and all were vancomycin susceptible. Six of the isolates were ST92, ST476 or contained *purK* allele 2 (RLA-5 and RLA-7 had an identical pulsotype while RLA-1, 2, and 3 had an identical pulsotype; data not shown and [27]), which indicated that they were not related to the CC17 genogroup. The hyl_{Efm} and esp_{Efm} genes were not present and the *fms* gene profile showed these isolates were missing the predicted pilus operon *fms11-19-16*, putative accessory pilin *fms20*, and putative adhesin *fms18*. One isolate was ST280 (within the CC17 genogroup) and contained all 14 of the *fms* genes; the exact year (between 1981–87) this organism was isolated is unknown.

We also found that CC17 genogroup isolates from the second study during this time frame were responsible for an *E. faecium* outbreak in a neonatal intensive care unit in 1982 at the Medical College of Virginia in Richmond [17,27]. This outbreak was the first report describing bacteremia and meningitis due to multi-resistant *E. faecium*. Seven isolates were studied from this outbreak and we found that all belonged to ST17 by MLST or harbored the *purK* allele 1 (MCV161 and 130 had an identical pulsotype while MCV264, 211, and 255 had an identical pulsotype; data not shown and [27]). This outbreak was different from the California outbreak in that ampicillin MICs were slightly higher, ranging from 32 to 64 μg/ml and this is the first time that *E. faecium* with high-level resistance to gentamicin was observed in our study, and to our knowledge, in the US. These early isolates contained all of the *fms* genes; only one isolate from this outbreak had the esp_{Efm} gene and none had the hyl_{Efm} gene.

We examined ten isolates recovered in four different states between the years 1986 and 1988. Two isolates were from two different hospitals in Boston, Massachuchetts [20,22], one from a nosocomial outbreak of *E. faecium* at Miriam Hospital in Providence, Rhode Island [28], three from Charlotte Memorial Hospital in North Carolina [29], and four from the University of Wisconsin at Madison clinics [30]. One isolate from the Children's Hospital Medical Center in Boston, MA had been obtained because of its reported high-level resistance to gentamicin [20]. This isolate was found to be ST112 and therefore did not belong to CC17; it was isolated in 1986, had an intermediate ampicillin MIC of 8 μg/ml and lacked the ebp_{fm} operon *fms1–5–9* but had the esp_{Efm} gene. Another Boston isolate, recovered in 1988 at New England Deaconess Hospital, had a new ST, ST473, with *purK* allele 8, was ampicillin resistant (MIC 32 μg/ml) and lacked the putative adhesins *scm* (*fms 10*), *fms15* and *fms18*. Four of the ten isolates in this time period were within the CC17 genogroup, specifically ST18, ST19, or *purK* allele 1 (M-1634 and W-27358 had an identical pulsotype; data not shown). The others

were ST10, ST25 or a new ST, ST474. Four isolates from locations other than Boston were ampicillin resistant (three within the CC17 genogroup and one not) with MICs between 16 and 64 μg/ml. The majority of the ten isolates showed high-level resistance to streptomycin (seven out of ten), but only one to gentamicin. All four CC17 genogroup isolates contained all *fms* genes, three contained the esp_{Efm} gene and two contained hyl_{Efm}. The two isolates that contained the hyl_{Efm} gene were isolated in 1986; this is the first appearance of the hyl_{Efm} gene in this study.

In addition to the ten isolates described above, six isolates from the Medical College of Pennsylvania in Philadelphia isolated in the 1988 were also studied [16]. These isolates were from a surveillance study showing an increase in penicillin resistance and an inability to treat some of these isolates with combination gentamicin/penicillin therapy. We found that all six isolates were within the CC17 genogroup with ST16, ST17, or *purK* allele 1 (FA295 and FA287 had an identical pulsotype while FA232 and FA191 had a similar pulsotype; data not shown and [27]) and had all 14 *fms* genes. They were all ampicillin resistant and vancomycin susceptible with ampicillin MICs ranging between 32 and 128 μg/ml. High-level resistance to streptomycin was present in five of the isolates while high-level resistance to gentamicin was found in three of the isolates during this time period. Five of the isolates contained the esp_{Efm} gene but none contained the hyl_{Efm} gene.

We also had available isolates from two published outbreaks occurring during the years 1990–1991 [21,22]: four isolates were obtained from Boston (two from New England Deaconess Hospital and two from the 22-year review study at Massachusetts General Hospital) [22] and eight isolates were obtained from a multi-resistant *E. faecium* nosocomial outbreak at Beth Israel Hospital in New York [21]. This was the first time we documented vancomycin resistant isolates in this study. Isolates from both outbreaks were all representatives of the CC17 genogroup, specifically ST18, ST16, ST17, or with the *purK* allele 1 (SH-4, 5 and 10 had identical pulsotypes while SH-6, 9, 11, 13 and 14 had similar pulsotypes; data not shown and [21]). The majority of isolates were ampicillin resistant with even higher MICs than the outbreaks in the late 1980's, ranging from 32–256 μg/ml. The isolates from Boston were vancomycin susceptible while the isolates from New York exhibited vancomycin resistance conferred by the *vanA* gene with MICs between 256–512 μg/ml. Both outbreaks had isolates with high-level resistance to both gentamicin and streptomycin. Interestingly, all isolates in both outbreaks contained hyl_{Efm} and esp_{Efm}, highlighting a marked increase in the presence of these two genes. Furthermore, most contained all 14 *fms* genes except that none of the New York isolates had the putative accessory pilus encoding gene, *fms20*.

Lastly, we also studied eight isolates recovered in Houston, Texas at two different hospitals between 1992 and 1994 [31]. Five isolates were within the hospital-associated CC17 genogroup with ST20, ST17, and *purK* allele 1 represented (VREH-1 and 4 had similar pulsed-field types; data not shown). These isolates exhibited ampicillin resistance with MICs between 32–128 μg/ml and two exhibited *vanA*-type of vancomycin resistance (MIC 512μg/ml). The majority of isolates exhibited high-level resistance to streptomycin (5 out of 8), but only one to gentamicin. The Texas CC17 genogroup isolates had the majority of *fms* genes, except for *fms20* and *fms18*. The hyl_{efm} and esp_{efm} genes were detected in only one of these isolates. We also identified a new CC17 genogroup related ST, ST475, in this group of isolates represented by isolate TX1399, a single locus variant of ST17 in the *ddl* allele. This isolate was susceptible to both ampicillin and vancomycin but had the same *fms*, hyl_{Efm} and esp_{Efm} gene profile as the other Texas CC17 isolates. Two of the strains isolated were not within the CC17 genogroup (ST71 and *purK* allele 8). Both were ampicillin and vancomycin susceptible and one showed high-level resistance to streptomycin; these two isolates lacked the hyl_{Efm} and esp_{Efm} genes and contained only seven or eight of the fourteen *fms* genes.

DISCUSSION

In this study, MLST was performed on clinical isolates to determine the clonality of early US ampicillin resistant isolates and the earliest hospital-associated CC17 genogroup isolate in our collection. For the isolates in this study, eight out of the forty-one ampicillin resistant isolates were not CC17 genogroup related isolates, showing that not all ampicillin resistant isolates were clonally related. However, the presence of ampicillin resistance in CC17 isolates versus non-CC17 isolates was statistically significant ($P = 0.0007$). While the earliest CC17 genogroup isolate may have been recovered in 1981 in California, ST17 isolates were clearly present in U.S. hospitals by 1982. This predates the earliest published CC17 genogroup isolate, which was recovered in Great Britain, by more than seven years and predates the previously earliest known US CC17 genogroup isolate (DO/ TX0016) by nine years (http://efaecium.mlst.net). The earliest CC17 genogroup isolates recovered in this study were ampicillin resistant and most exhibited high-level resistance to streptomycin as well. Interestingly, all of the earliest CC17 genogroup isolates contained all fourteen *fms* genes but the majority lacked esp_{Efm} and hyl_{Efm} genes.

Although *E. faecium* has been shown to be relatively intrinsically resistant to β-lactam antibiotics [32] leading to sporadic reports of such isolates as early as 1965 [15], reports of infections caused by highly ampicillin resistant *E. faecium* did not occur until the 1980's [16–18,28,33]. The fact that ampicillin resistance existed in the earliest CC17 genogroup isolates is consistent with the hypothesis that ampicillin resistance may have been one of the first steps of hospital-adaptation by the hospital-associated CC17 genogroup [4]. Only two of the CC17 genogroup isolates in this study were ampicillin susceptible. Within the CC17 genogroup isolates, ampicillin MICs progressively increased over time, a finding that is consistent with the fact that events such as further mutations or overexpression of the *pbp5* gene might have evolved in these isolates, further decreasing the affinity for ampicillin. Evidence suggests that particular substitutions present in combination amplify levels of resistance [34,35]. Additionally, there is some evidence that indicates that emergence of high-level ampicillin resistance among US *E. faecium* strains is due, at least in part, to the transfer of low-affinity *pbp5* between clinical isolates, a situation that we cannot rule out in this study [36]. Our data confirm that ampicillin resistance preceded the emergence of vancomycin resistance within the CC17 genogroup in the United States, as has been shown in European studies [15,37]. The earliest known vancomycin resistant *E. faecium* strain was isolated in Europe in 1986 [38,39]. Among our isolates, vancomycin resistance ($P = 0.044$ for CC17 versus non-CC17) first appeared among CC17 genogroup isolates in 1990 by acquiring the *vanA* gene cluster. Curiously, vancomycin resistance conferred by *vanB* in US *E. faecium* was not reported until around 1992 but numerous cases/outbreaks of *E. faecium* expressing the *vanB* type were described in the US thereafter; at least some of which are known to be CC17 [40–43].

We also found that all the isolates within the CC17 genogroup contained a similar *fms* gene profile with all isolates containing at least thirteen of the fourteen *fms* genes (with either *fms20* or *fms18* missing), a result that is clearly different from non-CC17 isolates where only five to nine *fms* genes were identified (*P*-values ranging from 0.001 to 0.029 for all *fms* genes except *fms20*) (Figure 3 and Supplementary Table 2). These data correspond with reports from Hendrickx et al. and Sillanpaa et al. demonstrating the enrichment of putative cell-wall anchored protein genes in clinical and outbreak-associated *E. faecium* isolates [9,11]. Indeed, the earliest CC17 genogroup isolates in the study contained all 14 *fms* genes.

Previous studies have indicated that esp_{Efm} is an important genetic marker for the previously designated *E. faecium* CC17 [8,44]. Our findings show that esp_{Efm}, whose presence in CC17 isolates versus non-CC17 isolates was statistically significant ($P = 0.0002$), was present in one of the earliest ST17 isolates (TX2038 isolated in 1982), however, it was not present in most

of the early published outbreak isolates. This finding suggests that esp_{Efm}, present in a putative pathogenicity island, was likely acquired at different times during this genogroup's evolution and that the *fms* genes are more likely to be early markers of the CC17 genogroup in the USA.

Interestingly, it has recently been shown that hyl_{Efm} is on a transferable plasmid which increases both the virulence and colonizing abilities of CC17 genogroup clinical isolates [45], (C.A.A. and B.E.M., unpublished data). This plasmid also appears to carry antibiotic resistance genes in certain CC17 genogroup clinical isolates, such as gentamicin and vancomycin resistance encoded by the *aac(6')-aph(2")* and *vanA* genes (C.A.A. and B.E.M., unpublished data). Indeed, in this study, almost all of the vancomycin-resistant strains also exhibited high-level resistance to gentamicin and contained the hyl_{Efm} gene. Our findings indicate that the presence of hyl_{Efm} ($P = 0.001$ for CC17 versus non-CC17) increased around the late 1980's in the evolution of the CC17 genogroup. Therefore, it is tempting to speculate that ancestral hospital-associated genogroup isolates in the USA carrying the *fms* genes, subsequently acquired esp_{Efm} and/or the transferable hyl_{Efm}-containing plasmid (which may or may not contain vancomycin and gentamicin resistance determinants) and these series of events may help explain the recent success of this organism as a nosocomial pathogen.

In summary, we demonstrated that hospital-associated isolates within the CC17 genogroup have been circulating in the United States as early as 1982. Our data indicate that even though most of the earliest ampicillin resistant isolates were part of the CC17 genogroup, ampicillin resistance seemed to be acquired independently of CC17. This is indicated by the fact that not all CC17 isolates are ampicillin resistant (two isolates), and not all ampicillin resistant isolates were within the CC17 genogroup (eight isolates). Furthermore, all vancomycin resistant isolates in this study were a part of the CC17 genogroup. Our data suggest that *fms* genes (putative pili or adhesins) were more associated with early CC17 genogroup-related STs than other putative virulence genes and, thus, the *fms* gene profile may be a more accurate marker of a CC17 genogroup isolate than the presence of ampicillin resistance, esp_{Efm}, or hyl_{Efm}. These data support the concept that hospital-adapted clones may not have evolved recently from a single founder, but that different hospital-adapted clones (STs) have evolved independently and become successful by acquiring antibiotic resistances and potential virulence factors such as the *fms* genes, esp_{Efm}, and hyl_{Efm}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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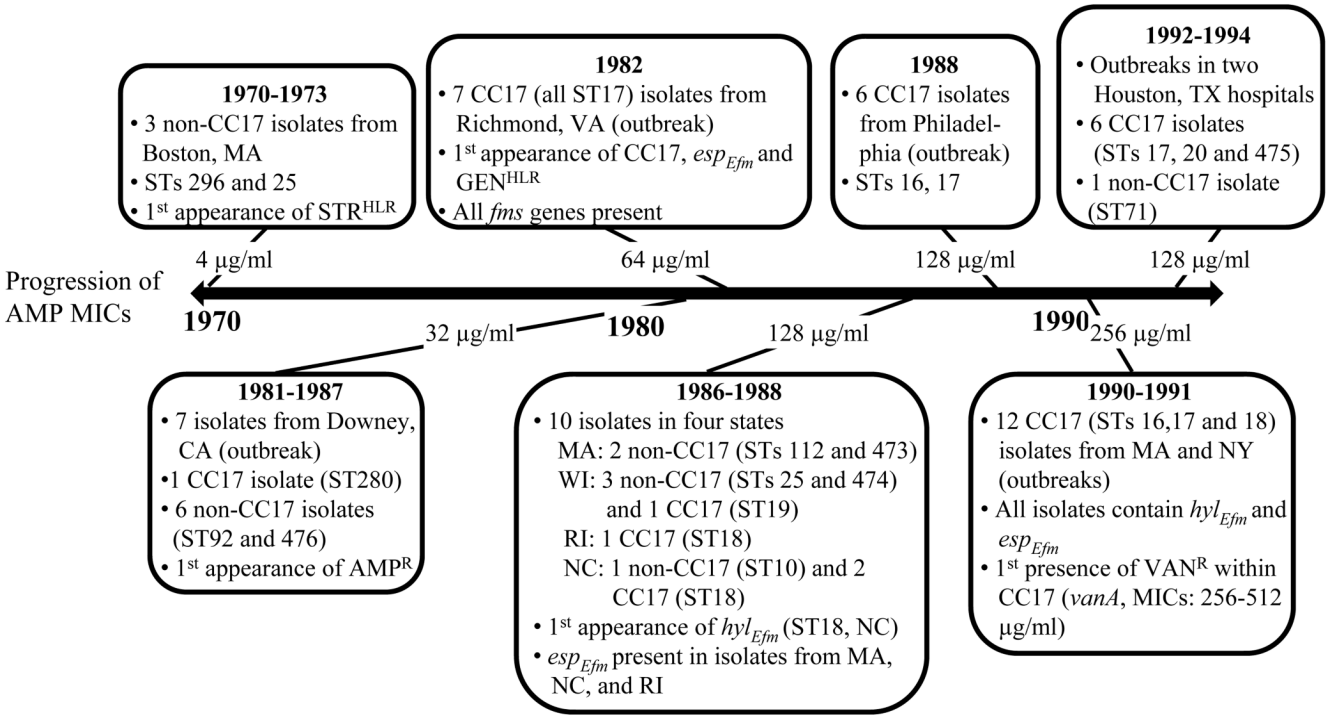

Figure 1.

Key events that occurred in the isolates of this study. The MICs listed correspond to the highest ampicillin MIC from each outbreak. The following abbreviations are used in this figure: high-level resistance to streptomycin (STR^{HLR}), high-level resistance to gentamicin (GEN^{HLR}), ampicillin resistance (AMP^{R}) and vancomycin resistance (VAN^{R})

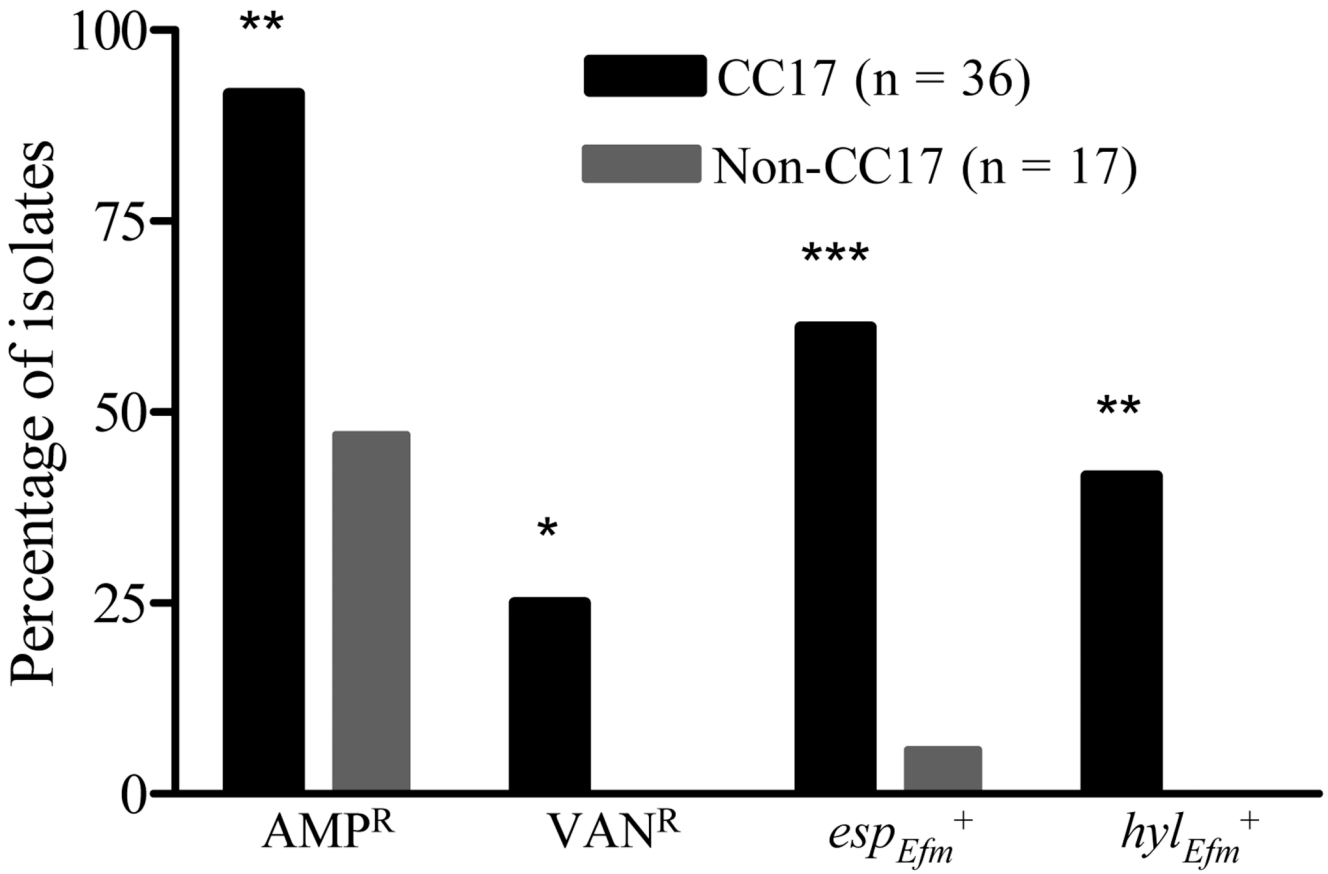

Figure 2.

A comparison between CC17 isolates and non-CC17 isolates for the presence of ampicillin resistance, vancomycin resistance, the esp_{Efm} gene, and the hyl_{Efm} gene. * $P < 0.05$ against non-CC17 isolates in our study. ** $P < 0.005$ against non-CC17 isolates in our study. *** $P < 0.0005$ against non-CC17 isolates in our study

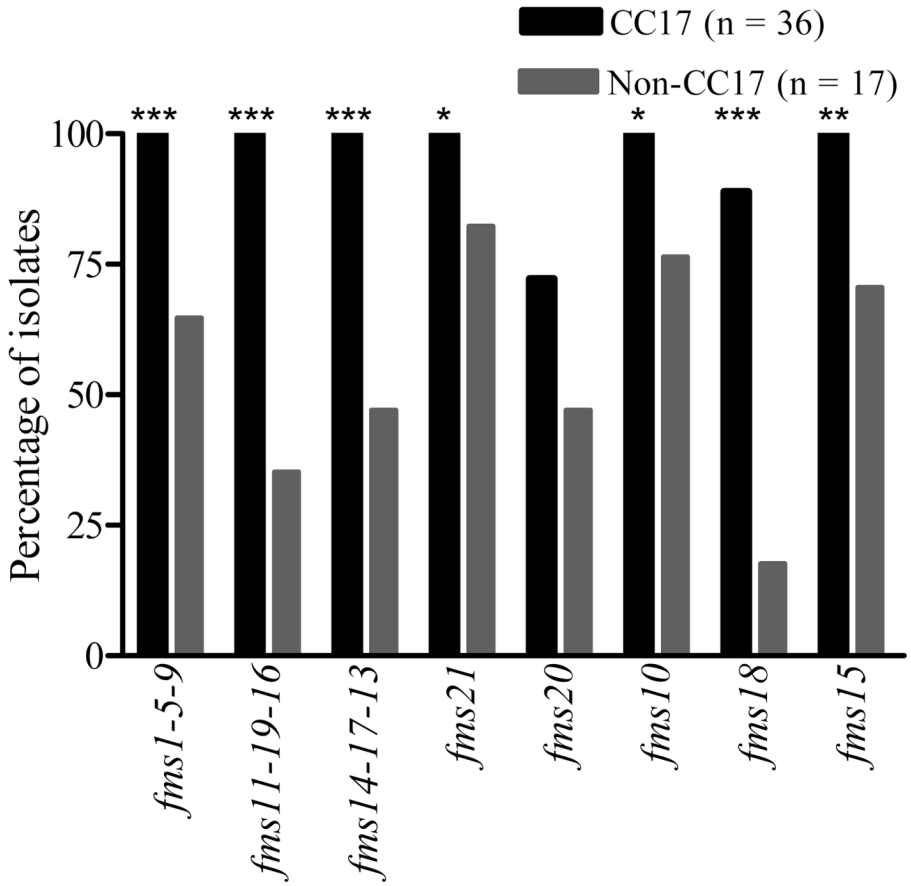

Figure 3.

A comparison between CC17 and non-CC17 isolates for the presence of the putative adhesins and pili (*fms*) genes. *fms1-5-9* is the ebp_{fm} operon while *fms11-19-16* and *fms 14–17–13* are putative pilus operons. *fms21* has been shown to encode pili while *fms20* is considered its accessory protein. *fms10*, also known as *scm*, is a collagen binding adhesin while *fms18* and *fms15* are thought to encode putative adhesins. * $P < 0.05$ against non-CC17 isolates in our study. ** $P < 0.005$ against non-CC17 isolates in our study. *** $P < 0.0005$ against non-CC17 isolates in our study

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TABLE 1

Relevant background and characteristics of the *E. faecium* isolates included in this study.

Isolate Name(s)	Location	Year	Antibiotic resistance(s)[a]	ST[b,c,d,e]	*purK*	References
NEDH 4901/TX2050	Boston, MA	1971	AMP^S, VAN^S, STR^{HLR}	296	8	[22,27]
NEDH 4586/TX2051	Boston, MA	1971	AMP^S, VAN^S	(296)	8	[22,27]
NEDH 758/TX2052	Boston, MA	1973	AMP^S, VAN^S, STR^{HLR}	25	6	[22,27]
RLA-1/TX2058	Downey, CA	1981–87	AMP^R, VAN^S, STR^{HLR}	476[e]	2	[18,27]
RLA-2/TX2059	Downey, CA	1981–87	AMP^R, VAN^S, STR^{HLR}	(476)[e]	2	[18,27]
RLA-3/TX2060	Downey, CA	1981–87	AMP^R, VAN^S, STR^{HLR}	(476)[e]	2	[18,27]
RLA-4/TX2061	Downey, CA	1981–87	AMP^R, VAN^S, STR^{HLR}	92	2	[18,27]
RLA-5/TX2062	Downey, CA	1981–87	AMP^R, VAN^S	92	2	[18,27]
RLA-7/TX2064	Downey, CA	1981–87	AMP^R, VAN^S	(92)	2	[18,27]
RLA-6/TX2063	Downey, CA	1981–87	AMP^R, VAN^S, STR^{HLR}	280[c]	1	[18,27]
MCV130/TX2029	Richmond, VA	1982	AMP^R, VAN^S	17[c]	1	[17,27]
MCV161/TX2031	Richmond, VA	1982	AMP^R, VAN^S	(17)[c]	1	[17,27]
MCV211/TX2033	Richmond, VA	1982	AMP^R, VAN^S, STR^{HLR}	17[c]	1	[17,27]
MCV255/TX2035	Richmond, VA	1982	AMP^R, VAN^S	(17)[c]	1	[17,27]
MCV/264/TX2036	Richmond, VA	1982	AMP^R, VAN^S, STR^{HLR}	(17)[c]	1	[17,27]
MCV266/TX2034	Richmond, VA	1982	AMP^R, VAN^S, STR^{HLR}	17[c]	1	[17,27]
MCV268/TX2038	Richmond, VA	1982	AMP^R,VAN^S, GEN^{HLR}, STR^{HLR}	17[c]	1	[17,27]
NEDH-87-P1/TX2053	Boston, MA	1986	AMP^S, VAN^S, GEN^{HLR}, STR^{HLR}	112	18	[20,27]
W-5586/TX2008	Charlotte, NC	1986	AMP^R, VAN^S, STR^{HLR}	10	6	[29]
W-27358/TX2016	Charlotte, NC	1986	AMP^R, VAN^S	18[c]	1	[29]
M-1634/TX2000	Charlotte, NC	1986	AMP^R, VAN^S, STR^{HLR}	(18)[c]	1	[29]
UWHC-9802/TX2041	Madison, WI	1987–88	AMP^S, VAN^S, STR^{HLR}	25	6	[27,30]
UWHC-4827/TX2042	Madison, WI	1987–88	AMP^S, VAN^S, STR^{HLR}	19[c]	1	[27,30]
UWHC-112/TX2043	Madison, WI	1987–88	AMP^S, VAN^S, STR^{HLR}	25	6	[27,30]
UWHC-2145/TX2046	Madison, WI	1987–88	AMP^S, VAN^S	474[e]	4	[27,30]
NEDH SF-76/TX2054	Boston, MA	1988	AMP^R, VAN^S	473[e]	8	[22,27]
875-D/TX2048	Providence, RI	1988	AMP^R, VAN^S, STR^{HLR}	18[c]	1	[28]
FA287/TX2024	Philadelphia, PA	1988	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	16[c]	1	[16,27]
FA295/TX2023	Philadelphia, PA	1988	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	(16)[c]	1	[16,27]
FA280/TX2025	Philadelphia, PA	1988	AMP^R, VAN^S, STR^{HLR}	16[c]	1	[16,27]
FA191/TX2027	Philadelphia, PA	1988	AMP^R, VAN^S, STR^{HLR}	17[c]	1	[16,27]
FA232/TX2022	Philadelphia, PA	1988	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	(17)[c]	1	[16,27]
FA185/TX2026	Philadelphia, PA	1988	AMP^R, VAN^S	17[c]	1	[16,27]
A358/TX2067	Boston, MA	1990	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	17[c]	1	[46]
A490/TX2069	Boston, MA	1990	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	17[c]	1	This study
A360/TX2068	Boston, MA	1990	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	17[c]	1	This study
A491/TX2070	Boston, MA	1990	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	17[c]	1	This study
SH-4/TX2429	New York, NY	1990–91	AMP^R, VAN^S, GEN^{HLR}, STR^{HLR}	18[c]	1	[21]
SH-5/TX2430	New York, NY	1990–91	AMP^R, VAN^R	(18)[c]	1	[21]
SH-10/TX2435	New York, NY	1990–91	AMP^R, VAN^R, GEN^{HLR}, STR^{HLR}	(18)[c]	1	[21]
SH-11/TX2436	New York, NY	1990–91	AMP^R, VAN^R, GEN^{HLR}, STR^{HLR}	16[c]	1	[21]
SH-6/TX2431	New York, NY	1990–91	AMP^S, VAN^R, GEN^{HLR}, STR^{HLR}	(16)[c]	1	[21]
SH-9/TX2434	New York, NY	1990–91	AMP^R, VAN^R, GEN^{HLR}, STR^{HLR}	(16)[c]	1	[21]
SH-13/TX2438	New York, NY	1990–91	AMP^R, VAN^R, STR^{HLR}	(16)[c]	1	[21]
SH-14/TX2439	New York, NY	1990–91	AMP^R, VAN^R, GEN^{HLR}, STR^{HLR}	(16)[c]	1	[21]
VREH-1/TX2416	Houston,TX	1992	AMP^R, VAN^R, GEN^{HLR}, STR^{HLR}	20[c]	1	[47]
VREH-4/TX2419	Houston, TX	1992	AMP^R, VAN^R	(20)[c]	1	This study

Isolate Name(s)	Location	Year	Antibiotic resistance(s)[a]	ST[b,c,d,e]	*purK*	References
VREH-5/TX2420	Houston, TX	1992	AMP^R, VAN^S, STR^{HLR}	17[c]	1	This study
SEH1a/TX1361	Houston, TX	1993–94	AMP^S, VAN^S, STR^{HLR}	71	2	[31]
SEH7/TX1371	Houston, TX	1993–94	AMP^R, VAN^S, STR^{HLR}	17[c]	1	[31]
SEH33/TX1399	Houston, TX	1993–94	AMP^S, VAN^S	475[c,e]	1	[31]
SEH41/TX1405	Houston, TX	1993–94	AMP^R, VAN^S, STR^{HLR}	17[c]	1	[31]
SEH36/TX1401	Houston, TX	1993–94	AMP^S, VAN^S	ND[d]	8	[31]

[a] Superscript “R” denotes resistance, “S” denotes susceptible, and “HLR” denotes high-level resistance

[b] ST numbers in parenthesis denote the inferred sequence type based on having the same *purK* allele and an identical pulsotype to a typed isolate

[c] ST numbers that are considered to be within the CC17 genogroup

[d] ND denotes not done

[e] New STs designated in this study