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Vancomycin-resistant *Enterococcus faecium* infection in three children given allogeneic hematopoietic stem cell transplantation: clinical and microbiological features

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ABSTRACT

Background and Objectives. **The emergence of vancomycin-resistant enterococci (VRE) as nosocomial pathogens is a major problem in the US; in Europe, VRE nosocomial infections are uncommon and only rarely have been reported in Pediatric or Neonatal Units. The aim of this study is to report on the clinical and microbiological features of VRE infections in 3 children given hematopoietic stem cell transplantation (HSCT).**

Patients and methods. **Five episodes of vancomycin-resistant *Enterococcus faecium* (VREF) infection were diagnosed in 3 children given an allogeneic HSCT. Molecular methods, such as random amplification of polymorphic DNA (RAPD) fingerprinting and automated ribotyping, were used in order to define the circulation of strains.**

Results. **All the isolates were resistant to all commercially available agents and showed the VanA genotypic profile. All children were successfully treated with the combination of quinupristin/dalfopristin (QD) plus teicoplanin (TEC), although treatment was not sufficient to eradicate the micro-organism promptly from the gastrointestinal tract. All our children are still alive. After the first isolation of VRE, a surveillance protocol was started and we documented that the rate of colonization in children and their mothers was less than 1.5%. The RAPD method demonstrated the possible nosocomial transmission of one strain.**

Interpretation and Conclusions. **Our experience demonstrates that VRE infection is a life-threatening complication in children given HSCT. Prompt diagnosis of this infection and its treatment with the combination of QD and TEC can successfully manage this severe infection in profoundly immunocompromised patients.**

Key words: vancomycin-resistant enterococci, bone marrow transplantation, teicoplanin, quinupristin/dalfopristin, RAPD, automated ribotyping

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Patients given hematopoietic stem cell transplantation (HSCT) after myeloablative therapy experience an unavoidable period of severe neutropenia, usually limited to the first 2-4 weeks after transplantation.[1] Associated with neutropenia, there is almost always disruption of mucosal surfaces of variable duration, and profound impairment of humoral and cellular immunity, lasting for months after transplantation.[2,3] In addition, patients receiving an allogeneic HSCT may develop graft-versus-host-disease (GvHD) of variable severity, leading to further immune suppression due to both GvHD itself and immunosuppressive drugs needed for treating this complication (corticosteroids, anti-thymocyte globulin, etc.).[4] It is, therefore, not surprising that such patients are exposed to an increased risk of life-threatening infections. Although infection-related mortality has been reduced over the past two decades by the introduction of highly effective antibiotic therapy, infections still represent one of the major causes of death in HSCT recipients.[5,6]

Enterococci are facultative anaerobic, Gram-positive cocci, existing in humans as normal commensals of the gastrointestinal and vaginal tracts, as well as of the oral cavity. The recent emergence of vancomycin-resistant enterococci (VRE) seems to be related, at least in the United States (US), to the increasing use of antibiotics, such as cephalosporins and quinolones, for prophylaxis of Gram-negative infections, and more directly attributable to the use of vancomycin, particularly in the hospital setting.[7] In Europe, colonization appears to occur frequently in people outside the health care settings and evidence suggests that foodborne VRE may cause human colonization. The zoonotic reservoir of VRE seems to be in animals which received, at subtherapeutic dosages, the glycopeptide avoparcin as a growth-promoting agent.[8] After the first outbreak report in 1988,[9] a rapid increase of VRE infections has been

reported from US hospitals; to date, almost 15% of enterococci causing nosocomial infections are vancomycin-resistant.[10] In Europe, VRE nosocomial infections are rare[11] and, to our knowledge, only rarely have outbreaks of VRE infections have been reported in Pediatric or Neonatal Units. We report our experience in a Pediatric HSCT unit with five episodes of vancomycin-resistant *Enterococcus faecium* (VREF) infections occurring in 3 children given an allograft. The children were successfully treated with the combination of quinupristin/dalfopristin (QD) and teicoplanin (TEC).

Design and Methods

The patient's clinical characteristics are shown in Table 1.

Patient #1

An 18-month old male with hemophagocytic lymphohistiocytosis (HLH) developed two sequential episodes of VREF septicemia after HSCT from an unrelated donor. On day +60 following HSCT, after the marrow had been successfully engrafted (WBC = 3.5×10^9/L; Hb = 10.9 g/dL; Plt = 28×10^9/L), the patient, while being treated with cyclosporine and methylprednisolone for cutaneous grade II acute GvHD, developed fever, abdominal pain and hemorrhagic enteritis. VREF was isolated from all blood cultures performed. The central venous line was removed and the patient was treated with QD (7.5 mg/kg every 8 hours) for 21 days and with TEC (10 mg/kg/day) for 21 days. This treatment was well tolerated, with the exception of mild nausea, and led to resolution of clinical symptoms; blood cultures became negative.

Twelve days after antibiotic discontinuation, the child re-experienced fever and bacteremia in the absence of any other clinical manifestations observed during the first episode. VREF was again isolated from blood cultures and the patient was retreated with QD at the same dosage for 53 days and with TEC for 7 days, until blood cultures became negative. Stool cultures always remained negative. The child was discharged from hospital. During outpatient follow-up, VREF was isolated from feces ten days after the patient's discharge. No antibiotic treatment was prescribed and no further isolation of VREF from different clinical samples was obtained. The child is presently alive and well, in complete remission of the primary disease.

Patient #2

A 20-month old female with acute lymphoblastic leukemia received an unrelated cord blood transplant. A few days after HSCT, a strain of VREF was isolated from a rectal swab. Four months later, after having been discharged from the hospital in good hematologic condition with stable engraftment (WBC = 7.3×10^9/L; Hb = 9 g/dL; Plt = 37×10^9/L), she developed a spiking fever (over 39°C), associated with abdominal pain, vomiting and diarrhea. Physical examination revealed a tense abdominal wall and X-ray showed a picture of ileus with intestinal pneumatosis, localized to the distal small bowel and proximal colon. VREF was isolated from two of the three blood cultures performed and from feces. Supportive management included abdominal decompression with rectal and nasogastric aspiration; the central venous line was removed. Treatment with QD (7.5 mg/kg every 8 hours) for 22 days and TEC (10 mg/kg/die) for 12 days led to resolution of clinical symptoms and disappearance of the organism from blood and stool. VREF was again isolated from a rectal swab 10 days after completing treatment. The child was then in a good clinical state and antibiotic treatment was withheld; at present, she is in complete remission of the primary disease 18 months after transplant. VREF was no longer isolated from surveillance cultures.

Table 1. Patient characteristics.

	AL	*PG*	*BG*
Age at HSCT (months)	18	20	25
Diagnosis	HLH	ALL	AML
Sex	Male	Female	Male
Conditioning regimen	TBI-TT-CY	TBI-TT-CY	TBI-TT-CY
Source of stem cells	Bone marrow	Cord blood	Bone marrow
Type of donor	Unrelated donor	Unrelated donor	Unrelated donor
GvHD prophylaxis	Cs-A+MTX+ALG	Cs-A+PDN+ALG	Cs-A+MTX+ALG
Nr. VRE isolates/sites	3 (blood 2, rectal swab 1)	7 (blood 1, rectal swabs 3, feces 3)	9 (blood 1, rectal swabs 5, feces 1, abdominal drainage 1, conjunct. swab 1)

HSCT = hematopoietic stem cell transplantation; HLH = hemophagocytic lymphohistiocytosis; AML = acute myeloid leukemia; ALL = acute lymphoblastic leukemia; TBI = total body irradiation; TT = thiotepa; CY = cyclophosphamide; GvHD = graft-versus-host disease; Cs-A = cyclosporin-A; MTX = methotrexate; PDN = prednisone; ALG = antilymphocyte globulin.

Patient #3

A 25-month old boy with acute myelogenous leukemia received a bone marrow allograft from an unrelated donor. One month after HSCT, VREF was simultaneously isolated from rectal and conjunctival swabs and, a few days later, while the child was on antibiotic treatment with TEC and imipenem-cilastatine, from blood cul-

tures. At that time, the patient had already engrafted (WBC = 11.5×10⁹/L; Hb = 9.5 g/dL; Plt = 14×10⁹/L) and was receiving cyclosporine and methylprednisolone for grade II acute GvHD. The infection was characterized by fever, abdominal pain and hemorragic enteritis. The boy was treated with QD (7.5 mg/kg every 8 hours) for 40 days and with TEC (10 mg/kg/die) for 21 days, with resolution of clinical symptoms and clearance of the organism from blood and stool. The central venous line was removed. Conjunctivitis was mild and was treated with topical application of ofloxacin.

Fifty days after complete resolution of the first episode, the child had recurrence of fever, with abdominal pain and isolation of *E. faecium* from stools. He was again treated with QD (7.5 mg/kg every 8 hours) for 20 days, with resolution of clinical symptoms and stool cultures becoming negative. The child was discharged from the hospital at the end of treatment. During follow-up, VREF was again isolated from feces two months later. He did not receive antibiotic treatment and no further isolation of VREF was obtained. Fifteen months after HSCT he is in good clinical condition and complete remission of the primary disease.

Bacterial strains

Nineteen enterococcal strains were isolated from patients (see Table 2). All the isolates were identified as enterococci by phenotypic methods. Identification was also confirmed using the automated RiboPrinter® microbial characterization system (Qualicon, Wilmington, DE, USA). Susceptibility tests were performed on all enterococcal strains using the disk diffusion method on Mueller-Hinton agar with a 24-hour incubation at 35°C; the results were expressed as susceptible, intermediate or resistant according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) for the modified Kirby-Bauer method. We also evaluated the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for vancomycin, teicoplanin, quinupristin/dalfopristin, ampicillin, penicillin and gentamicin, using a broth macrodilution method according to the NCCLS criteria.[12] On three representative strains for any patient (i.e. strains isolated from different sites) we evaluated the type of genotypic resistance and their similarity using random amplification of polymorphic DNA (RAPD), fingerprinting and automated ribotyping. The tested strains are shown in Table 2.

Genotypic resistance

We performed amplification of VREF DNA through polymerase chain reaction (PCR) with selected primers. Total enterococcal DNA was extracted using standard procedures (lytic agents, extraction with phenol-chloroform and precipitation with ethanol) and then amplified as described by Dutka-Malen *et al.*[13]

Table 2. List of the strains analyzed for similarity pattern with RAPD and ribotyping and their MIC-MBC for different antibiotics.

Pt.	Site (date)	VAN		TEC		Q/D		AMP		P		CN
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	HLR
AL1	blood (06.15.98)	>64	>64	16	32	0.25	0.5	64	>64	>64	>64	≤500
AL2	blood (07.21.98)	>64	>64	16	32	0.25	0.5	64	>64	>64	>64	≤500
AL3	feces (10.02.98)	>64	>64	16	64	0.25	0.5	64	>64	>64	>64	≤500
PG1	rectal swab (06.19.98)	>64	>64	16	64	0.25	0.25	>64	>64	>64	>64	≤500
PG2	blood (09.29.98)	>64	>64	16	64	0.25	0.5	>64	>64	>64	>64	≤500
PG3	feces (10.02.98)	>64	>64	16	64	0.25	0.25	>64	>64	>64	>64	≤500
BG1	conj. swab (02.02.99)	>64	>64	8	64	0.25	0.5	>64	>64	>64	>86	≤500
BG2	rectal swab (02.05.99)	>64	>64	16	64	0.25	0.5	>64	>64	>64	>64	≤500
BG3	blood (02.16.99)	>64	>64	16	64	0.25	0.5	>64	>64	>64	>64	≤500
IC	rectal swab	>64	>64	32	64	16	32	16	64	32	64	>500

VAN = vancomycin; TEC = teicoplanin; Q/D = quinupristin/dalfopristin; AMP = ampicillin; P = penicillin; CN = gentamicin; HLR = high level resistance (500 μg/mL). All values are expressed in μg/mL. IC is a strain of VRE previously isolated in our Laboratory that was used as internal control.

RAPD fingerprinting

We used six purified primers, ranging in size from 10 to 25 bases, with different nucleotide-proportions (G+C content), used in previous experiments[14] and supplied by Life Technologies, Italy.

Amplification reactions were performed in a 50 μL volume containing PCR buffer, 1.5 μM $MgCl_2$, 200 μM of each dNTP, 1 μM of each single primer, 20 ng of enterococcal DNA and 2.5 U of Stoffel Fragment Taq polymerase (Perkin Elmer Europe). After a first step at 94°C for 2 min samples were cycled 50 times through the following temperature profile: 94°C for 5 sec., 36°C for 30 sec., 72°C for 1 min. The samples were finally incubated at 72°C and at 60°C (10 minutes per step). Each PCR was repeated at least three times in independent experiments. Ten microliters of each amplification reaction were then loaded onto 3% (w/v) agarose gel with TAE buffer containing 0.5 μg/mL (w/v) ethidium bromide. RAPD markers were scored as present or absent and coded as two-state characters in order to calculate a matrix based on the Dice similarity index.[15]

Automated ribotyping

In order to confirm the strain identification and to type isolates, we used the automated RiboPrinter®.[16,17] The obtained fingerprints are normalized digital representations of the genetic data of the isolated organism. The process

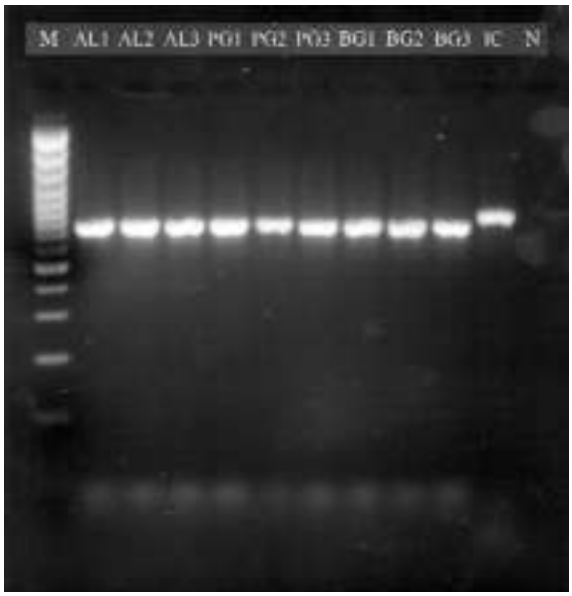

Figure 1. Genomic DNA PCR analysis of our isolates. Lane 1: phage fX174 digested with *Hae*III (used as molecular weight marker); lanes 2-10: the 732 bp amplification product of the nine isolates, described in Table 2, that is specific for *Van*A genotype; lane 11: a strain of *E. gallinarum* (internal control) shows an amplification product of 822 bp; lane 12: negative control.

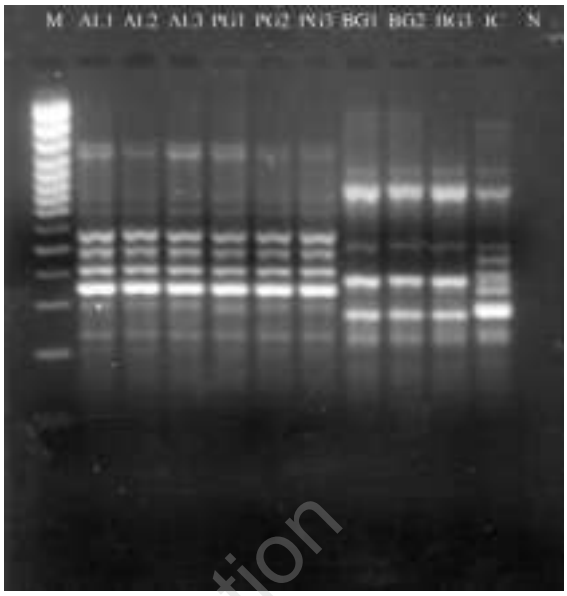

Figure 2. RAPD fingerprinting of the nine isolates obtained with primer AB-15. See Table 2 for further details. Lane 1: phage fX174 digested with *Hae*III (used as molecular weight marker); lanes 2-4: patient AL (samples 1-3); lanes 5-7: patient PG (samples 1-3); lanes 8-10: patient BG (samples 1-3); lane 11: internal control; lane 12: negative control.

has been previously described in detail.[16,18] The standard process performs restriction enzyme digestion with *Eco*RI and hybridization with a rRNA gene probe (*E. coli* region encoding the rRNA 16S-23S-5S genes and the spacer region including Glu-tRNA). In order to enhance the discriminatory power of the method, we performed further digestion analysis using other restriction enzymes (*Pst*I, *Bam*HI and *Ase*I).

Results

Susceptibility tests showed resistance of all strains tested to macrolides, floroquinolones, ampicillin, amoxicillin/clavulanate, glycopeptides, aminoglycosides, cotrimoxazole and carbapenems. Only for chloramphenicol was a profile of intermediate sensitivity was obtained. All strains appeared to be susceptible to quinupristin/dalfopristin. The results of MIC-MBC performed on our isolates are shown in Table 2. All strains showed the *Van*A profile of high resistance to glycopeptides, which was confirmed by detection in all strains of the VanA genotypic profile (Figure 1). Two of the 6 different primers chosen for RAPD analysis (whose 5'-3' sequences are GGAGGGTGTT and AGGGAACGAG) allowed the most reproducible patterns to be obtained. RAPD fingerprinting produced by the selected primers are shown in Figure 2. Automated ribotyping with *EcoRI* identified strains as *Enterococcus faecium*. No differences were noted between the several digestion patterns obtained using a number of restriction enzymes (*Eco*RI, *Pst*I, *Bam*HI and *Ase*I, Figure 3). After the first isolation of VRE, a surveillance protocol was started: in particular, rectal swabs were collected weekly from children and their mothers. The rate of colonization was less than 1.5%. The health-care workers were checked every six months and no case of colonization was found.

Discussion

Over the past decade, improvement of supportive care in hematology units has led to better prevention and more effective treatment of infections in neutropenic patients. Since introduction of oral antibacterial prophylaxis with quinolone derivatives, which are selectively active against Gram-negative organisms, Gram-positive cocci have become the dominant pathogens.[6] In fact, about 50% of microbiologically documented infections are due to this type of pathogen. The policy of employing glycopeptide antibiotics (either vancomycin or teicoplanin) as empirical first-line antimicrobial treatment bears the risk of spreading resistance to these antibiotics among enterococci, thus increasing the potential danger represented by a wide clinical variety of

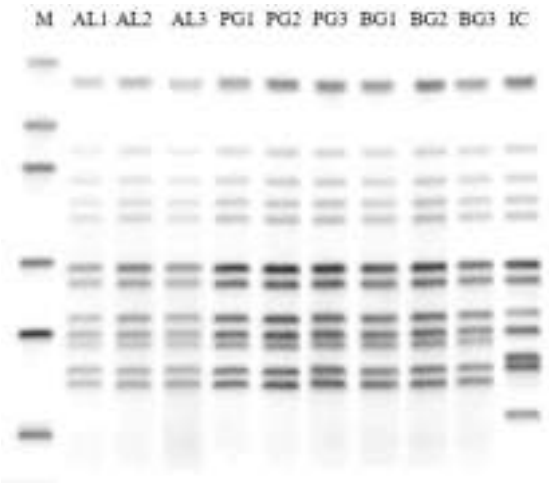

Figure 3. Automated ribotyping obtained with *Pst*I restriction enzyme (modified from the image generated by Riboprinter®).

VRE. The source of these micro-organisms is usually considered to be endogenous and nosocomial transmission of VRE, possibly by means of fecal spread, has been described among pediatric patients.[19,20] However, VRE infections have been reported in only small numbers of children, and only rarely has life-threatening infection due to VRE been described in these patients.[21-24]

Our children had profound impairment of immune function; they were VRE stool carriers at the time of bacteremia and all had a prolonged stay in hospital prior to first culture positivity. They also had been treated with broad-spectrum cephalosporines for various infections in the past. All these factors increase the risk of developing VRE infections.[25] In one patient, VRE was isolated from a conjunctival swab. Notably, this localization has not been previously described in literature. Transmission of the micro-organism via the hands of the patient can be hypothesized. In this case, the low pathogenicity of VRE was demonstrated by the rapid eradication of infection by means of topical therapy.

The optimal treatment for severe infections caused by these multiresistant organisms has yet to be determined and represents a major challenge for the clinician. The micro-organisms isolated in our patients were resistant to all commercially available antimicrobial agents; only chloramphenicol showed a profile of intermediate susceptibility. QD, an antibiotic not yet available in Italy, appears effective *in vitro*. Many studies have proposed various treatments for VRE infections, using for example the association vancomycin, penicillin and gentamicin,[26] quinolones,[27] chloramphenicol,[28,29] and QD.[30,31] New promising drugs under investigation include oxazolidinones and fluoroquinolones, but no exhaustive clinical trials are available. We decided to treat our patients with a combination of QD and teicoplanin, postulating a synergistic effect similar to that described for methicillin-resistant *Staphylococcus aureus*. In all cases, treatment was well tolerated. The only treatment-related adverse event we observed was mild nausea during drug infusion, none of the patients requiring discontinuation of therapy because of this symptom. Recently, two reports on patients who developed VRE infection after either autologous or allogeneic HSCT have been published.[32,33] In the large study on patients given autologous transplant of peripheral blood progenitors, 10 out of 321 recipients developed bacteremia during the first 2 weeks after transplantation and one patient died of VRE endocarditis.[32] By contrast, 17 out of 20 patients with VRE bacteremia reported in the study on both allogeneic and autologous HSCT recipients died, this suggesting that patients given an allograft seem to be exposed to an increased risk of VRE-related death.[33] Moreover, an increased risk of graft failure was observed in this latter population. Despite the fact they received allogeneic HSCT from unrelated donors, all our children are still alive and none experienced graft failure. The success in the treatment of our patients may be attributed to the timely diagnosis of VREF infection (which can be confused with gastrointestinal GvHD in allograft recipients) and to the combined therapy with QD and TEC. Prompt removal of the central venous line, which is easily colonized by this micro-organism, may have contributed to the resolution of VREF bloodstream infection, as well. Since after treatment discontinuation VRE were isolated from surveillance cultures, it can be concluded that treatment was usually not sufficient to eradicate the micro-organism from the gastrointestinal tract. Nevertheless, in this high-risk population, prompt treatment of severe VRE infection with QD and TEC was highly successful and, although prolonged therapy was required in all cases, toxicity was minimal. Even though these data should be confirmed in a larger population, they suggest that the combination of QD and TEC can be effective for treatment of life-threatening VRE infection in immunocompromised children.

In our study RAPD typing showed that two different genotypes of VREF were involved. This could suggest a cross-infection between patients AL and PG; it is noteworthy that the two children were hospitalized during the same period. Patient BG was hospitalized six months later and

the RAPD fingerprinting of enterococci isolated from this patient is different from those obtained from the other two patients. RAPD typing of genomic DNA is easy to perform; however, this technique needs experimental calibration.[34] Previous reports have emphasized that selected groups within species, such as VRE, may be groups with lower ribotyping discrimination.[35] Our study confirms that, in the case of VRE, ribotyping is less discriminating than RAPD, even if using different restriction enzymes. In our experience, *Pst*I, *Bam*HI and *Ase*I showed no great advantage in typing VRE over *Eco*RI. Other experiments with different restriction enzymes are needed in order to evaluate the most discriminating enzyme for VRE.

Contributions and Acknowledgments

EC performed the molecular analysis experiments, was involved in the data analysis and wrote the paper. DB performed the molecular analysis experiments. FL co-ordinated the transplant procedures and wrote the paper. EG and NP were responsible for the management of the patients. LP performed the susceptibility tests on isolates. PG and PM contributed to the clinical and laboratory management of VRE infection. FB was responsible for the management of the patients and wrote the paper.

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Disclosures

Conflict of interest: none.

Redundant publications: no overlapping with previously published papers.

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Potential implications for clinical practice

- Our report confirms the efficacy of QD plus teicoplanin in treating VRE infections in children. Once VRE is isolated, a surveillance protocol, based on molecular techniques, is needed.

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