First vancomycin-resistant blood isolate of *Enterococcus faecium* in a children's hospital and molecular analysis of the mechanism of resistance

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SUMMARY: Altun B, Cengiz AB, Kara A, Ceyhan M, Ünal S, Seçmeer G, Gür D. First vancomycin-resistant blood isolate of *Enterococcus faecium* in a children's hospital and molecular analysis of the mechanism of resistance. Turk J Pediatr 2008; 50: 554-558.

The first clinical isolate of vancomycin-resistant *Enterococcus* spp. in Hacettepe University Children's Hospital was isolated from a blood culture of a patient hospitalized in the intensive care unit. He had been on vancomycin therapy for the last four months for consecutive pneumoniae and sepsis. The isolate was identified as *Enterococcus faecium (E. faecium)* and minimal inhibitor concentration (MIC) values were determined as >256 µg/ml and 256 µg/ml for vancomycin and teicoplanin, respectively, with E-test. The isolate was shown to carry the *van*A gene with polymerase chain reaction (PCR). Twelve colonizing strains were isolated from the surveillance cultures during the same period and identified as *E. faecium,* and were also shown to carry the *van*A gene. However, arbitrarily-primed-PCR and pulsed-field gel electrophoresis results could not confirm the source of the resistant strain nor did they suggest a clonal spread in the hospital.

Key words: E. faecium, vancomycin-resistant enterococci, pulsed-field gel electrophoresis, childhood.

Enterococci are intrinsically resistant to many antibiotics and readily become resistant either by mutation or by acquisition of resistance genes from other bacteria[1]. Glycopeptides are important therapeutic alternatives against multiple-resistant enterococci; however, vancomycin-resistant enterococci (VRE) have emerged as nosocomial pathogens and these isolates are usually resistant to multiple antimicrobial agents, a condition that causes difficulties in treatment[2]. Six phenotypes of vancomycin resistance have been identified in enterococci (VanA, VanB, VanC, VanD, VanE, VanG)[3,4]. Van A-type resistance is the most common phenotype and causes high-level resistance to vancomycin and teicoplanin.

Enterococci are normal inhabitants of the human gut. The reservoirs of VRE are mostly environmental surfaces, contaminated equipment and the patient skin[3]. Nosocomial outbreaks caused by glycopeptide-resistant enterococci are increasingly being reported in Turkey[5-9]. The first clinical isolate of vancomycin-resistant *Enterococcus* spp. in Hacettepe University Children's Hospital was isolated from a blood culture of a patient in January 2003. The purpose of this study was to investigate the mechanism of resistance in the first vancomycin-resistant clinical isolate of *Enterococcus faecium* (*E. faecium*) in Hacettepe University Children's Hospital and the potential reservoirs and modes of spread.

Material and Methods

Clinical Isolate

An *Enterococcus* spp. was recovered from the blood culture of a patient in January 2003. He had been diagnosed as a ventricular septal defect and pulmonary hypertension at two months of age and underwent pulmonary binding operation at three months of age. One

month after operation he was re-admitted with pneumonia and was treated with sulbactam ampicillin initially. During his hospitalization he developed urinary tract infection with *Klebsiella pneumoniae* and his treatment was modified as meropenem and amikacin. On the 15th day of hospitalization because of progressive pneumonia with atelectasis, vancomycin was added to his therapy and he was transferred to the pediatric intensive care unit. In the 7th week of hospitalization, he had been on vancomycin therapy for the last four months for consecutive pneumoniae and sepsis. Blood culture yielded VRE. The isolate was identified by Gram's stain, catalase reaction, esculin hydrolysis, and growth in NaCl 6.5%. Identification was confirmed as *E. faecium* with BBL Crystal Identification System (Becton, Dickinson and Company, Maryland, USA).

Surveillance Cultures and Susceptibility Tests for Vancomycin and Teicoplanin

Screening tests for VRE were performed in patients from different wards of the hospital during the same period. From January 2003 to December 2003, 300 perianal and 115 environmental cultures were taken. Perianal swabs were directly plated onto D-coccosel agar (Bio Merieux, France) supplemented with 6 µg/ml of vancomycin and 64 µg/ml of ceftazidime. Minimal inhibitor concentrations (MIC) for vancomycin and teicoplanin were determined by E-test (AB Biodisk, Solna, Sweden) method. CLSI breakpoints were used in the interpretation of the results[10].

Molecular Tests

Bacterial DNA was prepared as described by McLaughlin[11]. Resistance genotypes were determined by polymerase chain reaction (PCR). The amplification of the *van*A gene by PCR was performed as described by Perlada[12]. The oligonucleotide primers used for amplification of *van*A genes were: Van A 1: (5'- ATG AAT AGA ATA AAA GTT GCA ATA C-3') and Van A 2: (5' CCC CTT TAA CGC TAA TAC GAT- 3'). PCR products were then separated by electrophoresis in 1.5% agarose gels with 1 xTAE (40mM Tris-acetate, 1mM ethylenediamine tetraacetate) running buffer. After the gels were stained with ethidium bromide, they were visualized on UV transilluminator (KODAK Gel Logic 200,

New York, USA) and photographed. 100 bp and φx174 were used as molecular weight standards. Isolates with Van A phenotype showed a 1029 bp band.

Arbitrarily-primed PCR (AP-PCR) was performed to examine the clonal similarity of these strains. The AP-PCR primer used was MT 1 (5'-CCT GCG AGC GTA GGC GTC GG-3').

Pulsed-field gel electrophoresis (PFGE) was performed on all isolates to examine the clonal relation of these isolates. *E. faecium* DNA embedded in agarose blocks was prepared as described by Murray[13]. The DNA fixed in the agarose disk was incubated in 20 h in 45 µl of restriction buffer and 20 U Sma I (Takara, Japan). Gels with 1.1% agarose (Genexis Spech bach, Germany) were prepared in 0.5 X TBE buffer (50mM boric acid, 0.2mM EDTA), and the DNA macro-restriction fragments were separated by PFGE (General Navigator, Pharmacia, Uppsala, Sweden).

Results

The index patient was treated with linezolid for five days. The patient's consecutive blood cultures after the first day of linezolid therapy were all negative. He was discharged with tracheostomy tube and home mechanical ventilation for chronic lung disease. His three-year follow up was uneventful with de-cannulation.

Susceptibility Tests

Minimal inhibitor concentrations (MIC) of vancomycin and teicoplanin were >256 µg/ml and 256 µg/ml respectively, suggesting the Van A phenotype.

Screening Tests for VRE

Of 300 perianal cultures, 12 isolates were vancomycin-resistant *E. faecium*. Seven of the 12 patients had congenital heart disease. None of the VRE-colonized patients were in the same ward with the index case. Their details are given in Table I.

Minimal inhibitor concentration values of vancomycin and teicoplanin were >256 µg/ml and 256 µg/ml, respectively, for all of these isolates. None of the environmental cultures yielded *E. faecium*.

Table I. VRE-Colonized Patients

Age	Sex	Underlying disease	Previous vancomycin therapy	Ward	Follow up
5 months	Male	Down syndrome, ventricular septal defect and pulmonary hypertension	Yes Infective endocarditis	Pediatric Unit	Discharged
7 months	Male	Ventricular septal defect	No	Pediatric Cardiac Surgery	Discharged
6 years	Male	Patent ductus arteriosus	No	Pediatric Cardiac Surgery	Discharged
6 months	Female	Tetralogy of Fallot	No	Infant Unit	Discharged
14 months	Male	Down syndrome, Morgagni hernia	Yes	Pediatric Surgery	Discharged
6 months	Female	Pulmonary venous return anomaly, atrial septal defect, pulmonary hypertension	Yes	Infant Unit	Discharged
1 year	Male	Patent ductus arteriosus	No	Infant Unit	Discharged
6 months	Male	Hydrocephalus, Ventriculoperitoneal shunt	Yes	Pediatric Neurosurgery	Exitus
9 months	Female	Encephalocele	No	Pediatric Neurosurgery	Discharged
4 years	Male	Meningomyelocele	No	Pediatric Neurosurgery	Discharged
6 months	Male	Spinal muscular atrophy	No	Infant Unit	Discharged
4 years	Male	Wilms tumor	Yes Febrile neutropenia	Pediatric Unit	Discharged

VRE: Vancomycin-resistant enterococci.

Molecular Tests

All of the vancomycin-resistant *E. faecium* isolates were shown to carry the *van*A gene (Fig. 1). The AP-PCR analysis revealed four different groups (Fig. 2).

Pulsed-field gel electrophoresis results: Isolates showed six distinct patterns in PFGE. Lanes 1, 7, 8 pattern A, lanes 2, 3, 4, 6, 11, 12 pattern B, lane 5 pattern C, lanes 9, 13 pattern D (clinical isolate), lane 10 pattern E, and lane 14 pattern F (Fig. 3).
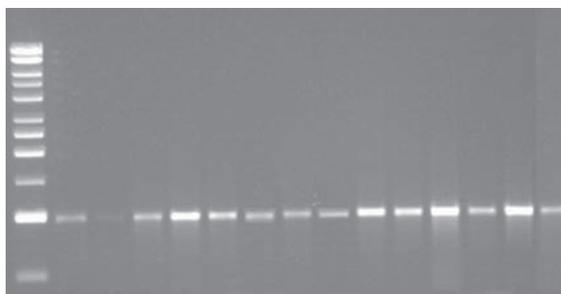

Fig. 1. Positive results. Lane 1, molecular weight marker; lanes 2-15, positive results; lane 12, clinical isolate.
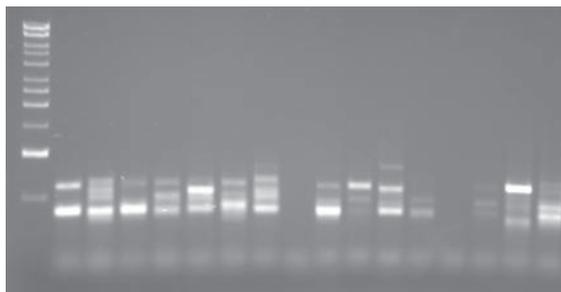

Fig. 2. AP-PCR results. Lane 1, molecular weight marker; lanes 2, 3, 5, 6, 17, different clones; lanes 2, 4, 6, 10, 12, 13, 15, clone A; lanes 5, 7, 11, 16, clone B; lanes 3, 8, clone C; lane 17, clone D; lane 11, clinical isolate.
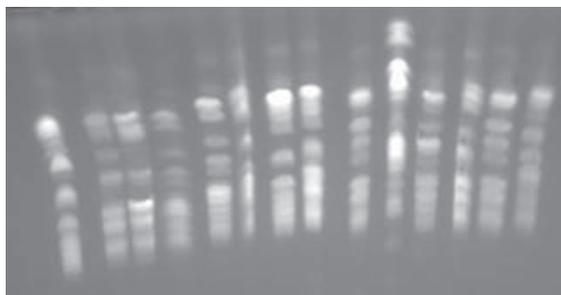

Fig. 3. PFGE results. Isolates showed 6 distinct patterns in PFGE. Lanes 1, 7, 8, pattern A; lanes 2, 3, 4, 6, 11, 12, pattern B; lane 5, pattern C; lanes 9, 13, pattern D (clinical isolate); lane 10, pattern E; lane 14, pattern F.

Discussion

Enterococci are responsible for serious nosocomial infections including endocarditis, bacteremia, urinary tract and neonatal infections. Emergence of resistance to multiple antimicrobial agents among enterococci poses an important problem in treatment. In Europe, VRE was first reported from England and France in 1988[14,15]. In Turkey, the first clinical isolate was reported from Antalya in 1999[16]. The first outbreak with VRE was reported in Europe in 1988, and since then, VRE have been identified in many other countries worldwide, including Turkey[3,5,6,8,9,12,17,18].

Selective antibiotic administration is a risk factor for VRE colonization or infection[3]. Vancomycin and third-generation cephalosporin use has been accepted as a risk factor for VRE infection or colonization[4]. In this study, vancomycin-resistant clinical isolate of *E. faecium* was isolated from the blood culture of an infant who had been treated with vancomycin and meropenem for four months. Five of 12 patients who were colonized with VRE had also received vancomycin treatment before the isolation date. These five patients who were colonized with VRE had also received meropenem with vancomycin. Five of seven patients had not received any antibiotics and the other two patients had only received meropenem and ampicillin.

Enterococci may have both intrinsic and acquired mechanisms of antibiotic resistance. The Van A type resistance is acquired and inducible and causes a high-level resistance to vancomycin and teicoplanin[17]. All the isolates in this study showed high-level resistance to vancomycin (MIC >256 µg/ml) and teicoplanin (MIC=256 µg/ml), which suggests a Van A phenotype. They were also shown to harbor the *van*A gene by PCR with primers specific for *van*A.

Enterococci are common inhabitants of the gastrointestinal tracts of humans and animals[19]. Resistance genes for glycopeptides can be disseminated either by clonal dissemination of the resistant isolates or by the spread of resistance genes between different strains[4]. There seems to be genetic heterogeneity in VRE isolates in Hacettepe University Children's Hospital. Thirteen isolates showed six distinct PFGE patterns, which suggests that there are at least six different VRE clones in our hospital. None of the colonizing VRE shared a common clone with the clinical isolate. The isolates with the same clonal pattern were isolated from patients who were in different wards. A common ward could not be shown for VRE isolates.

Vancomycin-resistant enterococci are transmitted mostly via direct contact through the hands of healthcare workers, colonized patients and contaminated equipment[3]. In this study, environmental cultures were all negative for VRE and there was no clonal relationship between the isolates, which suggests that the clinical isolate was not acquired from the hospital. These results suggest that in our hospital, infection control measures taken according to the recommendations of the Hospital Infection Control Practice Advisory Committee have been appropriate. The first clonal cases from Turkey were reported in 2007 by Cömert et al.[20]. In these reports, the first isolate was obtained from a wound culture of a patient in an intensive care unit and five more isolates were from screened rectal swabs (n: 205), skin swabs (n: 67) and environmental samples (n: 123)[20].

According to the results of this study, the reservoir of the clinical isolate of VRE could not be determined and the glycopeptide-resistant genes found in the colonizing enterococci from different wards do not suggest the spread of a single resistant clone in our hospital. Routine surveillance cultures are beneficial for monitoring the spread of VRE isolates and for avoiding nosocomial outbreaks in the future.

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