Identification of a Novel Clone, ST736, among *Enterococcus faecium* Clinical Isolates and Its Association with Daptomycin Nonsusceptibility

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Resistance to daptomycin in enterococcal clinical isolates remains rare but is being increasingly reported in the United States and worldwide. There are limited data on the genetic relatedness and microbiological and clinical characteristics of daptomycin-nonsusceptible enterococcal clinical isolates. In this study, we assessed the population genetics of daptomycin-nonsusceptible *Enterococcus faecium* (DNSE) clinical isolates by multilocus sequence typing (MLST) and whole-genome sequencing analysis. Forty-two nonduplicate DNSE isolates and 43 randomly selected daptomycin-susceptible *E. faecium* isolates were included in the analysis. All *E. faecium* isolates were recovered from patients at a tertiary care medical center in suburban New York City from May 2009 through December 2013. The daptomycin MICs of the DNSE isolates ranged from 6 to >256 μg/ml. Three major clones of *E. faecium* (ST18, ST412, and ST736) were identified among these clinical isolates by MLST and whole-genome sequence-based analysis. A newly recognized clone, ST736, was seen in 32 of 42 (76.2%) DNSE isolates and in only 14 of 43 (32.6%) daptomycin-susceptible *E. faecium* isolates (*P* < 0.0001). This report provides evidence of the association between *E. faecium* clone ST736 and daptomycin nonsusceptibility. The identification and potential spread of this novel *E. faecium* clone and its association with daptomycin nonsusceptibility constitute a challenge for patient management and infection control at our medical center.

Daptomycin is a cyclic lipopeptide with *in vitro* bactericidal activity against Gram-positive bacteria. Daptomycin is approved by the U.S. Food and Drug Administration (FDA) for the treatment of complicated skin and soft-tissue infections, bacteremia, and right-sided infective endocarditis caused by certain Gram-positive microorganisms (1–3). Given its potent bactericidal activity against enterococci, low risk of serious side effects, and minimal drug-drug interactions, daptomycin is increasingly used in the United States and other countries to treat serious staphylococcal and enterococcal infections. Daptomycin is also used "off label" as the antibiotic of choice for serious infections caused by vancomycin-resistant enterococci (VRE) (1, 4–6), despite the high cost of the drug and some toxicity issues such as myopathy and eosinophilic pneumonia (7).

In vitro antimicrobial susceptibility testing of Gram-positive microorganisms worldwide suggests that resistance to daptomycin in *Enterococcus* spp. is rare (8–12). Daptomycin-nonsusceptible enterococci, however, are emerging as a cause of health careassociated infection (4, 6) and may be fairly common; they comprised 3.9% of 2,029 *Enterococcus faecium* isolates from U.S. hospitals over the period 2007 to 2010 (13) and 15.2% among VRE blood isolates at a New York cancer center (14). In addition to some earlier case reports (reviewed in reference 4), a notable increase in the number of patients infected or colonized with daptomycin-nonsusceptible enterococci has been reported from different geographic regions in the United States (14–19).

The majority of daptomycin-nonsusceptible enterococci documented in the literature are vancomycin-resistant *E. faecium*, although vancomycin-resistant *E. faecalis* and vancomycin-susceptible *E. faecium* have been reported (4, 6). Most patients develop resistance during daptomycin therapy (18, 20, 21). *De novo*

resistance is also reported for some patients without prior use of daptomycin (16, 22). The results of a recent case-control study suggested that immunosuppression, the presence of comorbid conditions, and prior exposure to antimicrobials are independent predictors of infections caused by daptomycin-nonsusceptible enterococci (17). The mechanism of daptomycin resistance in enterococci, especially in *E. faecium*, remains to be fully elucidated. Based on whole-genome analysis of a very few clinical and laboratory-derived strains, mutations in several genes have been implicated in the development of daptomycin resistance in enterococci (15, 23–26). The emergence and spread of daptomycin-nonsusceptible enterococci in distinct patient populations or geographic regions are likely caused by a diverse population of enterococci (14, 18). Such studies are largely restricted due to the lack of sufficient daptomycin-nonsusceptible clinical isolates. In this study, we determined the genetic relatedness and clinical and microbiological characteristics of daptomycin-nonsusceptible *E. faecium* (DNSE) isolates from patients in a tertiary care medical center in suburban New York City. A novel *E. faecium* clone asso-

ciated with daptomycin nonsusceptibility was identified and characterized.

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MATERIALS AND METHODS

Bacterial isolates. Forty-two nonduplicate, daptomycin-nonsusceptible *E. faecium* (DNSE) clinical isolates were included in this study. These DNSE isolates were recovered from patients of a tertiary medical center in suburban New York City from May 2009 through December 2013. The Institutional Review Board of New York Medical College approved this study. For patients with multiple DNSE isolates, only the first confirmed isolate was selected. In this report, DNSE was defined as an *E. faecium* isolate with a daptomycin MIC of >4 μg/ml determined by a MicroScan WalkAway system (Siemens, Tarrytown, NY) and confirmed by Etest (bioMérieux, Durham, NC) and/or the reference broth microdilution method per the Clinical Laboratory and Standards Institute (CLSI) guidelines (27). Forty-three nonduplicate daptomycin-susceptible *E. faecium* (DSE) isolates from the same study period were randomly selected and analyzed for comparison. All DNSE and DSE clinical isolates were identified on the basis of colony morphology and conventional biochemical tests and were confirmed by sequence analysis of 16S rRNA genes. Antimicrobial susceptibility of enterococcal isolates to daptomycin and other antimicrobial agents was determined routinely using MicroScan dried Gram-positive panels by the Prompt inoculation method with a MicroScan WalkAway system. Confirmatory Etest was performed for DNSE isolates reported by MicroScan (i) if the isolates were recovered from blood or other sterile body sites, (ii) if the isolates were recovered from patients in the intensive care units and oncology wards, or (iii) if the test was requested by clinicians. Eleven DNSE isolates confirmed by Etest in 2009 and 2010 were also sent to Laboratory Specialists (Westlake, OH) and validated by the CLSI reference broth microdilution method (27).

DNA preparation. Bacterial isolates were subcultured on blood agar or Trypticase soy broth from cryovials stored at −80°C or from refrigerated nutrient slants. DNA from pure bacterial culture was extracted by using a protocol for isolation of genomic DNA from Gram-positive bacteria with a QIAamp Mini DNA kit (Qiagen, Germantown, MD) per instructions of the manufacturer. DNA concentration was measured by using a Qubit fluorometric quantitation system (Life Technologies, Grand Island, NY).

Whole-genome sequencing (WGS). DNA libraries from clinical isolates were prepared by using a Nextera XT sample preparation kit according to instructions of the manufacturer (Illumina, San Diego, CA). WGS was performed on an Illumina MiSeq system by using paired-end methods (2 × 250 bp) with either 24 or 96 indices incorporated. Taken as a whole, the data set had a mean fold coverage of 59× and a median fold coverage of 53× for the chromosomal DNA.

MLST analysis. Multilocus sequence typing (MLST) data were obtained using both classic Sanger DNA sequencing and WGS methods. For classic MLST analysis, 7 loci (*atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS*, and *adk*) of each *E. faecium* isolate were amplified using PCR primers and conditions as described by Homan et al. (28). PCR amplicons were sequenced using a BigDye Terminator v1.1 cycle sequencing kit (Life Technology, Foster City, CA) on an ABI 3500xl Genetic Analyzer.

For WGS-based MLST analysis, WGS sequence data were aligned to *E. faecium* sequence type 17 (ST17) reference strain Aus0004 (NC_017022.1) (29) using the BWA alignment software (30, 31). Consensus base calls for the aligned nucleotides were derived using SAMtools (32). MLST profiles for *E. faecium* were downloaded from an online MLST database (http://efaecium.mlst.net). The consensus genome sequences generated from the alignment were then analyzed using a locally configured version of the BIGSdb framework to call MLST types (33).

Phylogenetic analysis. Single nucleotide variations (SNVs) were called from the BWA alignment to *E. faecium* Aus0004 chromosome 1

using varscan. Phylogenetic trees were obtained using a maximum-likelihood method (34). Trees were visualized and annotated using the Interactive Tree Of Life online tool (35). The genetic relatedness of different enterococcal isolates and STs was explored using the goeBURST program (36).

Statistical analysis. The Fisher's exact test from the Prism software (version 5.0) was used to determine the statistical significance of the results of comparisons of different groups of enterococci with distinct clinical and microbiological characteristics.

RESULTS

Microbiological and clinical characteristics of DNSE isolates. From May 2009 through December 2013, 42 nonduplicate *E. faecium* clinical isolates were confirmed as DNSE by Etest and/or reference broth microdilution. The 42 DNSE isolates were recovered from urine (*n* = 17, 40.5%), blood (*n* = 13, 30.9%), surgical wounds (*n* = 7, 16.7%), and peritoneal fluid (*n* = 5, 11.9%). Thirty-five of 42 (83.3%) isolates were from patients with distinct signs and symptoms suggestive of infection associated with these enterococcal isolates, including 13 patients with bacteremia. The average age of the patients was 56.5 (range, 23 to 84) years. Twenty-two (52.4%) of these patients were immunocompromised due to underlying malignancy and/or ongoing immunosuppressive therapies. Twenty-four of 42 (57.1%) patients had had exposure to treatment with daptomycin within the past 12 months prior to the DNSE isolation. It is noteworthy that 17 of 21 (81.0%) patients with DNSE colonization or infection from 2009 to 2012 had a prior exposure to daptomycin, in contrast to only 7 of 21 (33.3%) patients in 2013 (*P* = 0.0044). The microbiological characteristics of 42 DNSE isolates and clinical features of patients are summarized in Table 1.

Sequence types of DNSE and DSE isolates. MLST data were derived from WGS of 85 *E. faecium* (42 DNSE and 43 DSE) isolates. Of these, the MLST sequence types of 25 *E. faecium* isolates were also determined using Sanger DNA sequencing. Identical alleles and STs were confirmed for all but one isolate, for which the Sanger DNA sequencing and the WGS method called different *ddl* alleles, and the ST determined by the Sanger DNA sequencing was used in the final data analysis.

For the 42 DNSE isolates, six different STs were observed (Table 2). Surprisingly, 32 of 42 (76.2%) DNSE isolates possessed a novel sequence type, ST736. Of the remaining DNSE isolates, ST18 (*n* = 4) and ST412 (*n* = 3) were observed in 7 of 10 non-ST736 DNSE isolates, while three other isolates each represented a distinct ST (ST282, ST750, and ST893).

To determine if *E. faecium* ST736 is the predominant clone that is endemic among patients at our study site, we also sequenced the whole genomes of 43 additional DSE clinical isolates randomly selected from the same study period. The MLST data from these DSE isolates are summarized in Table 2. Three major *E. faecium* clones (ST18, ST412, and ST736) were identified among these DSE isolates. While ST736 was recognized as the predominant clone in DNSE isolates, it was seen in only 14 of 43 (32.6%) of the daptomycin-susceptible *E. faecium* clinical isolates. Twenty-nine of 43 (67.4%) DSE isolates were ST18 (*n* = 14), ST412 (*n* = 9), and other non-ST736 (*n* = 6) (DNSE versus DSE, *P* < 0.0001). This demonstrates that the ST736 is variably distributed among DNSE and DSE isolates and is not the sole dominant enterococcal clone at our study site.

Antimicrobial susceptibility of DNSE isolates. As shown in Table 1, the daptomycin MIC of 42 DNSE isolates ranged from 6

TABLE 1 Clinical and microbiological characteristics of DNSE isolates[a]

Isolate no.	Year	Isolate designation	ST	VAN MIC[b] (µg/ml)	DAP MIC[c] (µg/ml)	Source	Underlying disease(s) or condition(s)	Age (yrs)	Sex	No. of days prior to DNSE isolation[d]	Prior DAP exposure
1	2009	E39	ST736	>16	96	Blood	ALL	28	F	79	Y
2	2009	E49	ST750	≤2	16	Blood	Cholangiocarcinoma	72	F	29	Y
3	2009	E7	ST18	1	32	Urine	Liver transplant, abdominal abscess	63	F	83	Y
4	2009	E8	ST282	>16	16	Urine	AML	45	M	52	Y
5	2010	E13	ST736	1	16	Blood	ALL	72	M	17	Y
6	2010	E14	ST736	>16	8	Blood	ALL	46	F	24	Y
7	2010	E17	ST736	>16	16	Wound	Hepatitis C, DM	58	M	42	Y
8	2010	E20	ST893	2	6	Urine	Hepatitis B, lymphoma	58	M	43	Y
9	2010	E34	ST736	>16	48	Urine	Multiple myeloma, DM	54	M	9	Y
10	2010	E50	ST412	>16	64	Blood	Heart transplant	65	F	53	Y
11	2010	E53	ST736	>16	24	Peritoneum	Trauma, bowel perforation	48	M	38	Y
12	2010	E78	ST18	>16	6	Peritoneum	Fulminant hepatic failure, liver transplant	66	M	15	N
13	2011	E91	ST736	>16	64	Blood	ALL, HSCT, GVHD	24	M	41	N
14	2011	E146	ST736	>16	32	Urine	Cholangitis/peritonitis	69	F	88	Y
15	2011	E165	ST736	>16	6	Urine	Hepatitis C, DM, ovarian carcinoma	58	F	22	N
16	2011	E176	ST736	>16	12	Blood	Chronic osteomyelitis, decubitus ulcer, paraplegia	54	M	238	Y
17	2012	E166	ST736	2	8	Urine	Cirrhosis, enterocutaneous fistula	56	M	26	Y
18	2012	E168	ST736	>16	32	Urine	Scrotal/testicular cancer, spina bifida	39	M	75	Y
19	2012	E169	ST736	>16	8	Blood	Endocarditis, necrotizing fasciitis	61	F	262	Y
20	2012	E173	ST736	>16	32	Wound	Burn, osteomyelitis	23	M	162	Y
21	2012	E203	ST736	>16	8	Blood	ALL	41	F	11	N
22	2013	E207	ST736	>16	12	Urine	Cirrhosis, autoimmune hepatitis	62	F	13	N
23	2013	E208	ST736	>16	6	Peritoneum	Cirrhosis, ESRD	66	F	5	N
24	2013	E209	ST736	>16	8	Urine	AML, DM	61	M	0	N
25	2013	E211	ST412	>16	12	Urine	AML, colon cancer	38	M	86	Y
26	2013	E214	ST736	>16	8	Blood	Hepatitis C, liver/kidney transplant	56	M	5	N
27	2013	E218	ST736	>16	8	Urine	CABG, MVR, ureteral stent	84	F	19	N
28	2013	E222	ST736	>16	8	Urine	Lymphoma, HSCT	48	M	1	N
29	2013	E225	ST736	>16	6	Blood	Cirrhosis, ESRD, liver transplant	69	F	139	Y
30	2013	E226	ST736	>16	6	Peritoneum	Liver transplant	71	M	5	N
31	2013	E231	ST736	>16	6	Wound	Necrotizing fasciitis	41	M	40	Y
32	2013	E236	ST736	>16	6	Wound	s/p LVAD, osteomyelitis	69	M	16	N
33	2013	E237	ST736	>16	8	Blood	Lymphoma	66	M	12	N
34	2013	E241	ST18	>16	12	Urine	Multiple myeloma	56	F	36	N
35	2013	E242	ST736	>16	8	Wound	Necrotizing fasciitis	52	M	32	Y
36	2013	E243	ST736	>16	>256	Peritoneum	Endometrial carcinoma	56	F	21	Y
37	2013	E245	ST18	>16	12	Urine	Ovarian cancer, AML	66	F	18	N
38	2013	E249	ST736	>16	8	Urine	Vulvar cancer, wound infection	61	F	34	N
49	2013	E251	ST736	>16	6	Urine	Trauma, bowel perforation	75	F	29	N
40	2013	E252	ST412	>16	8	Blood	AML	56	F	30	Y
41	2013	E253	ST736	>16	>256	Wound	ESRD, prosthetic knee infection	74	F	83	Y
42	2013	E261	ST736	>16	8	Urine	SLE, kidney transplant, DM	49	F	11	N

[a] n = 42. ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; HSCT, hematopoietic stem cell transplant; DM, diabetes mellitus; ESRD, end-stage renal disease; GVHD, graft versus host disease; CNS, central nervous system; SLE, systemic lupus erythematosus; CABG, coronary artery bypass graft; MVR, mitral valve repair; s/p LVAD, status post-left ventricular assist device; CHF, congestive heart failure; F, female; M, male; Y, yes; N, no.
[b] Vancomycin (VAN) MICs were determined using MicroScan Gram-positive MIC panels.
[c] Daptomycin (DAP) MICs were determined by Etest. DNSE for isolates 1 to 11 was also confirmed by CLSI reference broth microdilution.
[d] Duration of hospital stay prior to DNSE isolation.

TABLE 2 Distribution of sequence types among DNSE and daptomycin-susceptible *E. faecium* clinical isolates determined by multilocus sequence typing analysis

ST	No. (%) of isolates		*P* value
	DNSE	DSE	
ST736	32 (76.2)	14 (32.6)	<0.0001
ST18	4 (9.5)	14 (32.6)	0.0155
ST412	3 (7.1)	9 (20.9)	0.1171
ST282	1 (2.4)	1 (2.3)	
ST750	1 (2.4)	0	
ST117	0	1 (2.3)	
ST584	0	1 (2.3)	
New[a]	1 (2.4)	3 (7.0)	
Total	42 (100)	43 (100)	

[a] The four new STs first described in this study have been assigned the designations ST893 (isolate E20), ST894 (isolate E179), ST895 (isolate E238), and ST896 (isolate E293).

to >256 μg/ml, with a geometric mean MIC of 14 μg/ml. Notably, 10 of 42 (23.8%) DNSE isolates had a high-level daptomycin MIC (> = 32 μg/ml). Two of these isolates (E243 and E253, both belonging to ST736) were resistant to daptomycin, with an *in vitro* MIC of >256 μg/ml. All 42 DNSE isolates examined were resistant to ampicillin as typically seen in North American *E. faecium* isolates (9). Thirty-seven (88.1%) DNSE isolates were resistant and 5 (11.9%) DNSE isolates were susceptible to vancomycin. Forty of 42 (95.2%) DNSE isolates were susceptible to linezolid and quinupristin-dalfopristin. Similar susceptibility profiles were seen among DNSE and DSE isolates for almost all antibiotics. The antimicrobial susceptibility profiles of DNSE and DSE isolates are summarized in Table S1 in the supplemental material.

Population genetics of DNSE and DSE isolates. Based on MLST data, 76 of 85 (89.4%) *E. faecium* isolates analyzed belonged to three major STs (ST18, ST412, and ST736). Of the remaining 9 *E. faecium* isolates, 5 isolates each possessed a distinct ST, while 4 isolates had new STs (assigned as ST893 to ST896). To further determine the population genetics of these *E. faecium* clinical isolates, a phylogenetic tree based on the SNVs of WGS data, compared to the reference genome from isolate Aus0004, was generated. Collectively, the 85 isolates analyzed had a mean SNV call rate of 1.96 SNVs/kb on the reference genome. As shown in Fig. 1, three major clusters, corresponding to ST18, ST412, and ST736, were observed for the 83 *E. faecium* clinical isolates included in the analysis. DNSE isolate E20 and DSE isolate E179 (not shown in Fig. 1) showed a larger genetic distance from the reference isolate
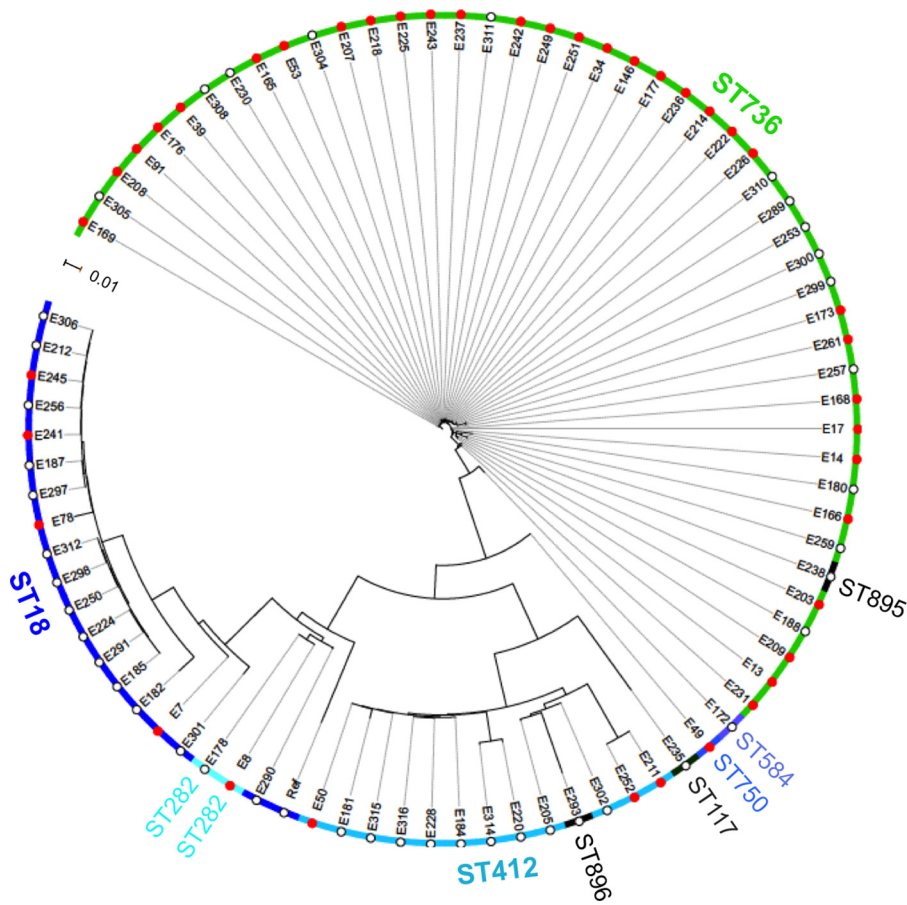
FIG 1 Whole-genome-based phylogenetic tree of daptomycin-nonsusceptible (red solid circles; *n* = 42) and daptomycin-susceptible (white empty circles; *n* = 43) *E. faecium* clinical isolates. The phylogenetic tree was generated using a maximum-likelihood method based on single nucleotide variations of genomes compared to a reference strain, Aus0004 (29). Two outlier isolates (E20 and E179) were excluded from the phylogenetic tree for improved visualization of the genetic distance between different subgroups of *E. faecium*.

as well as from other *E. faecium* clinical isolates and may represent two unique *E. faecium* clones. Although bearing a new ST, DSE isolates E238 and E293 showed high genetic similarity and clustered in the ST736 and ST412 groups, respectively.

Genetic relatedness of DNSE with VRE isolates. To explore the genetic relatedness of *E. faecium* isolates from this study to other VRE clones reported worldwide, comparative clustering analysis was performed using the goeBURST algorithm. Data from ~500 different STs of *E. faecium* in clonal complex 17 (CC17) that are currently available in the MLST database were included in the analysis. Interestingly, the two major STs (ST18 and ST412) seen in DSE isolates have been widely reported in other geographic areas of North America and South America (37–39). *E. faecium* clone ST736 that was seen in 76.2% of DNSE and 32.6% of DSE isolates appears to represent a novel emerging group within CC17. *E. faecium* ST736 is most closely related to ST280, showing a single allele variation in 1 of the 7 alleles (*purK*). Two allelic differences (*ddl* and *purK*) between ST736 and ST17, the presumed founder of CC17 enterococci, were noticed (see Fig. S1 in the supplemental material).

DISCUSSION

Vancomycin-resistant enterococci belonging to CC17 have been emerging globally since the 1990s and are now in the predominant group of enterococci causing nosocomial infections (5, 40). Our findings in 42 DNSE clinical isolates from patients of a tertiary medical center in suburban New York City, together with those of several recent studies from other U.S. institutions (14–19), demonstrate the emergence and spread of DNSE in the U.S. health care setting. However, there are only limited data available on the population genetics of DNSE isolates. It is also unknown if an association exists between daptomycin nonsusceptibility and a particular genetic group or clone of enterococci.

In this report, we describe the distribution of various STs among *E. faecium* clinical isolates and their distinct associations with daptomycin susceptibility. A total of six STs have been identified from the 42 DNSE isolates. Strikingly, 32 of 42 (76.2%) DNSE isolates exhibited high genetic similarity by MLST and WGS analysis and were defined as a single novel *E. faecium* clone, ST736. In contrast, among daptomycin-susceptible *E. faecium* isolates, three major clones (ST18, ST412, and ST736) were recognized. *E. faecium* ST18 and ST412 isolates have been well documented in North America, including the United States, Canada, and Mexico (37–39). They are among the most common STs found in VRE isolates from Canada since 2006 (37). Similarly, *E. faecium* ST18 and ST412, together with other non-ST736 isolates, account for approximately two-thirds of the DSE isolates analyzed in this study, demonstrating that *E. faecium* clone ST736 is not the overall dominant endemic clone at our study site. Conversely, ST736 is the predominant clone among DNSE isolates and is therefore strongly associated with daptomycin nonsusceptibility.

Ten of 42 (23.8%) DNSE isolates in this study showed a high level (MIC ≥ 32 μg/ml) of daptomycin resistance. Two of these DNSE isolates exhibited a daptomycin MIC of >256 μg/ml. Both *de novo* resistance and development of resistance during daptomycin treatment have been reported. A possible community source of *de novo* DNSE has also been proposed (41, 42). It is unclear if this predominance of the ST736 clone, especially among DNSE clinical isolates in our institution, is attributable to nosocomial clonal spread or whether the ST736 clone carries unique genetic and microbiological characteristics that predispose it to daptomycin nonsusceptibility. Twelve of 17 (70.6%) patients infected with DNSE ST736 isolates in 2013 had no prior exposure to daptomycin. It is likely that nosocomial transmission and clonal spread of *E. faecium* ST736 isolates occurs among some patients, if not all, at this study site. Our finding is similar to data from a study at a New York City hospital (14) but is distinct from data from a recent study in Ohio in which a diverse population of DNSE was reported (i.e., 16 pulsed-field gel electrophoresis [PFGE] types and 24 subtypes among the 29 patient isolates available for molecular typing) (18). Due to the lack of sufficient strain typing data for comparison, we cannot conclude if the observed differences between the two studies are due to the dissimilarity in the subtypes of the infecting enterococci, their transmission modes among discrete patient populations, or mechanisms of development of resistance to daptomycin.

Daptomycin nonsusceptibility has been shown in other non-ST736 *E. faecium* clones as well, including ST18, ST412 in this study, and ST203 (referred from the published whole-genome sequence of confirmed DNSE isolate R446 [GenBank accession numbers AMAJ01000001 to AMAJ01000323] from Texas). The limitations of our study are that all of the DNSE isolates were from a single institution and that most isolates were selected from patients in the intensive care and oncology units. It will be meaningful and worthwhile to assess the clonality of DNSE isolates from other institutions and geographic regions and compare them to a larger collection of DSE isolates to better define the population genetics of daptomycin-nonsusceptible enterococcal infections.

In conclusion, this report provides evidence of the association between *E. faecium* clone ST736 and daptomycin nonsusceptibility. Our findings offer new insights on the population genetics of DNSE clinical isolates and a possible direction in which to explore the mechanisms of daptomycin resistance in enterococci. The identification of novel *E. faecium* clone ST736 and its predominance in DNSE isolates may imply a potential clonal outbreak of this organism in our institution. Recently, *E. faecium* ST736 isolates have been recovered from the cerebrospinal fluids of two patients in a U.S. hospital in Bethesda, MD (http://efaecium.mlst.net/; accessed 15 May 2014). This also raises concern on its further spread in U.S. hospital settings as well as the associated patient management and infection control challenges. Further studies will be undertaken to determine the genomic similarities and differences between different STs of *E. faecium* clones and their relative likelihood in developing resistance to daptomycin.

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