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Spread of Vancomycin-Resistant *Enterococcus faecium* Isolates Despite Validated Infection Control Measures in an Italian Hospital: Antibiotic Resistance and Genotypic Characterization of the Endemic Strain

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An alarming increase of vancomycin-resistant *Enterococcus faecium* (VREfm) isolates was detected in an Italian referral hospital subjected to policies of infection control validated by the Joint Commission International. Analysis of the population structure of 122 consecutive, nonreplicate VREfm isolates collected over an 18-month period identified a single major clone that spread around the whole hospital, rapidly establishing an endemic state. It belonged to sequence type (ST) 17 and showed a highly multidrug-resistant phenotype, being resistant to all antimicrobial classes for the carriage of several resistance determinants. Furthermore, some strains with decreased susceptibility to daptomycin were detected. Eighteen out of the 122 isolates did not group in the major clone. They showed a low spreading potential inside the hospital wards, even if most of them displayed a multidrug-resistant phenotype and belonged to a hospital-adapted lineage. Causes that led to the VREfm endemic state have not been fully elucidated. However, it is conceivable that the increase in systemic antibiotic consumption and the use of selective digestive tract decontamination, including vancomycin in critically ill patients during the period before 2014, may have played a role in the ST17 clone dissemination, but additional traits conferring high fitness in hospital environment cannot be excluded.

Keywords: resistant enterococci, endemic state, molecular typing, surveillance, antibiotic use

Introduction

Enterococci are commensal of the gastrointestinal tract that can cause healthcare-associated infections, including endocarditis, bloodstream infections, and urinary tract infections, particularly in immunocompromised patients. They have shown to be disseminated in hospitals both from colonized patients and healthcare workers, and can persist on surfaces and medical devices for long time.[1]

The most common *Enterococcus* species isolated from clinical samples, *Enterococcus faecalis* and *Enterococcus faecium*, are particularly worrisome since they are intrinsically resistant to different classes of antibiotics (*e.g.*, β-lactams, aminoglycosides) and can acquire resistance to other classes, including glycopeptides.

Since 1980s, vancomycin-resistant enterococci (VRE) have been increasingly reported worldwide in the hospital environment, mostly sustained by three major *E. faecium* (VREfm) lineages, originating from sequence types (STs) 17, 18, and 78. These lineages were originally grouped into a single clonal complex (CC17) but have been recently separated by a phylogenetic approach called Bayesian Analysis of Population Structure (BAPS),[2] highlighting a distinct evolutionary history for lineage 78, assigned to BAPS group 2-1, and lineages 17 and 18, both assigned to group 3-3.[3]

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Their successful spread in the hospital environment has probably been facilitated by the acquisition of mobile genetic elements and putative virulence determinants, such as *esp* gene, coding for an enterococcal surface protein promoting the initial attachment to biotic and abiotic surfaces, and hyl_{Efm} gene, coding for a glycosyl hydrolase that seems to facilitate intestinal colonization and peritoneal invasion.[4,5]

The rate of VREfm detection has always shown to be high in the United States, whereas great variability exists among different geographic areas, including European countries. In 2015, the European Antimicrobial Resistance Surveillance System (EARSS) reported rates of invasive VREfm ranging from <1% in the Baltic region to >40% in Ireland. Italy showed a low–medium rate (11%) of VREfm, with an increasing trend observed since 2012 (6%),[6] with a prevalence of lineage ST78, by far the most represented, followed by ST17 detected to a lesser extent.[7,8]

In the present study, we report a dramatic increase of VREfm detection in the Trieste University Hospital (Northeastern Italy). Since 2008, this hospital implemented the practices of infection control according to the Joint Commission International (JCI) accreditation. Despite such infection control practice, since March 2014, the increase in VREfm led to an endemic state, without a contemporary increase in detection of other multidrug-resistant organisms, such as extended spectrum beta-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae, which are endemic in most of the Italian hospitals.[9]

The object of this study was the phenotypic and genotypic characterization of the VREfm strains isolated in this hospital to define a tentative strategy for controlling this phenomenon.

Materials and Methods

Description of the endemic setting and isolates of the study

The Trieste University Hospital is an 840-bed hospital admitting nearly 24,000 adult patients each year and including various medical and surgical specialties. There are 2 intensive care units (ICUs), with a total of 36 beds, and no pediatric or transplant units.

It is noteworthy that since 1990s the ICUs in our hospital routinely practice selective digestive tract decontamination (SDD) in all patients with both expected ICU stay longer than 72 hours and mechanical ventilation longer than 48 hours. An enteral mixture of colistin sulfate, tobramycin, amphotericin B deoxycholate, and vancomycin (the latter antibiotic has been added more recently in patients colonized by methicillin resistant *staphylococcus aureus* [MRSA]), and a short-term parenteral cefotaxime are administered to these patients.

Regarding consumption of systemic antibiotics, the total consumption grew significantly from 80 defined daily doses (DDD) in 2008 to 97 DDD/100 patient-days in 2014.[9]

The presence of multidrug-resistant organisms in rectal swabs of patients admitted to the ICUs is checked weekly. A very small number of VRE (<10 isolates/year) were detected by such surveillance programs until 2013, but since May 2014, a rapid increase of VRE isolates was reported, leading to a collection of 122 consecutive nonreplicate VREfm over the next 18 months. Most of them were responsible for colonization but five isolates causing infection were collected from various body samples (namely blood and peritoneal fluid).

Microbial identification and antimicrobial susceptibility testing to vancomycin, teicoplanin, ampicillin, levofloxacin, linezolid and tigecycline were routinely performed using the VITEK2 automated system (bioMérieux). Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-approved breakpoints.[10]

For selected isolates, a more complete antibiotic resistance profile was achieved. The Kirby–Bauer disk diffusion test was performed, according to the CLSI guidelines,[11] to evaluate resistance to ampicillin (10 μg/disc), cefotaxime (30 μg/disc), ampicillin–sulbactam (10/10 μg/disc), amoxicillin–clavulanate (20/10 μg/disc), imipenem (10 μg/disc), penicillin (10 U), erythromycin (15 μg/disc), clindamycin (2 μg/disc), lincomycin (2 μg/disc), and tetracycline (30 μg/disc; (Oxoid, Thermo Scientific, Milan, Italy).

Confirmatory minimum inhibitory concentration (MIC) testing for teicoplanin (Aventis, West Malling, United Kingdom), vancomycin, quinupristin–dalfopristin, gentamicin, streptomycin (Sigma Chemical, St. Louis, MO), tigecycline, linezolid (Pfizer, Inc., New York, NY), and daptomycin (Novartis, Basel, Switzerland) was carried out by the broth microdilution method, following standard criteria.[11]

Clonal relatedness of the isolates

Genomic DNA, extracted from cells embedded in agarose plugs as previously described,[12] was digested with *Sma*I (Roche Molecular Biochemicals, Mannheim, Germany) and separated by pulsed-field gel electrophoresis (PFGE) performed in 0.5× TBE buffer in a CHEF DR III apparatus (Bio-Rad), with pulse times ranging from 2 to 25 seconds, 6 V/cm, and a 120° switch angle for 23 hours at 12°C.

DNA patterns were analyzed by the GelCompar II v. 6.6 software (Applied-Maths, Kortrijk, Belgium) using Dice coefficient for pairwise comparison. Strains with pattern similarity >85% were considered strictly related and grouped into clusters, indicated by capital letters (*e.g.*, A). Different patterns inside clusters were considered subtypes and indicated by numbers (*e.g.*, A1).

Representative isolates for different subtypes of each cluster were subjected to multilocus sequence typing (MLST) analysis.[13]

Screening of antibiotic resistance and virulence determinants

vanA, *vanB*, hyl_{Efm}, and *esp* genes were screened by dot blot hybridization[14] on the whole set of isolates. Digoxigenin-labeled probes were obtained by amplification of an internal fragment of each gene in the presence of 70 μM dig-11-dUTP (Roche Molecular Biochemicals).

Genomic DNAs from *E. faecium* strains GUC[15] and C68[16] were used as the template and as positive control. Genomic DNA of *E. faecium* GE-1 strain[16] was included as negative control.

Hybridization, carried out either at 68°C (*vanA*, *vanB*) or at 64°C (hyl_{Efm}, *esp*) on the basis of preliminary experiments, and hybrid detection were performed as recommended by the manufacturer.

On selected isolates, the presence of other resistance determinants [*ermB*, *tetM*, *aadE*, *aac-aphD*, *aph(2″)C*, *aphA3*]

was detected by polymerase chain reaction, as previously published.[17]

Plasmid profiling and localization of vanA, vanB, *and* hyl$_{Efm}$ *determinants*

DNA in agarose plugs was digested with 20 U of S1 nuclease (Sigma Chemical) for 20 minutes at 37°C and separated by PFGE for 22 hours at 12°C, with pulse times ranging from 2 to 35 seconds, 6 V/cm, and a 120° switch angle.

Direct in-gel hybridization[18] with dig-labeled probes was performed to identify plasmids harboring the determinants.

Results

Clonal relationships among isolates

The clonal structure of the 122 VREfm isolates was evaluated by macrorestriction analysis, which was performed for the whole set of isolates. The computer-assisted clustering based on the Dice similarity coefficient allowed identification of seven PFGE types, named A to G, formed at 85% similarity (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/mdr).

The large majority of the isolates, 104 out of 122 (85%), appeared strictly related and were included in the cluster A. Only 5 out of 104 isolates caused infection, which resulted to be deadly in three cases; the other 99 isolates caused just colonization. Cluster A consisted of 15 subtypes, corresponding to patterns that differed by 1–5 bands. Fifteen isolates, representatives of each different subtype, were analyzed by MLST and assigned to ST17, one of the major human lineages detected in the hospital environment although not the most frequent in the Italian hospitals until now.

A smaller group (11 isolates distributed into 6 subtypes) was named cluster B and 3 representative isolates were assigned to ST780. Remaining isolates were distributed into groups D to G, each formed by one or two strains. MLST analysis revealed that five of them belonged to ST117, one to ST552, and one to ST561. None of the latter isolates caused infection.

Characterization of phenotypic and genotypic antibiotic resistance profiles

Besides antimicrobial susceptibility testing performed by the VITEK2 automated system, additional analysis of the antibiotic susceptibility profile was performed on selected representatives of the different clusters. All of them showed a multidrug-resistant phenotype, due to their resistance to at least one agent in three or more antimicrobial classes (Table 1).

Unusually, eight VREfm strains, mainly belonging to cluster A (seven out of eight), showed daptomycin MIC values of 8 mg/L; these data need further analyses to identify the mechanism responsible for this decreased susceptibility.

Resistance determinants carried by selected isolates are shown in Table 2. Resistance to erythromycin was always associated with *ermB* gene. Tetracycline resistance was associated with *tetM* (105; 86%) and was detected to a lesser extent among the strains belonging to the VanB phenotype.

The major cluster A showed a highly multidrug-resistant phenotype, being resistant to all antimicrobial classes tested, and carrying all the resistance genes analyzed. High-level-resistance to streptomycin and gentamicin was mainly associated with the presence of *aadE*, *aphA3*, and/or *aac-aphD* genes, respectively; 35 strains carried *aadE* and *aphA3*; 1 strain carried *aadE* and *aac-aphD*. In a single isolate, we found *aph(2″)C* gene. In six isolates belonging to the other clusters, the presence of *aph*A3 did not correlate with gentamicin resistance.

Description of the clusters

Isolates of cluster A, mostly detected in ICUs at the beginning of the study, rapidly spread around the hospital and established an endemic state, being detected in patients of 18 different wards (Fig. 1) and generating a very alarming situation, even if mainly associated with colonization. All isolates of this cluster carried the *esp* gene, but only one resulted positive for *hyl*$_{Efm}$. They showed resistance to vancomycin (MIC >256 μg/ml) and teicoplanin (MIC values between 16 and >256 μg/ml; Table 1), due to the carriage of the *vanA* determinant. Besides, several other resistance determinants, included *tetM* and *aacA-aphD*, were detected (Table 2).

Compared with cluster A, cluster B showed different features. Nine out of the 11 isolates, divided into 4 strictly related subtypes (>94% similarity), were mostly detected in a single ward during a limited period of the study (Fig. 1). They displayed a VanB phenotype, almost all of them were resistant to gentamycin (high-level gentamycin resistance [HLGR]) but not to tetracycline (Table 1) and all carried both *esp* and *hyl*$_{Efm}$ genes (Table 2).

The remaining two isolates of the cluster were detected months later in a different ward (Fig. 1). Their PFGE patterns had a slightly lower similarity with isolates detected before (91%) but carried the *vanA* determinant. Both were positive for *esp* gene but only one carried *hyl*$_{Efm}$ (Table 2).

MLST analysis performed on three isolates (both of those carrying *vanA* and one representative of *vanB*) assigned them to ST780, an ST never described before as responsible for hospital spread (only one isolate belonging to this ST is described in the database of the *E. faecium* MLST website—http://pubmlst.org/efaecium/), differing from the hospital-adapted ST117 for a single-point mutation (452 A → G in the *pstS* gene).

Therefore, according to the eBURST analysis proposed by Willems *et al.*,[3] ST780 can be considered a direct descendant of ST117, belonging to the ST78 lineage. After all, it is worth mentioning that different strains belonging to ST117 are circulating in the hospital, as shown by the characterization of five out of the seven isolates neither included in cluster A nor in cluster B (Table 2).

The two remaining isolates, carrying neither *esp* nor *hyl*$_{Efm}$ determinants, belonged to two STs seldom detected till now (ST552 and ST561). They carried few resistance determinants and, for their sporadic nature, were considered not relevant for the study.

Plasmid profiles and localization of vanA, vanB, *and* hyl$_{Efm}$ *determinants*

S1 nuclease digestion of total DNA revealed different plasmid profiles among strains of the different PFGE types, and differences were detected even among subtypes of the same cluster (Supplementary Fig. S2). The *vanA* determinant was mostly harbored by plasmids of about 50 kb (Supplementary Fig. S2a), with two subtypes of cluster A (A8 and

Table 1. Antibiotic Resistance Profiles of One Representative Isolate for Each Pulsed-Field Gel Electrophoresis Subtype

Cluster/ subtype	*Representative isolate*	*Antibiotype*[a]										*MICs,*[b] *mg/L*							
		AMP	*CTX*	*SAM*	*AMC*	*MY*	*ERY*	*IPM*	*PEN*	*CLI*	*TET*	*VAN*	*TEC*	*DAP*	*TGC*	*Q-D*	*LZD*	*HLSR*	*HLGR*
A1	VRE 13	R	R	R	R	R	R	R	R	R	S	>256	64	4	0.12	>32	2	>1,024	>512
A2	VRE 108	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.06	>32	4	>1,024	>512
A3	VRE 24	R	R	R	R	R	R	R	R	R	S	>256	16	8	0.06	>32	2	>1,024	>512
A4	VRE 128	R	R	R	R	R	R	R	R	R	S	>256	32	4	0.12	>32	4	>1,024	>512
A5	VRE 89	R	R	R	R	R	R	R	R	R	R	>256	32	8	0.12	>32	4	>1,024	>512
A6	VRE116	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.12	>32	4	>1,024	>512
A7	VRE 62	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.12	>32	2	>1,024	>512
A8	VRE 132	R	R	R	R	R	R	R	R	R	R	>256	64	8	0.12	>32	2	>1,024	>512
A9	VRE 9	R	R	R	R	R	R	R	R	R	R	>256	32	8	0.12	>32	2	>1,024	>512
A10	VRE 130	R	R	R	R	R	R	R	R	R	R	>256	>256	8	0.12	>32	4	>1,024	>512
A11	VRE 61	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.06	>32	2	>1,024	>512
A12	VRE 8	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.12	>32	4	>1,024	>512
A13	VRE 60	R	R	R	R	R	R	R	R	R	R	>256	64	8	0.06	>32	4	>1,024	>512
A14	VRE 76	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.03	>32	4	>1,024	>512
A15	VRE 93	R	R	R	R	R	R	R	R	R	R	>256	32	8	0.12	>32	4	>1,024	>512
B1	VRE 1	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.12	>32	2	>1,024	512
B2	VRE 124	R	R	R	R	R	R	R	R	R	R	>256	64	4	0.06	>32	2	>1,024	>512
B3	VRE 65	R	R	R	R	S	R	R	R	R	S	16	1	4	0.12	>32	2	1,024	512
B4	VRE 48	R	R	R	R	R	R	R	R	R	S	64	1	4	0.12	>32	2	>1,024	4
B5	VRE 77	R	R	R	R	R	R	R	R	R	S	16	1	8	0.03	>32	4	>1,024	>512
B6	VRE 67	R	R	R	R	S	R	R	R	R	S	>256	1	4	0.12	4	2	>1,024	512
C	VRE 6	R	R	R	R	R	R	R	R	R	R	>256	32	4	0.12	>32	2	>1,024	4
D	VRE 37	R	R	R	R	R	R	R	R	R	R	>256	>256	4	0.12	>32	2	>1,024	512
E	VRE 23	R	R	R	R	R	R	R	R	R	R	>256	0.5	4	0.25	4	4	>1,024	512
F	VRE 44	R	R	R	R	R	R	R	R	R	S	16	0.5	4	0.06	4	4	>1,024	4
G1	VRE 47	R	R	R	R	R	R	R	R	R	S	>256	32	2	0.06	>32	1	>1,024	128
G2	VRE 91	R	R	R	R	R	R	R	R	R	S	16	0.5	4	0.03	8	2	>1,024	4

Light and dark gray background indicates susceptibility and intermediate resistance levels, respectively. Levofloxacin resistance was detected in all isolates by the VITEK2 automated system.

[a]Antibiotype was obtained by the Kirby–Bauer disk diffusion method.

[b]MICs were obtained by the microbroth dilution method.

AMC, amoxicillin–clavulanate; AMP, ampicillin; CLI, clindamycin; CTX, cefotaxime; DAP, daptomycin; ERY, erythromycin; HLGR, high-level gentamycin resistance; HLSR, high-level streptomycin resistance; IPM, imipenem; LZD, linezolid; MICs, minimum inhibitory concentrations; MY, lincomycin; PEN, penicillin; Q-D, quinupristin–dalfopristin; SAM, ampicillin–sulbactam; TEC, teicoplanin; TET, tetracycline; TGC, tigecycline; VAN, vancomycin; VRE, vancomycin-resistant enterococci.

Table 2. Genetic Characterization of the Clusters Identified by Macrorestriction Analysis

PFGE subtype	*ST*	*No. of isolates*	*Virulence genes*	*Resistance determinants*[a]
A1	17	1	*esp*	*vanA*, *ermB*, *aadE*, *aphA3*
A2		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aac-aphD*, *aph(2″)C*, *aphA3*
A3		3	*esp*	*vanA*, *ermB*, *aadE*, *aphA3*, *aac-aphD*
A4		1	*esp*, hyl_{Efm}	*vanA*, *ermB aadE*, *aphA3*, *aac-aphD*
A5		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A6		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aac-aphD*
A7		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A8		14	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A9		26	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*
A10		3	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A11		2	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A12		7	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*
A13		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*
A14		41	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
A15		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
B1	780	1	*esp*, hyl_{Efm}	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*
B2		1	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
B3		5	*esp*, hyl_{Efm}	*vanB*, *ermB*, *aadE*, *aphA3*, *aac-aphD*
B4		1	*esp*, hyl_{Efm}	*vanB*, *ermB*, *aadE*, *aphA3*
B5		2	*esp*, hyl_{Efm}	*vanB*, *ermB*, *aadE*, *aphA3*, *aac-aphD*
B6		1	*esp*, hyl_{Efm}	*vanB*, *ermB*, *aadE*, *aphA3*, *aac-aphD*
C	117	2	*esp*	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*
D		1	*esp*, hyl_{Efm}	*vanA*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
E		1	*esp*	*vanB*, *ermB*, *tetM*, *aadE*, *aphA3*, *aac-aphD*
G1		1	*esp*, hyl_{Efm}	*vanA*, *ermB*, *aadE*, *aphA3*
F	552	1	/	*vanB*, *ermB*, *aadE*, *aphA3*
G2	561	1	/	*vanB*, *ermB*, *aadE*, *aphA3*

[a]*vanA* and *vanB* were screened on the whole set of isolates. Other resistance determinants were detected in the representative isolates listed in Table 1.

PFGE, pulsed-field gel electrophoresis; ST, sequence type.

A12) carrying a second copy of this gene on a bigger plasmid of about 230 kb.

All strains but a sporadic one contained at least one megaplasmid higher than 200 kb. As expected, hyl_{Efm} gene was always harbored by one of them, ranging from 250 to 350 kb (Supplementary Fig. S2c) and, in isolates belonging to cluster B, the same plasmid carried also the *vanB* determinant (Supplementary Fig. S2b). In one isolate (subtype B6), a second *vanB* gene was detected on a smaller plasmid of about 100 kb (Supplementary Fig. S2b). On the contrary, in the sporadic isolates (types E, F, and G2), the *vanB* probe did not recognize any plasmidic band, suggesting a chromosomal localization.

Discussion

Dissemination of VREfm isolates in hospital environments has been a worldwide problem since the 2000s. Such strains are often multidrug resistant, because they can easily acquire resistance determinants by horizontal transfer[19] and have been shown to be capable of persisting for months on dry surfaces.[1]

Many recent reviews pointed out that major risk factors for VREfm are lack of contact precautions by healthcare workers, suboptimal cleaning of patient care areas, and exposure to antimicrobials, in particular to glycopeptides.[20,21] These risk factors are consistent with polyclonal spread of resistant strains that have been described in Europe till now, mostly due to mobilization of extrachromosomal genetic elements.[4,22] However, some outbreaks caused by clonal dissemination of single clonal lineages have been described too.[23,24]

This study reports an alarming increase in the detection of VREfm among patients admitted to the Trieste University Hospital since 2014, which rapidly led to an endemic setting sustained by the spread of a single clonal lineage in patients admitted to most of the hospital wards. We assume that the transfer of some colonized patients from ICUs, where these isolates were initially detected, to other wards might explain the spread of such clusters. Indeed, during the first 7 months of the study, 5 out of 18 colonized patients have been effectively moved from ICUs to other hospital units.

The epidemic strain belonged to ST17, one of the most diffuse human lineages although not the one detected to a major extent in Italian hospitals until now. It showed a multidrug-resistant phenotype combined with high-level aminoglycoside resistance for the presence of several resistance determinants. Besides, some of these isolates displayed nonsusceptibility to daptomycin.

Analysis of the whole plasmid content showed different patterns among the subtypes, as expected in Enterococci that easily acquire mobile elements and undergo recombination events.[19] However, all of them carried the *vanA* determinant

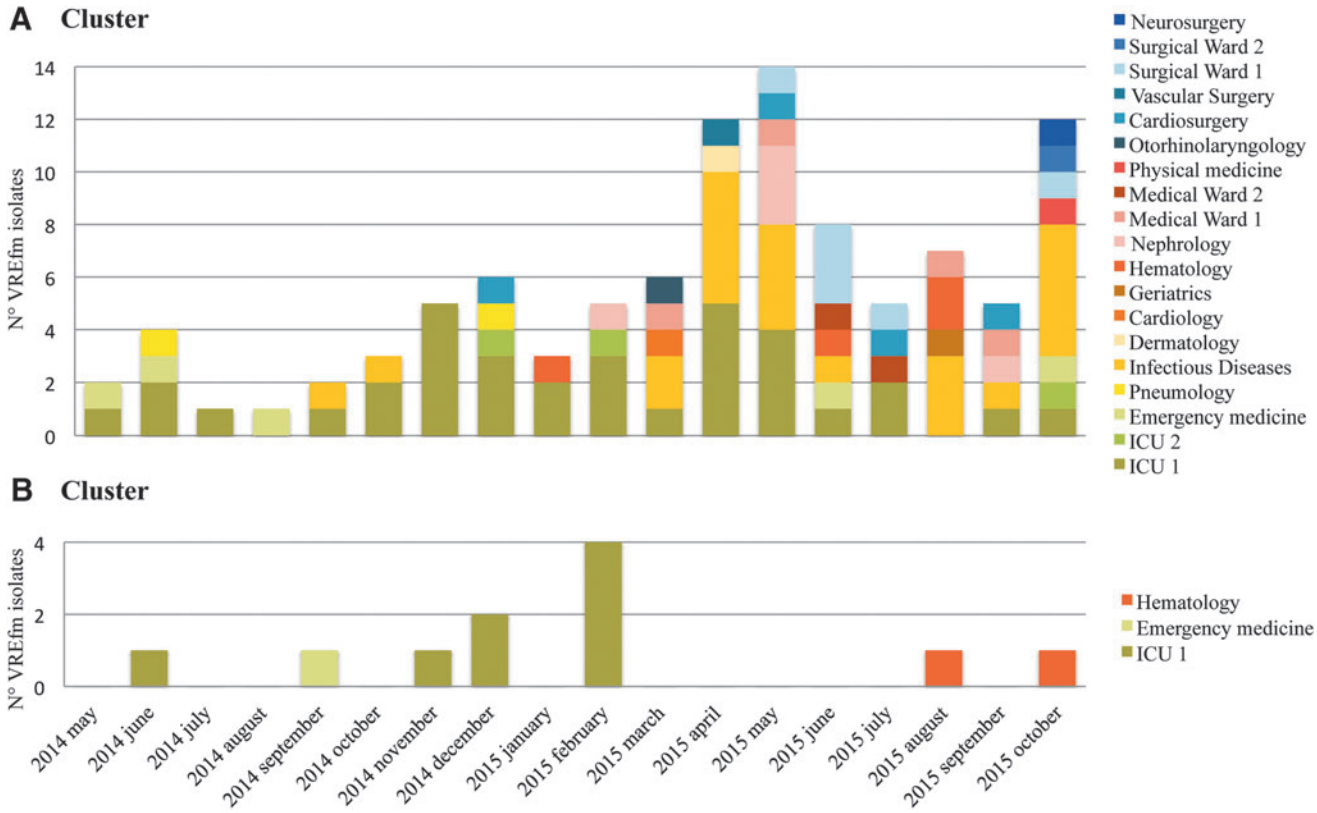

FIG. 1. Monthly distribution of isolates of cluster **(A)** and cluster **(B)** in the different wards. *Green shades* indicate emergency departments; *yellow-red* and *blue shades* indicate medical and surgical departments, respectively. ICU, intensive care unit.

on plasmids of about 50 kb, although in some cases (subtypes A8 and A12) another copy of *vanA* was detected on different plasmids. It is noteworthy that in a recent outbreak that occurred in Poland, some strictly related isolates harboring *vanA* on a 50 kb plasmid were described.[25]

The epidemic strain carried the *esp* gene, coding for a surface protein putatively involved in surface colonization, but not the virulence determinant hyl_{EFM}, which was detected in only 1 out of the 104 isolates of the cluster due to the acquisition of a megaplasmid higher than 350 kb. This single isolate was collected in May 2015 and did not further disseminate, suggesting that the genetic content of the megaplasmid does not further enhance the spreading potential of the bacterial host.

Fortunately, the epidemic strain did not seem very virulent to date, since only 5 out of the 104 VREfm patients got infected by it. However, this finding might be associated with the relatively small number of severely immunocompromised patients such as neutropenic and transplant patients in this hospital.

In fact, up to the emergence of multidrug-resistant strains in 1970s, enterococci have been considered relatively innocuous organisms.[26] Nevertheless, both vancomycin-susceptible and vancomycin-resistant strains of *E. faecium* causing bacteremia have been associated with high mortality in the era before the advent of VREfm effective therapy (*i.e.*, quinupristin–dalfopristin, linezolid, and daptomycin).[27] In particular, VREfm bacteremia has been associated with an attributable mortality of 40%, reaching up to 67% and 100% in cases with severe sepsis and septic shock, respectively.[28]

Moreover, despite the availability of effective VRE therapy, a recent meta-analysis showed that VRE bacteremia remains associated with increased risk of morbidity and mortality when compared with vancomycin-susceptible enterococcal bacteremia (odds ratio, 1.80; 95% confidence interval, 1.40–2.32; $I^2 = 0\%$; $n = 12$).[29] Indeed, three out of the five infected patients of this study died. It is noteworthy that neither of them (two oncologic patients with bloodstream infections and one with tertiary peritonitis) could be treated with a targeted anti-VREfm therapy because the diagnosis of VREfm infection arrived postmortem.

During the period of the study, 18 VREfm isolates not related with cluster A were detected too, indicating the contemporary presence, in the hospital, of different VREfm lineages that, on the contrary of the epidemic strain, showed a low-potential spreading inside the hospital wards. Most of them belonged to the BAPS group 2-1 (78 lineage), which is frequent in Italian hospitals. Nevertheless, except for one strain that caused a small, self-limited, outbreak that interested a single ICU, they remained sporadic.

Causes that led to the VREfm endemic state described in this study have not been fully elucidated. From 2008 to 2014, the hospital received the JCI accreditations and no major changes in the practices for infection control have been reported. However, a significant increase in the annual consumption of some classes of antibiotics, including vancomycin, has been described during this 6-year period.[9] As a consequence, we believe that the increased usage of systemic antimicrobials as well as SDD practice, including vancomycin in

ICU patients, may have played a role in the spread of the major epidemic strain in this hospital.

Isolates belonging to the ST17 lineage were the most resistant among those described in the study, mainly for the carriage of the *ermB*, *tetM*, and aminoglycoside resistance determinants. Besides, one isolate carried the *aph(2″)C* determinant. However, this cause alone probably cannot exhaustively explain the massive diffusion of this single clonal lineage, which possibly expresses one or more features conferring a higher fitness to the bacterial host in the hospital environment.

So, despite the low virulence potential displayed until now by the epidemic strain, its dissemination is alarming both for the community, because colonized patients are important contributors to environmental contamination,[30] and patients themselves, because they should be considered at risk of progression toward infection.[21]

For this reason, further implementation of control measures has been recently introduced, consisting in reducing antibiotic exposure, in particular, systemic and SDD vancomycin use, more accurate environmental cleaning of rooms, enlargement of the screening program to patients who share the room with the colonized ones, and more extensive information for visitors.

On the contrary, however, this clonal lineage would deserve further analysis. It might be worthwhile comparing some of these isolates by molecular techniques based on whole-genome sequencing, allowing a more accurate comparison both of isolates relatedness and of the plasmids they harbor. Besides, identification of putative, still unknown, epidemic markers carried by this clonal lineage might be achieved.

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Disclosure Statement

No competing financial interests exist.

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