

## RESEARCH ARTICLE

# Clonal distribution of vancomycin-resistant *Enterococcus faecium* in Turkey and the new singleton ST733

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## Abstract

**Background:** The aim of this study was to provide information about the spread and characteristics of the vancomycin-resistant *Enterococcus faecium* isolates (VREfm) in Turkey.

**Methods:** Seventy-one nonduplicate consecutive isolates of VREfm were obtained from various clinical specimens of inpatients treated at university or training hospitals in seven regions of Turkey. Further characteristics included antibiotic susceptibility testing, pulsed-field gel electrophoresis (PFGE) of SmaI-digested genomic DNA, and multilocus sequence typing (MLST) of selected isolates. The presence of vancomycin resistance and virulence genes (*esp* and *hyl*) was investigated by polymerase chain reaction (PCR).

**Results:** All VREfm isolates had MICs to vancomycin of  $\geq 32$  mg/L and contained the *vanA* gene. The presence of *esp* gene was identified in 64 and *hyl* in eight VREfm isolates. All VREfm showed the multiresistance phenotype, including ampicillin (99%), penicillin (99%), imipenem (99%), ciprofloxacin (87%), moxifloxacin (87%), erythromycin (97%), streptomycin (86%), gentamicin (82%), tetracycline (70%), and teicoplanin (99%). All were susceptible to tigecycline while quinupristin-dalfopristin (97%) and linezolid (93%) were the most active other agents. Analysis of the PFGE profiles showed that 53 (74.6%) VREfm isolates shared a similar electrophoretic profile, designed as type 1, and were closely related (>85%). The sequence type was identified by MLST in 44 VRE isolates with unrelated or closely related PFGE patterns. MLST revealed that nosocomial spread of VREfm resulted from dissemination of lineage C1 *E faecium* clones. Sequence types ST78, ST203, and ST117 were the most frequently isolated. This is the first report of ST733 around the world.

**Conclusions:** Lineage C1 clones are disseminated among clinical VREfm isolates in seven different regions in Turkey. Regarding VREfm isolates, the worldwide epidemic strains are in circulation in Turkey.

## KEYWORDS

CC17, MLST, PFGE, risk factors, ST733, VRE

VRE Study Group authors are listed in Appendix A.

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## 1 | INTRODUCTION

The most common infections associated with VRE are bacteremia, endocarditis, and urinary system infections.<sup>1,2</sup> Various risk factors which significantly contributed to the invasive VRE infections and caused mortality were described before. Long period of hospitalization in the intensive care, transplant, hematology, or oncology units, receiving hemodialysis, contact with patients diagnosed with VRE, enteral feeding, corticosteroid use, administration of antineoplastic treatment, sucralfate use, and the history of the use of antibiotic (vancomycin, second- or third-generation cephalosporins, metronidazole, clindamycin, imipenem, ticarcillin-clavulanic acid) were reported as the risk factors.<sup>1-5</sup> Allogenic bone marrow transplant, neutropenia, use of central venous line, and hypoalbuminemia were described as the independent risk factors in the development of the VRE bacteremia in the multi-variant analyses.<sup>6-9</sup>

Although seven different resistance genotypes (VanA to VanE and VanG) have been described in VRE, VanA and VanB are of greatest clinical relevance and VanA is the most frequent genotype detected in the world.

The molecular epidemiologic investigation of resistant microorganisms is important in terms of infection control and epidemiology. *Enterococcus faecium* attracts more and more attention due to its capacity of acquiring multiple antibiotic resistance determinants, especially those encoding glycopeptide resistance and its potential to spread among the nosocomial setting. It has been suggested that the virulence gene *esp* is a characteristic feature of isolates involved in nosocomial outbreaks. Hospital-adapted VRE exhibit relatively high pathogenicity by expressing factors like enterococcal surface protein (Esp), which facilitates epidemic spread. *Enterococcus faecium* consists of different clonal complexes as demonstrated by multiple-locus sequence typing (MLST). Clonal complex-17 *E faecium* are enriched in the *purK1* allele and the *esp*-containing pathogenicity locus. The *esp* gene encodes the enterococcal surface protein Esp, enabling adhesion to epithelial cells, allowing biofilm formation. As VRE infections appear to be more deadly and more costly than infections caused by vancomycin-susceptible strains, epidemiological data concerning occurrence and spread of these microorganisms have to be compiled, and VRE isolates have to be epidemiologically investigated. Several molecular typing schemes have been developed to study the epidemiology of VRE. Of these, pulsed-field gel electrophoresis (PFGE) of genomic restriction fragments has been considered the gold standard because of its high degree of isolate differentiation.<sup>10,11</sup> Multilocus sequence typing (MLST) and multiple-locus variable-number tandem repeat analysis (MLVA) have been developed recently to recognize genetically related and potential epidemic isolates of *E faecium*. MLST was recommended for strain characterization and long-term epidemiological investigations. It was shown that few clones emerged recently carrying the vancomycin resistance determinant. MLST confirmed the unrelatedness of human and nonhuman. Several authors used MLST for outbreak

investigations. CC17 *E faecium* are responsible for a significant portion of hospital-associated infections, which can cause severe morbidity and mortality.<sup>12,13</sup> In addition, providing the same genetic data can be used in single nucleotide polymorphism (SNP) analysis or core genome MLST. Whole genome sequencing could be an alternative in the molecular epidemiological investigation of VRE.<sup>10</sup>

The aim of this study was to characterize and elicit the genetic relatedness of emerging vancomycin-resistant *E faecium* (VREfm) and to provide a comprehensive overview of prevalence and risk factors for VREfm in patients admitted to Turkish hospitals.

## 2 | MATERIALS AND METHODS

### 2.1 | Data collection

At the participating hospital, a standardized questionnaire was used to collect basic demographic and clinical information of the patient including site of infection, age, ward, gender, and risk factors such as comorbid condition, infection, surgical history, invasive procedures, indwelling device use, hospitalizations, previous antibiotic consumption, or antibiotic treatment in the prior 3 months. The confirmed VRE isolates from the participating hospitals were then shipped by courier to the collection center, Department of Clinical Microbiology, Istanbul Faculty of Medicine, Istanbul University, for further analysis. The VRE isolates were received from participating hospitals at different times during the study period at the collection center in a nutrient agar transport medium. They were stored in  $-70^{\circ}\text{C}$  until further analysis.

Bacterial isolates: In January-December, 71 nonduplicate consecutive isolates of vancomycin-resistant VREfm were obtained from different clinical specimens such as blood, urine, wound swabs, and other clinical samples of inpatients treated at university or training hospitals including Istanbul Faculty of Medicine, Istanbul University Cerrahpaşa Faculty of Medicine, Haydarpaşa GATA, Ankara GATA, Ege University, Akdeniz University, and Van Yüzüncü Yıl University located in various regions of Turkey. The identification of isolates was done by conventional methods, API systems, and automated testing (VITEK2, BioMérieux).

### 2.2 | Antimicrobial susceptibility testing

The in vitro activity of ampicillin, penicillin, ampicillin/sulbactam, erythromycin, vancomycin, imipenem, chloramphenicol, ciprofloxacin, moxifloxacin, quinupristin/dalfopristin, tetracycline, teicoplanin, and tigecycline, and high-level resistance (HLR) to gentamycin (120  $\mu\text{g}$ ) and streptomycin (300  $\mu\text{g}$ ) were evaluated against these non-repeat clinical isolates of VREfm. The minimum inhibitory concentration (MIC) tests were performed with Epsilon meter (E-test) strips (AB Biodisk). The gradient test was performed on Mueller-Hinton agar supplemented with 50 mg/L

calcium (Difco, USA), and MIC values were interpreted according to the CLSI guidelines.<sup>14</sup> The reference strain *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 were used as quality control for susceptibility testing.

### 2.3 | Amplification of resistance genes and sequencing

The genomic DNA of each VREfm isolate was extracted using the HiPure Bacterial DNA Kit, according to the manufacturer's instructions, and stored at  $-20^{\circ}\text{C}$  until use. The presence of *vanA* and *vanB* resistance genes and virulence genes (*esp* and *hyl*) was investigated by polymerase chain reaction (PCR).<sup>15,16</sup> *Enterococcus faecium* strain C68 (*hyl*<sub>Efm</sub> and *esp*<sub>Efm</sub>) was used as the positive control. A 100-bp DNA ladder (Bio-Rad) was used as a molecular size marker. PCR amplicons were sequenced using a BigDye Terminator v1.1 cycle sequencing kit (Life Technologies) on a Beckman DNA genetic analyzer.

### 2.4 | Molecular epidemiology investigation

Molecular epidemiology investigation of selected isolates was assessed and by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

### 2.5 | Pulsed-Field Gel Electrophoresis (PFGE)

Molecular epidemiology of selected isolates was assessed by PFGE.<sup>17,18</sup> Briefly, bacterial cells embedded in 1.6% low-melting-point agarose plugs were lysed with lysozyme and proteinase K and then chromosomal DNA was digested with 40 U Sma I (Fermentas). Fragmented DNA samples were electrophoresed in 1% pulsed-field certified agarose in 0.5× TBE buffer by the contour-clamped homogeneous electric field method with a CHEF-DRII drive module (Bio-Rad Laboratories Ltd.) with 10–40 seconds pulse times, for 21 hours at  $14^{\circ}\text{C}$  at 6 V cm. The gels were stained with ethidium bromide to detect the DNA band profiles, and the image was digitized with a Gel Doc 1000 system (Bio-Rad Laboratories). The DNA band profiles were analyzed with GelCompar software (version 3.0; Applied Maths). Band tolerances of 1.5% and 1% normalization were used for comparison of DNA profiles.

### 2.6 | Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) was performed by amplifying seven relatively conserved *E faecium* housekeeping genes (*adh*, *atp*, *ddl*, *ddh*, *gyd*, *purK*, and *pst*) according to the database methodology and guidelines available at <http://pubmlst.org/efaecium/>. MLST sequences were then queried into the MLST databases (ie,

<http://efaecium.mlst.net/>) to determine their sequence types (STs). An identified novel ST and alleles were submitted to the PubMLST database

## 3 | RESULTS

### 3.1 | Patients and bacterial isolates

The distribution of a total of 71 nonduplicate VREfm received from inpatients treated at university or training hospitals in seven various hospitals of Turkey were as follows: Istanbul Faculty of Medicine ( $n = 11$ ), Istanbul University-Cerrahpaşa, Cerrahpasa Faculty of Medicine ( $n=6$ ),<sup>6</sup> Haydarpaşa GATA ( $n=14$ ),<sup>14</sup> Ankara GATA ( $n=1$ ),<sup>1</sup> Ege University ( $n=17$ ),<sup>17</sup> Akdeniz University,<sup>11</sup> and Van Yüzüncü Yıl University.<sup>9</sup> All the 71 VREfm isolates were noted to have been recovered from hospitalized patients and thus represented healthcare-associated isolates and infections. A total of 71 patients including 18 pediatric patients were enrolled in this study, of which 53.6% were females with a mean age of 46.3, a range of 20 months–89 years. These isolates obtained from inpatients in several disciplines including surgical, medical, and intensive care units of the hospitals. These VREfm isolates were recovered from ICU ( $n = 25$ ), medical wards ( $n = 34$ ), and the surgical ward ( $n = 12$ ). No duplicate isolates from a single patient were included

The source of these isolates was as follows: majority of isolates obtained from blood 84.5% ( $n = 39$ ), urine ( $n = 21$ ), 15.5% from surgical wound specimens ( $n=3$ ),<sup>3</sup> cerebrospinal fluid ( $n=2$ ), abscess ( $n=2$ ),<sup>2</sup> bronchoalveolar lavage ( $n=1$ ),<sup>1</sup> catheter tube ( $n=2$ ),<sup>2</sup> and transtracheal fluid samples ( $n=1$ ), respectively.

### 3.2 | Antibiotic susceptibility and PCR results

All VREfm isolates had MICs to vancomycin of  $\geq 32$  mg/L and contained the *vanA* gene. The presence of *esp* gene was identified in 64 (90%), *hyl* in eight (11.2%), and co-presence *esp* and *hyl* genes were observed in five (7%) VREfm isolates. All VREfm strains showed the multi-drug-resistance phenotype, including ampicillin (99%), penicillin (99%), imipenem (99%), ciprofloxacin (87%), moxifloxacin (87%), erythromycin (97%), streptomycin (86%), gentamicin (82%), tetracycline (70%), and teicoplanin (99%). All were susceptible to tigecycline while quinupristin-dalfopristin (97%) and linezolid (93%) were the most active other agents.

### 3.3 | The clinical results and risk factors of VRE infection in patients

The causes of hospitalization, significant risk factors, and frequency of underlying diseases associated with VRE infections are demonstrated in Table 1. The anamnesis of the patients showed that 32% had malignancy and 26% had neutropenia. Renal failure was detected

in 16% and diabetes mellitus was detected in 14.4% of the patients. The investigation of the risk factors of hospital demonstrated that 52 patients (73.2%) were hospitalized in the last 3 months. Twenty-five patients (35.2%) were hospitalized in the intensive care unit in the study period, and the total number of patients hospitalized in hematology (6 patients), nephrology (3 patients), transplantation (2 patients), and oncology (1 patient) units was found as 12 (16.9%). The number of patients who received intensive care treatment was identified as 32 (46.3%). The number of patients who were inserted intravenous catheter was 36 (52.1%), who were inserted urinary catheter was 26 (37.7%), and who were inserted ventriculo-peritoneal shunt was 2 (2.8%). Twenty-three (35.7%) patients received mechanic ventilation, six patients underwent kidney dialysis, two patients underwent colonoscopy, and nasogastric catheter was inserted to one patient. Two patients received solid organ transplant, and one patient received bone marrow transplant.

In study period, 55 (79.7%) patients received antibiotic treatment in the last 3 months. The most frequently used antibiotics were identified as cephalosporins (72.4%), carbapenems (55%), linezolid (39%), teicoplanin (27.5%), and vancomycin (21.7%) (Table 2). The use of immunosuppressive drug was detected in 15 (21.7%) patients, and the use of gastrointestinal system targeting drugs was detected in 12 (17.4%) patients (Table 1).

Thirty-three per cent of the patients underwent surgical procedure (8.6% of the surgeries were intra-abdominal surgeries)

(Table 1). The prevalence of enterococci infections among patients with blood infections (54.9%) and UTIs (29.6%) was higher than the other infections. We detected the VREfm associated mortality rate as 21.7% in this study. Higher mortality was significantly associated with illness severity (sepsis and/or preexisting comorbidities). The median age of died patients was 68 years (IQR 20-89 years). All patients were hospitalized for more than 48 hours (range, 4-5 112 days). All patients who had been admitted to the intensive care unit mostly required mechanical ventilation, and majority of patients had intravenous and indwelling urinary catheter. There were various treatment regimens, but linezolid-containing regimens were generally used.

### 3.4 | Results of PFGE analysis

The cluster analysis was achieved by the BioNumerics software (Applied Maths). Percentages of similarity were determined using the Dice correlation coefficient, and a dendrogram was produced via the unweighted pair group method with arithmetic mean clustering (UPGMA). The band tolerance was set at 1.5%, and the threshold cutoff value was set at 85%. The analysis of molecular typing demonstrated 29 PFGE genotypes among the 71 VREfm isolates. The predominant clones occurring in 78.8% (56/71) of the isolates were closely related (>85%). The predominant clone present in

**TABLE 1** Significant risk factors and frequency of underlying diseases associated with VRE-infected patients

Comorbidities	n (%)	Comorbidities	n (%)	Comorbidities	n (%)
Malignancy	22 (31.8)	Meningitis, hydrocephalus	2 (2.9)	Hospitalization (last 3 months)	52 (75.3)
Neutropenia	16 (23.1)	Congenital hydrocephalus	2 (2.9)	Intensive care unit (ICU)	32 (46.3)
Diabetes Mellitus	10 (14.5)	Dilated cardiomyopathy	2 (2.9)	Surgical operation	17 (24.6)
Pneumonia	9 (13)	Acute myocardial infarction	1 (1.4)	Intra-abdominal surgery	6 (8.7)
Bacteraemia	6 (8.7)	Parkinson/Behcet's Disease	1 (1.4)	Kidney dialysis	6 (8.7)
Chronic renal failure	6 (8.7)	Meningomyelocele, hydrocephalus, shunt inf.	1 (1.4)	Intravenous catheterization	36 (52.1)
Acute renal failure	5 (7.2)	Cerebral hemorrhage	1 (1.4)	Urinary catheterization	26 (37.7)
Hydronephrosis	1 (1.4)				
Chronic heart failure, respiratory failure	5 (7.2)	Graft infections	1 (1.4)	Mechanical ventilation/intubation	23 (33.3)
Pleural effusion	4 (5.8)	Acute pancreatitis	1 (1.4)	Immunosuppressive drug use	15 (21.7)
Respiratory failure, COPD, Aspiration pneumonia, Acute bronchiolitis	4 (5.8)	Infective endocarditis	1 (1.4)	Drug use for gastrointestinal system	12 (17.4)
Hypertension	2 (2.9)	Tuberculosis peritonitis	1 (1.4)	Ventriculo-peritoneal shunt	2 (2.9)
Neurological Diseases	3 (4.3)	Rectovaginal fistula	1 (1.4)	Nasogastric tube	1 (1.4)
Acute lymphoblastic leukemia (ALL)	3 (4.3)	Ataxia + immunodeficiency	1 (1.4)	Gastroscopy/Colonoscopy	2 (2.9)
Acute myeloblastic leukemia (AML)	1 (1.4)	Hemophagocytic syndrome	1 (1.4)	Transplantation Solid organ (liver, kidney)	3 (4.3)
Myelodysplastic syndrome	1 (1.4)	Bleeding in esophageal varices	1 (1.4)	Transplantation Bone marrow	1 (1.4)

**TABLE 2** Antibiotic treatment in the last 3 months received by the patients

Antimicrobials	Numbers (%)	Antimicrobials	Number (%)
Vancomycin	15 (21.7)	Fosfomycin	1 (1.4)
Teicoplanin	19 (27.5)	Daptomycin	1 (1.4)
Linezolid	27 (39.1)	Amikacin	14 (20.3)
Cefazolin	1 (1.4)	Gentamicin	3 (4.3)
Ceftriaxone	5 (7.2)	Netilmicin	2 (2.9)
Cefotaxime	5 (7.2)	Levofloxacin	2 (2.9)
Ceftazidime	8 (11.6)	Ciprofloxacin	4 (5.8)
Cefepime	3 (4.3)	Tigecycline	4 (5.8)
Cefoperazone	3 (4.3)	Colistin	5 (7.2)
Cefoperazone/ sulbactam	12 (17.3)	Amphotericin B	7 (10.1)
Piperacillin/ tazobactam	13 (18.8)	Voriconazole	2 (2.9)
Ampicillin	1 (1.4)	Caspofungin	2 (2.9)
Ampicillin/ sulbactam	7 (10.1)	Fluconazole	2 (2.9)
Imipenem	10 (14.5)	Metronidazole	2 (2.9)
Meropenem	24 (34.7)	Ornidazole	1 (1.4)
Doripenem	2 (2.9)	Ganciclovir	1 (1.4)
Ertapenem	2 (2.9)	Acyclovir	1 (1.4)
Co-trimoxazole	5 (7.2)	Valacyclovir	1 (1.4)
Clarithromycin	2 (2.9)		

all hospitals was as follows: Istanbul Faculty of Medicine (11/11), Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine (6/6), GATA Ankara (1), GATA Haydarpasa (15/15), Ege University (10/18), Akdeniz University (11/11), and Van Yüzüncü Yıl University (2/9) (Figures 1-7).

### 3.5 | MLST results

MLST was performed for selected VRE isolates ( $n = 44$ ). The isolates were selected based on their PFGE profiles. The sequence type was identified by MLST in 44 VREfm strains with unrelated or closely related PFGE patterns. Sequence types ST203 (34%), ST78 (27.2%), ST17 (15.9%), ST117 (15.9%), and ST280 (4.5%) were the most frequently isolated ones. ST17, ST203, and ST117 were found in Akdeniz University; ST203, ST78, ST17, ST117, and ST733 in Ege University; ST78 and ST203 in Istanbul University and Istanbul University-Cerrahpasa; ST 17, ST 203, and ST78 in Haydarpasa GATA; and ST17, ST117, ST78, ST203, and ST280 in Van Yüzüncü Yıl University. One of the isolates from Ege University revealed a new sequence type named as ST733 (atpA 15; ddl 1; gdh 11; purK 1; gyd 21; pst 1, and adk 1). All these STs were from the clonal complex 17 (CC17) ancestor which has a worldwide distribution.

## 4 | DISCUSSION

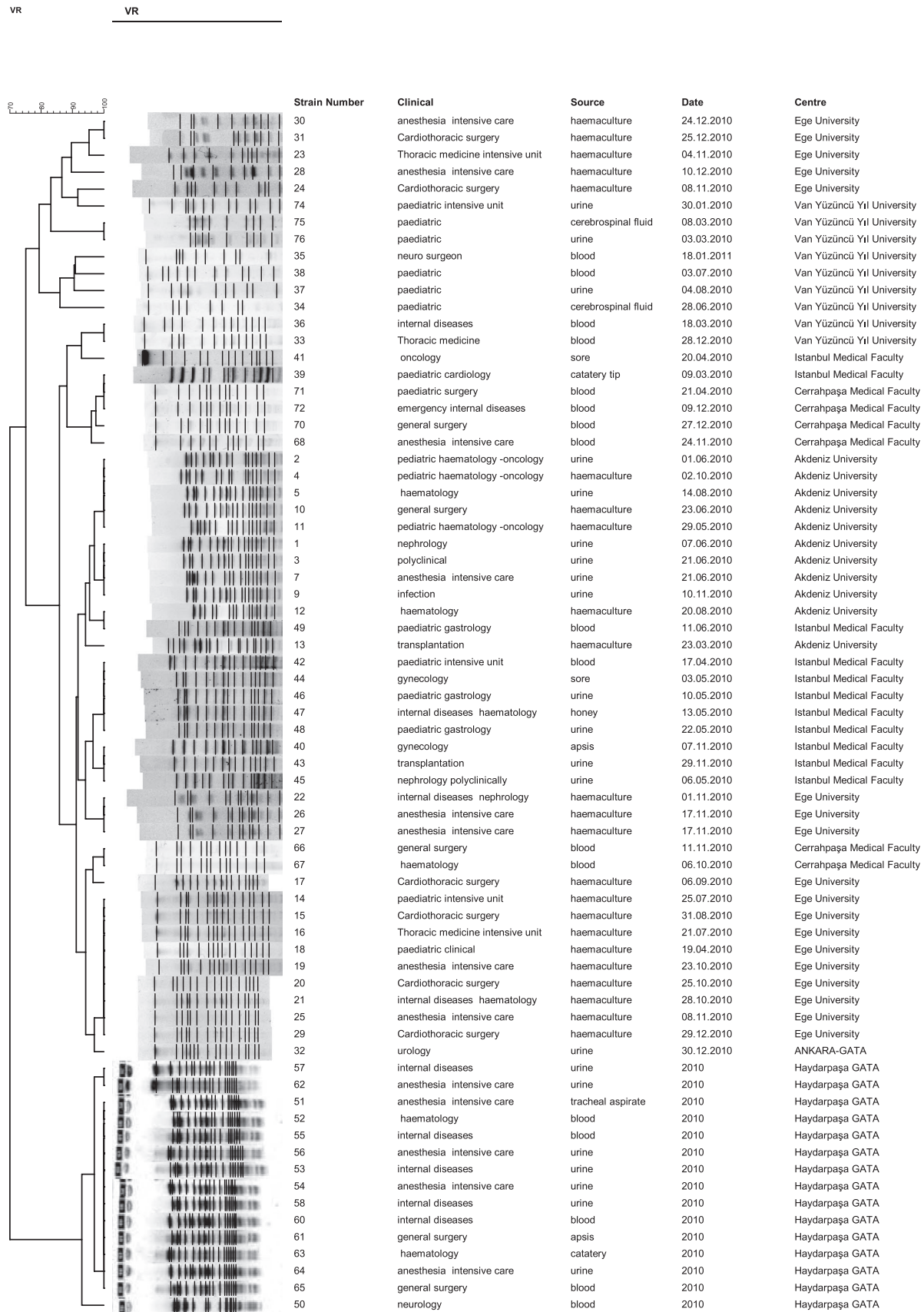
The aim of this study was to analyze clonal lineages and risk factors in the spread and persistence of vancomycin resistance among *E faecium* strains causing infections in different regions in Turkey.

The treatment of vancomycin-resistant enterococcus infections is difficult because of VRE strains are generally multi-drug-resistant. The mortality rate was reported as 13-27% in bacteremia with vancomycin sensitive strains; however, various studies detected the rate as 36-52% in VRE bacteremia.<sup>19</sup> Linezolid, tigecycline, quinupristin/dalfopristin, daptomycin, and chloramphenicol are recommended for use in VRE infections. Although linezolid resistance is rarely detected in VRE, resistance may develop due to the long-term use in patients. However, acquisition of the linezolid resistance was also reported with horizontal gene transmission in patients who had received no linezolid before. Corticosteroid use, the previous use of multiple antimicrobials, parenteral feeding, peripheral vascular diseases, and solid organ recipients were identified as the risk factors that caused linezolid resistance.<sup>20-22</sup> The SENTRY Antimicrobial Surveillance Program, the Linezolid Experience and Accurate Determination of Resistance (LEADER) initiative, ZAAPS (Zyvox® Annual Appraisal of Potency and Spectrum), and TEST concordantly report a sustained high potency of LZD against the entire spectrum of tested bacteria with very low rates of resistance (<1%) development over the last two decades.<sup>23</sup>

The most frequently detected resistance gene was *vanA* in the studies conducted in Turkey. The *vanA* gene was found positive in all VREfm strains with PCR method in this study, and these results were found compatible with the phenotyping results suggesting VanA positivity, except one strain (number 4) (teicoplanin MIC:1) which was found as VanA genotype but showed the VanB phenotype, which is the term known recently by some authors "vanA genotype-vanB phenotype" that is detected for the 1st time in Turkey. The cause of heteroresistance to teicoplanin in enterococcus isolates carrying the *vanA* gene is not well understood. Some authors explained such heterogeneity by the occurrence of mutations, either in the *vanA* gene cluster or in other regulatory elements. VanR and VanS form a two-component regulatory system. VanS comprises an N-terminal glycopeptide sensor domain with two membrane-spanning segments and a C-terminal cytoplasmic kinase domain that catalyzes transfer of the phosphate group to VanR. Amino acid substitutions in the VanS sensor of the VanA-type vancomycin-resistant enterococcus strains result in high-level vancomycin resistance and low-level teicoplanin resistance. Amino acid substitutions due to the three point mutations of *vanS* are responsible for impaired teicoplanin resistance among *vanA* genotype.<sup>24-26</sup>

The detection of *esp* gene as a specific virulence factor was suggested to be beneficial in the differentiation of epidemic strains from nonepidemic strains. In this study, the *esp* gene was detected in the majority of the strains (90%) in the PCR results. *hyl* gene was





**FIGURE 1** Dendrogram of Sma I PFGE typing of 71 VREfm isolates. 56/71—the isolates having a similarity coefficient 85%. The scale bar given on the top indicates similarity percentages detected for pulsotypes. The phylogenetic tree was constructed by the use of Dice coefficient and UPGMA clustering; the band tolerance was set at 1.5%, and the threshold cutoff value was set at 85%

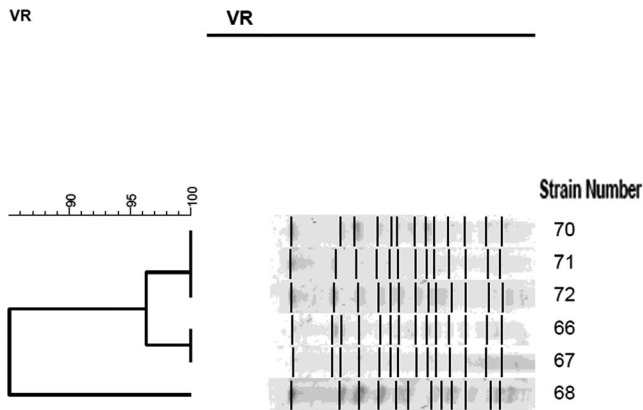


FIGURE 2 PFGE results of Istanbul University-Cerrahpasa (n = 6)

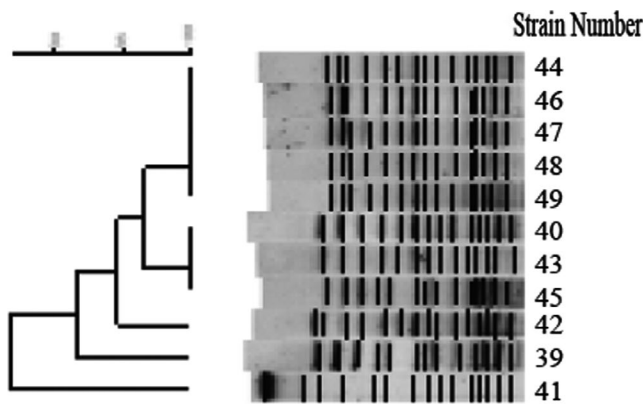


FIGURE 3 PFGE results of Istanbul Faculty of Medicine (n = 11)

detected in eight strains (11.2%), and the co-existence of *hyl* and *esp* genes was detected in five strains (7%).

In this study, the clonal association of the VREfm strains that were isolated from various centers in Turkey was identified. Also, the most common clones and the association of these clones with the other clones in Europe and worldwide were investigated using the MLST method. In our study, 78.8% of the strains were found clonally associated with the PFGE method (Figures 1-7). This was an important result and was the first study demonstrating the clonal dissemination between different regions in Turkey. One or two samples from the strains which were detected to be clonally associated were selected from each center as the representatives, and the allelic profiles of other strains which had no clonal association were identified using the MLST method. A total of 44 strains were investigated in this study and were typed as ST203 (n:15), ST78 (n:12), ST17 (n:7), ST117 (n:7), ST280 (n:2), and the newly encountered ST733 (n:1). All ST types were found to be associated with the epidemic CC17, and this is the first report worldwide for ST733.

Recently, infections and outbreaks of vancomycin-resistant enterococci (VRE) appear not to be rare in Turkey.<sup>27-29</sup> To our knowledge, despite this common ancestor and association of outbreaks of this lineage clones, no multicenter studies have been conducted in Turkey. There are some local studies. In a study

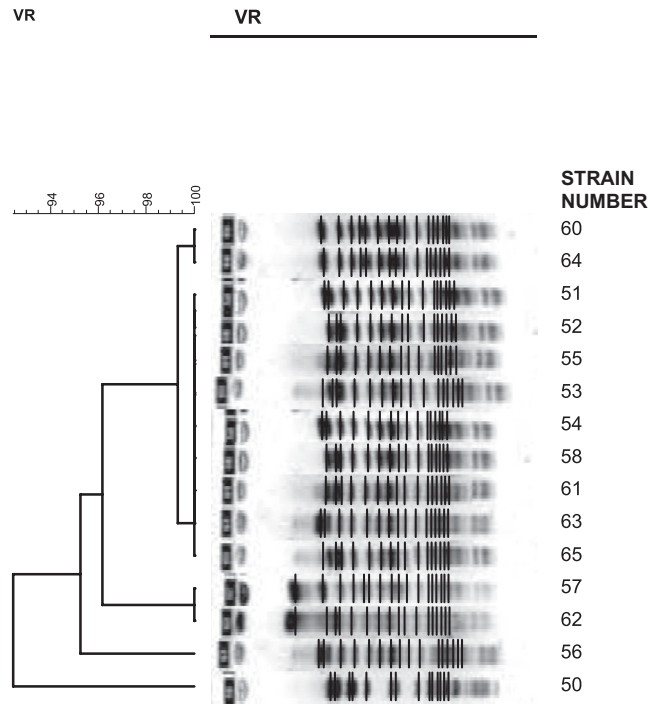


FIGURE 4 PFGE results of GATA Haydarpasa (n = 15)

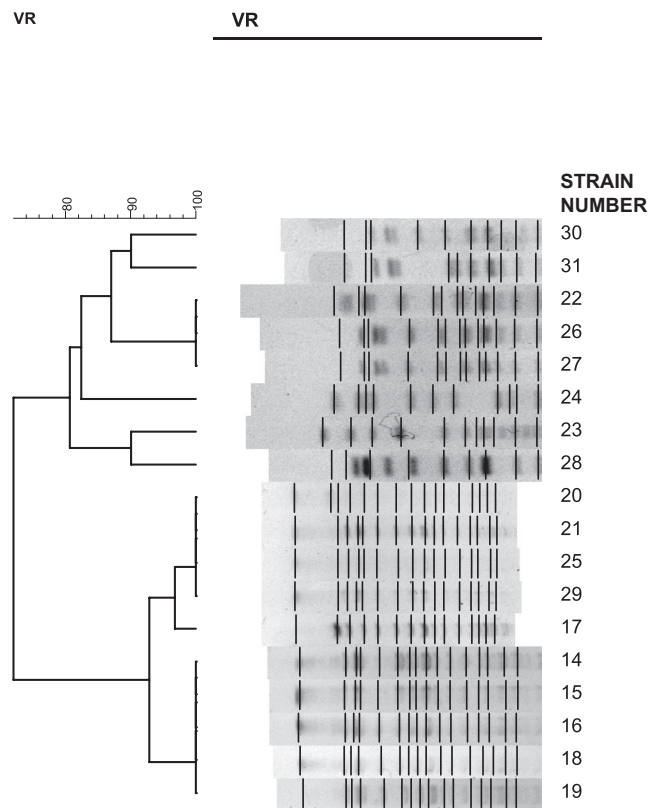
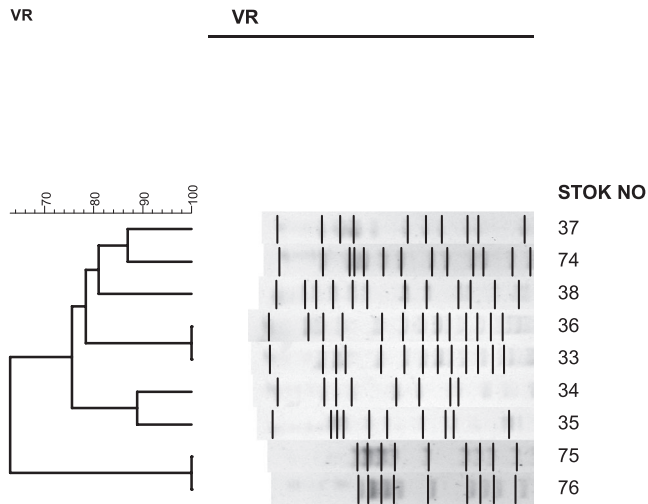


FIGURE 5 PFGE results of Ege University (n = 18)

conducted in Turkey, clonal relationship of 38 isolates *E faecium* carrying the *vanA* gene was determined by PFGE and MLST methods.<sup>28</sup> A pulsotype and its subtypes belonged to ST117 (76.3%), three B pulsotype belonged to ST280 (7.9%), two C pulsotype



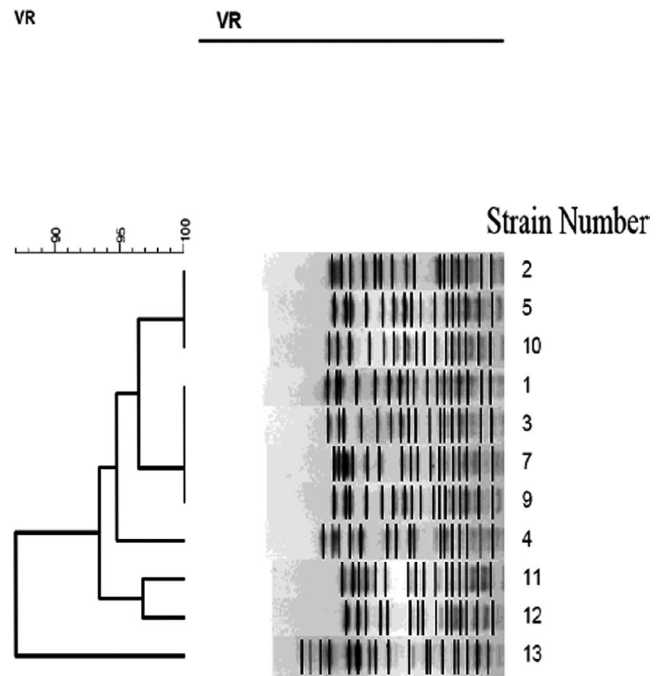
**FIGURE 6** PFGE results of Van Yuzuncu Yil University (n = 9)

belonged to ST18 (5.2%), and three D pulsotype belonged to ST17 (7.9%).<sup>30</sup> In another study, during an outbreak in a hematology unit of a training and research hospital in Turkey, ST17 and ST78 have been reported common ST types.<sup>31</sup>

In a well-conducted multicenter study in 1986-2009 in 13 countries in five continents (Germany, Greece, Hungary, Holland, Poland, Portugal, Serbia, Spain, Canada, USA, Saudi Arabia, Tunisia, and Australia), ST16, ST17, and ST18 were the types most frequently detected in these strains associated with CC17, while ST80, ST125, ST192, ST412, ST173, and ST280 were reported as the other types.<sup>32</sup> ST16, ST17, and ST18 are prevalent worldwide; ST192 and ST203 were reported from Germany, Spain, and Korea; ST280 was reported from Portugal, Singapore, and the United States; and ST412 was reported from Greece. ST78, ST117, ST203, ST316, ST362, ST363, ST364, and ST365 were reported as associated with CC17 from a single center in a study conducted in China.<sup>33,34</sup>

ST16, ST17, ST203, and ST65 types were reported as associated with CC17 in linezolid resistant *E faecium* strains in a study conducted in Greece.<sup>35</sup> Two new sequence types assigned to ST1463 and ST1464 were reported from Tunisia.<sup>36</sup> In this study, strains with linezolid resistance were found to be associated with ST203, ST78, and ST17 types, but as can be seen from the results, there is no homogeneous group to be associated with linezolid resistance. The investigation of the worldwide prevalence of the types detected in our study showed that ST78 was reported in Italy, Austria, Germany, Korea, Hungary, Holland, China, Japan, Lithuania, and Portugal; ST117 was reported in Portugal, Germany, America, and Holland; and ST203 was reported in Germany, Korea, Denmark, China, and Serbia ([www.mlst.net](http://www.mlst.net)).

The results showed that there was a major clone in the hospitals in Turkey. However, it is difficult to explain the clonal relationship between the strains with MLST method results. In the selection of the strains for the MLST method, one or two representatives from the strains which had 100% band similarity in PFGE method were selected, and other strains which had more than one band



**FIGURE 7** PFGE results of Akdeniz University (no = 11)

differences were analyzed. Interestingly, isolates (strain numbers 2, 3, 5, 8, 12, 13, 15, 21, 22, 27, 28, 33, 34, and 43) with differently related PFGE patterns had the same ST (ST16). This indicates that PFGE is more discriminatory than MLST for homology analysis of small areas in the short term, such as the examination of hospital or ward isolates. Although different ST types were detected in the strains with the same pattern in PFGE method, ST types which were found similar in MLST method were found in different PFGE patterns. No clonal association was detected with PFGE method in some strains that were identified as ST203. In another sample, three different ST types (ST17, ST117, and ST203) were detected using the MLST method in the strains (numbers 2, 5, and 10) with 100% band similarity which were isolated from Akdeniz University. These results showed that it was impossible to demonstrate the clonal association between the strains using the MLST analysis. Seven protected gene regions were investigated, and the mutations in these genes were identified with the MLST method. However, all chromosome was evaluated by intersecting with restriction enzymes in PFGE method, but again, the mutations of the genes could not be identified. Even one or two base changes in the alleles in MLST analysis resulted with the identification with different ST types. The strain number 25 that was isolated from a blood culture showed a new allelic combination (*atpA* 15; *ddl* 1; *gdh* 11; *purK* 1; *gyd* 21; *pst* 1; and *adk* 1) in the MLST analysis and was entitled as a new sequence type in the MLST database, being recorded as ST733 ([www.mlst.net](http://www.mlst.net)). This strain demonstrated a 100% band profile similarity in strains 20, 21, and 29 in the PFGE method. The strain 21 was typed as ST117 in the MLST analysis.

Calculating the economic costs spent for the prevention or treatment of VRE infections is very difficult but it is an undeniable fact that these losses bring huge burden for the hospital and for the



economy of the country. Its effect on the health, morbidity, and mortality of patient is also important in addition to the economic burden. Considering all these, the aim must be to obtain beneficial outcomes with the help of the precautionary measures, and to enable the national standardization.

This study has several limitations. First, we collected VRE isolates, only clinical samples which can give valuable insights in understanding the association of clonal lineages and risk factors in the spread and persistence of vancomycin resistance among *E. faecium* strains causing infections in different regions in Turkey. In the present study, 78.8% of the strains were found clonally associated with the PFGE method. However, causality cannot be proven and cannot investigate an ecological study design. Routine screening for VRE at hospital admission was not implemented. Patients may have been colonized on admission or may have acquired VRE in outside hospitals. When the majority of VRE acquisition is due to background acquisition, infection control measures other than active surveillance with contact isolation (such as antimicrobial stewardship, environmental cleaning, and hand hygiene) need to be optimized. We were not able to investigate the status of VRE colonization. We need further large, controlled prospective studies that could provide data. Second, MLST analysis was done only for selected 44 VRE isolates which based on their PFGE profiles (because of financial problems).

In conclusion, we documented the clonal backgrounds and resistance types of VRE<sub>fm</sub> in different hospitals and regions in Turkey. In this study, 78.8% of the strains were found clonally associated in PFGE method. This was an important result and was the first study demonstrating the clonal dissemination between the centers in Turkey. ST203, ST78, ST17, ST117, and ST280 were most frequently detected in these strains and were associated with CC17. The results showed that both methods must be evaluated separately, and there was a requirement for conducting MLST analysis for all strains to identify the accurate prevalence. It was suggested that more clear information can be obtained about the ST types in Turkey if MLST analysis is conducted for all strains. These results will form a basis to the data in Turkey for investigating the patterns of the families associated with evolutionary development, and to learn about the population structure of the VRE<sub>fm</sub>. Dissemination of VRE must be prevented with proper infection control measures and regular VRE screening. The current study had some limitations: We had a relatively short span of time (1 year) for the data and isolate collection, and a limited number of hospitals contributed to the study, although from different regions covering Turkey. Further surveillance studies are needed to obtain the ST map of VRE in Turkey.

#### CONFLICT OF INTEREST

None declared.

#### AUTHORS' CONTRIBUTIONS

FE, Z.A and O.O designed the study and drafted the article. CK participated in the organization of the study. Z.A and FE performed

molecular genetic analysis. A. K performed analysis of the PFGE. Study group participated in the collection of strains and clinical details.

#### ETHICAL APPROVAL

Ethical approval was obtained from Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (no: 2 008 919).

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## APPENDIX A

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