ORIGINAL ARTICLE

Surveillance of intestinal colonization and of infection by vancomycin-resistant enterococci in hospitalized cancer patients

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Objective: To study epidemiologic features of and risk factors for intestinal colonization and infection by vancomycin-resistant enterococci (VRE) in cancer patients.

Methods: During a 41-month period, over 7600 fecal samples and all samples from sterile sites from hospitalized cancer patients were screened for VRE. Species were identified and isolates analyzed by pulsed-field gel electrophoresis (PFGE) of *Sma*I DNA restriction fragments. Antibiotic resistance was characterized by MIC determinations, and polymerase chain reaction for *vanA*, *vanB*, and *vanC*$_1$ genes. Plasmid contents were analyzed before and after *Pst*I and *Hind*III restriction, and by Southern hybridization with a *vanA* probe. Two case-control studies were performed to identify risk factors for colonization or infection by VRE, respectively.

Results: Eighty-two isolates were recovered from 81 patients. Most (72%) isolates were *Enterococcus faecium* VanA/*vanA*, with 37 different PFGE types, each of which was found in only one to four patients, except for type P1, which was found in 20 patients hospitalized over a 3-month period in the pediatric wards. Plasmid analysis suggested that only two types of plasmid were carrying gene *vanA*, as part of a transposon related to transposon Tn*1546* from reference strain *E. faecium* BM4147. Seventy-seven patients were colonized during the study period. Six of them became infected. Four patients were infected but not colonized. Only one patient died during the course of infection, but intestinal colonization persisted for months in the survivors. Case-control analysis revealed that cephalosporin treatment was a significant risk factor for colonization. No significant risk factor for infection was found in colonized patients.

Conclusion: Colonization by VRE was mostly endemic and the colonized patients were not often infected. However, when clustered cases of colonization occurred, they were then associated with an increased rate of infection.

Key Words: *Enterococcus,* glycopeptide, resistance, colonization, cancer, genotyping

INTRODUCTION

The intestinal tract is a recognized major source of Gram-negative bacteremia in cancer patients. Enterococci are also natural inhabitants of the intestinal tract [1] and cause a significant number of cases of bacteremia in cancer patients [2,3]. Because of their increasing resistance to antimicrobial agents, enterococcal infections are often difficult to treat and require the use of glycopeptide antibiotics [4]. In addition, isolates of enterococci which are also resistant to glycopeptides have been described [5] and are referred to as vancomycin-resistant enterococci (VRE). The genetics and biochemistry of this resistance have been extensively studied and recently reviewed [6]. These VRE have been recognized as an increasing cause of infection in hospitalized patients [7–11].

Since vancomycin is often used empirically in cancer patients against Gram-positive microorganisms,

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VRE represent a major threat that this regimen may be ineffective.

In order to assess this risk, we performed a long-term surveillance study in patients hospitalized in a large cancer referral center and showed that although colonization was mostly endemic, clustered cases of colonization could occur and then be associated with an increased rate of infection.

MATERIALS AND METHODS

Surveillance of intestinal colonization

From 1 December 1989 to 30 April 1993, a surveillance system was established to detect VRE intestinal colonization in patients hospitalized at the Institut Gustave-Roussy, a 420-bed, tertiary-care, cancer referral treatment center located in the Paris (France) suburbs. Over that time, the clinicians were asked to obtain a fecal sample from their patients whenever they felt that they were at risk of infection. All fecal specimens arriving in the central microbiology laboratory were screened for the presence of VRE by plating the feces on bile esculin agar (Difco Detroit, Mich.) supplemented with 10 μg/mL of vancomycin (E. Lilly, Paris, France). This concentration was chosen because preliminary experiments (not shown) showed that: (1) it was highly selective for VRE, (2) the medium could be stored for up to 7 days at 4 °C before use without appreciable loss of selectivity, and (3) it allowed ample growth of reference strains *vanA Enterococcus faecium* BM4147 [5], *vanB Enterococcus faecalis* V583 [12], and *vanC Enterococcus gallinarum* BM4174 [13] (kindly provided by R. Leclercq and P. Courvalin, Institut Pasteur, Paris, France) (data not shown). In addition, this concentration of vancomycin has been used by others [14] for selection of VRE isolates.

After 48 h of incubation at 37 °C, species were identified using the API-20 Strep technique (Bio-Mérieux, La Balme les Grottes, France) according to the recommendations of the manufacturer.

Detection of cases of infection

During the study period, all microbiological samples arriving in the laboratory for diagnosis were screened for the presence of enterococci on non-selective agar and on bile esculin agar without antibiotics. Clones identified as enterococci using the API-20 Strep technique were further tested. Resistance to glycopeptide was suspected when a zone with a reduced diameter was observed in the disk susceptibility testing assay using vancomycin (30 μg) and teicoplanin (30 μg) disk, as recommended by the manufacturer (Diagnostic Pasteur, Paris, France).

Antimicrobial susceptibility testing

Susceptibility to gentamicin (500-μg disks) and ampicillin (30-μg disks) was assayed using the disk diffusion technique on Mueller-Hinton Agar (Diagnostic Pasteur). Results were interpreted as previously described [15]. Beta-lactamase production was assayed by the nitrocefin disk technique (Cefinase; BBL, Baltimore, Maryland). Minimum inhibitory concentrations of vancomycin and teicoplanin were determined by the agar dilution method [16]. VanA/VanB and VanC phenotypes were determined as described [6].

Detection of *van* genes

The presence of *vanA*, *vanB* or $vanC_1$ genes was detected using the polymerase chain reaction (PCR). The oligonucleotide primers chosen for amplification were selected from the published sequences [13,17,18] with the assistance of Bisance Software [19]. The primers were complementary to bases 777 to 796 and the sequences were CGT TGA CAT ACA TCG TTG CG and 1191–1172 (ATC CGT CCT CGC TCC TCT GC), 100–119 (GCG CAT CGC CGT CCC CGA AT) and 444–425 (ATC ATC GCA TTC TCT GAG CC) and 605–624 (TGG CTC TTG CAT CAA CTT GC) and 991–972 (TGG GAC AGT GAT CGT GGC GC) for genes *vanA*, *vanB* and $vanC_1$, respectively. DNA extraction was performed, as described elsewhere [20], and 1 ng of DNA was added in 25 μL of reaction mixture containing: 15 mM Tris (pH 8.3); 40 mM KCl; gelatin 100 mg/mL; 200 mM desoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP); 1.75 mM $MgCl_2$ for *vanA* PCR, 2.75 mM for *vanB* PCR, and 2 mM for *vanC* PCR; 1 mM (each) primer; and 0.75 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, NC). A Perkin-Elmer Cetus model 9600 DNA thermocycler was used for a 35-cycle amplification procedure with a first denaturation step for 2 min at 94 °C, and then a 15-s denaturation step at 94 °C, a 15-s annealing step at 58 °C for *vanA* PCR, and at 60 °C for both *vanB* and *vanC* PCRs, a 15-s extension step at 72 °C, with the exception of the last cycle, in which this step lasted 10 min, followed by a holding step at 4 °C, until analysis. PCR products were electrophoresed in a 2% agarose gel in 1× TBE buffer (89 mM Tris; 89 mM boric acid; 2 mM EDTA) for 90 min at 110 V, and stained with ethidium bromide.

Amplimers were characterized as the expected intragenic fragments in *vanA*, *vanB*, or $vanC_1$ genes both with respect to size (415 bp, 344 bp and 386 bp, respectively), estimated on agarose gel electrophoresis, and restriction patterns, as described elsewhere [13,17,18].

Determination of pulsed-field gel electrophoresis (PFGE) types

Genomic DNA was prepared by modifying a previously described procedure [21,22]. In the first step of suspension the cells were directly suspended in the lysis suspension without Big58, and the concentration of proteinase K was increased to 4 mg/mL. Genomic DNA was digested using restriction endonuclease *Sma*I (Boehringer Mannhein, Germany) following the recommendations of the manufacturer. Genomic DNA was then electrophoresed in a 1.5% agarose gel in ×0.5 TBE buffer by using a CHEF DR-II apparatus (Bio-Rad, Richmond, Va). Two runs were used, the first lasting 8 h with initial and final switch times of 4 s, and the second lasting 20 h, with initial and final switch times of 4 and 30 s, respectively. For both runs the start ratio was 1, the voltage 200 V, and the temperature, 15 °C. Gels were then stained with ethidium bromide. Reading of the gels was interpreted with the naked eye by two independent observers who matched their findings for agreement. Two isolates were considered identical when PFGE types differed from one another by only one or two visible bands, as previously described [22,23].

Determination of plasmid fingerprints and *vanA* hybridization

Plasmid contents were characterized using whole plasmid analysis [24] and Southern blot hybridization with a *vanA* probe, either on unrestricted plasmidic DNA, or after *Pst*I restriction to analyze the plasmidic background of *vanA* gene, or after *Hind*III restriction to analyze the structure of the *vanA* transposon, as recommended [25]. The *vanA* PCR procedure described above was used to prepare the *vanA* probe, except that 40 μM of digoxigenin 11-dUTP (Boehringer Mannheim, Germany) was added to the PCR mixture.

Hybridization and detection conditions were as recommended by the manufacturer (Boehringer), except that 2.5% of blocking reagent was used in the detection step.

Descriptive epidemiology and definitions

Colonized patients were defined as carriers of an intestinal VRE isolate in at least one stool culture. Infected patients were defined as those in whom a VRE was isolated from a normally sterile site. Colonized and infected patients were those in whom a VRE isolate was isolated both from a fecal sample and from a normally sterile site. The occurrence of cases of colonization, infection, and colonization and infection was plotted by month.

Case-control studies

A first case-control study was performed to analyze the risk factors for sporadic cases of intestinal colonization by VRE. Only cases of colonization which occurred during the first 36 months of the study were included, because, as will be described below, an outbreak of colonization and of infection occurred afterwards, and the risk factors might have been different during the two periods. A surveillance-for-detection period was defined as the time that elapsed between admission and the day on which the first positive stool culture was passed. Risks factors for colonization, including complete medical history of the underlying disease, and detailed treatments received during the 2 months preceding the first isolation of the VRE isolate, were recorded. Controls were chosen among patients who had not had any stool culture positive for VRE. In order to select such patients, we first defined a surveillance-for-detection period for the potential control patients as starting on the day of admission and ending after a number of days equal to that of the surveillance-for-detection period of the case patient to be matched. Then, the number of stool cultures actually performed in the potential controls during the surveillance-for-detection period was recorded and the final choice of controls was made after matching with the cases for underlying disease (leukemia versus solid tumor), ward, year and month of hospitalization, age (± 5 years), sex, duration of surveillance-for-detection period and number of stool cultures performed for VRE detection during the surveillance-for-detection period. This last item was included to ensure that the probability of detection of intestinal VRE colonization was not lower in non-colonized controls than in colonized cases. Two controls were searched for matching with each case. However, only one adequate control was available for 20 cases.

A second case-control study was performed to investigate risk factors for infection by VRE isolates among the colonized patients. Patients included as cases were those both colonized and infected (see above for definitions). Controls were chosen among patients who were only colonized, i.e. who were only carrying intestinal VRE and had no other positive culture. Two controls were matched with each case. Case patients and controls were matched for sex, age (± 5 years), underlying disease (leukemia versus solid tumor), ward, and year of hospitalization. The same risk factors used for the first case-control study were analyzed.

Clinical data for both studies were obtained by consulting the clinical charts and the main frame computer, and pertinent information was processed and analyzed using EpiInfo software [26]. The matched pairs odds ratio and the Mantel–Haenszel summary

chi-square were calculated for categorical data. The Kruskal–Wallis test was used for continuous variables.

RESULTS

Microbiological results

In all, 7672 stool cultures from approximatively 2250 patients (estimated from the actual number of patients from whom the last 1250 stool cultures were obtained) were screened during the study period. VRE isolates were isolated from 81 patients. In 71 (88%) a VRE isolate was isolated only from feces (one or multiple samples), in six (7%) a VRE isolate was isolated both from a fecal sample and another site (i.e. blood, four patients; pus, two patients), and lastly in four (6%) cases a VRE isolate was isolated only from a normally sterile site (i.e. blood, three patients; pus, one patient with a double infection with two different VRE species isolated 2 months apart) but not from feces. In all instances when a VRE isolate was isolated from a normally sterile site, no other microorganism was present concomitantly in the culture. The distribution of the VRE cases of colonization and of infection over time is shown in Figure 1.

The distributions of *Enterococcus* species, PFGE types and glycopeptide resistance phenotypes among the 82 VRE isolates are shown in Table 1. Seventy-five (91%) isolates were *E. faecium*. Seventy-two (88%) had a VanA phenotype and *vanA* genotype. The concordance between phenotype and genotype determinations of glycopeptide resistance was 100% (not shown). Forty-one isolates (50%) were resistant to ampicillin. None of them were penicillinase positive, and nor were they highly resistant to gentamicin (data not shown).

Analysis of genomic DNA by PFGE among the 79 isolates of *E. faecium* and *E. gallinarum* resulted in 41 different types numbered P1 to P41, examples of which are presented in Figure 2A (lanes 1 to 6). The number of isolates with the same type varied from 1 to 18 (data not shown).

Plasmid fingerprinting was performed on 21 *vanA* VRE isolates with 15 different PFGE types. Sixteen different unrestricted plasmid profiles were obtained (not shown), but results of Southern blot hybridization on plasmidic DNA, with or without *Pst*I restriction, showed that apparently only two types of *vanA* plasmids were present. The first type, referred to as pb20 plasmid, was present in all P1 and P16 PFGE type isolates, and appeared to be similar to plasmid pIP816 present in reference strain BM4147 (Figure 2B, lanes 1, 2, 3 and 6). The other one, referred to as pb10 plasmid, was present in all other tested isolates (for examples see Figure 2B, lanes 4 and 5).

After *Hind*III restriction the probe hybridized with a fragment of similar molecular weight irrespective of whether the plasmid was of pb10 or pb20 type (not shown). This suggested that the *vanA* gene was carried on a transposon related to transposon Tn*1546* in reference strain BM4147.

Epidemiologic results

Once all these data were taken into account, it was possible to distinguish two types of events from an epidemiologic standpoint.

The first type of event comprised cases of colonization which occurred during the whole study period in patients hospitalized in various wards of the hospital (medicine, 25 (41%) patients; intensive care, one (1%) patient; pediatrics, 36 (58%) patients). Among the 49 typed *E. faecium* strains isolated from such cases, no PFGE type was shared by more than four isolates. The epidemic distributions of the PFGE types of isolates which were found in two or more patients are shown in Figure 3. Except for isolates with P6, P18 and P22 PFGE types, which were isolated from patients

Table 1 Distribution of species, PFGE types, glycopeptide resistance phenotypes and sites of isolation among 82 vancomycin-resistant *Enterococcus* isolates from patients hospitalized at the Institut Gustave-Roussy from 1 December 1989 to 30 April 1993

Species	Number of isolates (%)		Number of different PFGE types	Phenotypes			Sites of isolation		
				VanA	VanB	VanC	Feces	Blood	Pus
E. faecium	75	(91.4)	37	71	4	0	72[a]	6[b]	3[a]
E. gallinarum	4	(5)	4	0	0	4	4	0	0
E. faecalis	1	(1.2)	–	0	1	0	1	0	0
E. casseliflavus	1	(1.2)	–	0	0	1	0	1	0
E. avium	1	(1.2)	–	1	0	0	0	0	1
Total	82	(100)	41	72	5	5	77	7	4

[a] Two strains were isolated both from the feces and from pus.
[b] Four strains were isolated both from the feces and from blood.

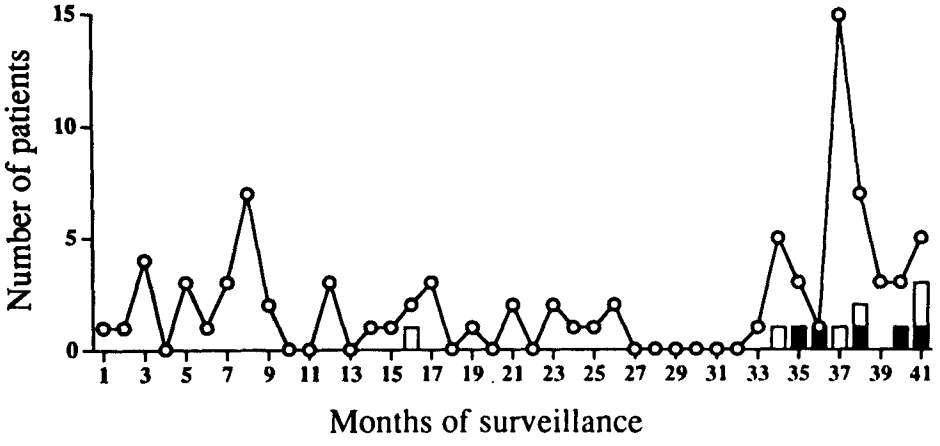

Figure 1 Epicurve of cases of intestinal colonization (○), infection in colonized patients (□) and infection in non-colonized patients (■), by vancomycin-resistant *Enterococcus* isolates in patients hospitalized at the Institut Gustave-Roussy from 1 December 1989 to 30 April 1993.

hospitalized several months apart, patients carrying isolates sharing a common PFGE type were to a large extent clustered in time, but only for isolates with P17 and P21 types were the patients hospitalized in the same wards, suggesting possible cross-contamination. In the other cases, patients were hospitalized in wards located on different floors of the hospital.

Results of plasmid typing showed that isolates with different PFGE types isolated from sporadic cases of colonization were apparently capable of harboring the same *vanA* plasmid. As shown in Figure 4, plasmid pb10 was found in eight vancomycin-resistant *E. faecium* isolates with six different PFGE types and isolated from patients hospitalized over a 30-month period.

Results of the first case-control study, which analyzed the risk factors for sporadic cases of intestinal VRE colonization, showed that the use of second- or third-generation cephalosporins (odds ratio=3.2, $p = 0.047$), and the number of days of treatment with cephalosporins ($p=0.006$), were significantly associated with intestinal carriage of VRE (Table 2). Pre-exposure to parenteral glycopeptides did not appear to be a significant risk factor (Table 2). Oral vancomycin, which was used in only five patients (two cases and three controls), was not significantly associated with VRE colonization.

During the second part of the study period an outbreak occurred. Throughout December 1992 and January 1993, 20 new cases of colonization by *E. faecium vanA* isolates were observed in the pediatric ward. All tested strains isolated from these patients shared the same P1 PFGE type and contained a pb20 plasmid carrying the *vanA* gene (for examples, see

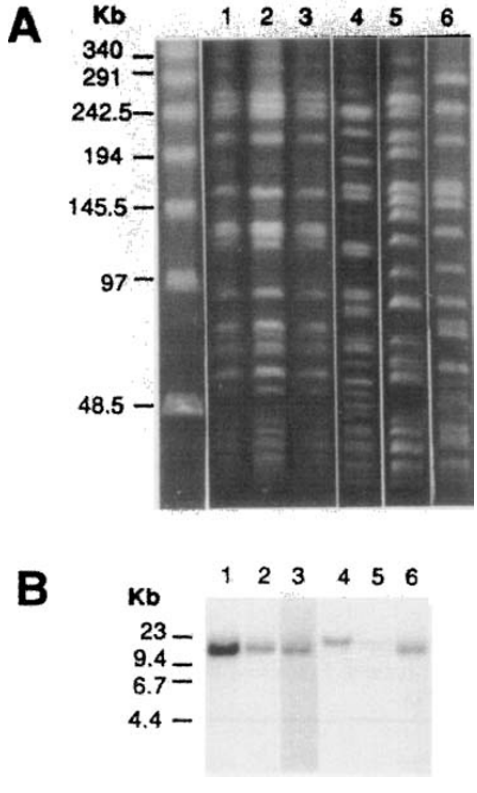

Figure 2 Examples of *Sma*I PFGE types (A) and *vanA*-probed blots of *Pst*I-digested plasmid DNA (B). Lanes 1 to 3, isolates with PFGE type P1 containing plasmid pb20; lane 4, isolate with PFGE type P11 containing plasmid pb10; lane 5, isolate with PFGE type P24 containing plasmid pb10; and lane 6, BM4147 strain containing plasmid pIP816. Lane without number is λ-ladder molecular weight.

Figure 2A, lanes 1 to 3). During this period, 10 cases of infection occurred concurrently in the hospital (Figure 1). In five (50%) of these cases, VRE intestinal carriage was also detected. At the peak of the epidemic, stringent hygiene measures were implemented throughout the hospital and enteric isolation precautions were applied to VRE-colonized patients. This policy was followed by a return to baseline (Figure 1), where VRE colonization and infection rates, have since remained (not shown).

Concerning the patients who were infected over the 42 months of VRE surveillance, 11 cases of infection occurred in 10 patients (six colonized and infected, five infected only). As stated above, one patient was infected twice. Table 3 shows the clinical features and outcomes of these infected patients. Seven patients were bacteremic (six with an *E. faecium* isolate and one with an *E. casseliflavus* isolate). All had a central venous catheter in place at the time of infection; however, none of them developed pain or erythema at the site of entry. Urine cultures were sterile in all seven, but stool cultures were positive in four with the same VRE *E. faecium* strain as that isolated in the blood. Two other patients had intra-abdominal infections. The stool cultures of these two patients were positive for VRE. In patient no. 6, however, the VRE isolated respectively from pus and feces had different PFGE types.

The length of hospital stay before infection ranged from 11 to 142 days (mean 45.5 days) in infected patients. All infected patients were neutropenic (< 500 polynuclear cell/mm^3) at the time of infection, and the duration of neutropenia before infection ranged from 4 to 129 days (means 32.5 days). However, the second case-control analysis found no significant risk factor for VRE infection in colonized patients, neutropenia included (data not shown).

Four *E. faecium*-infected patients received high doses of ampicillin, together with gentamicin. The *E. casseliflavus* bacteremic patient received teicoplanin. One *E. faecium* bacteriemic patient died 2 days after VRE isolation with symptoms consistent with septic shock, and death was directly attributed to VRE bacteremia, whereas three other patients ultimately died as a result of their underlying disease, respectively 20, 35 and 42 days after VRE infection was diagnosed. VRE infection was implicated to a minor degree, if any, in the possible causes of death, since blood cultures were repeatedly sterile several days before death (not shown). In the three colonized and infected patients who survived the VRE bacteremic episode, long-term intestinal carriage persisted even after discharge from hospital (Table 3).

DISCUSSION

Altogether, our results show that, during the 42 months of the study, intestinal colonization occurred endemically in cancer patients hospitalized in our institute. Colonization was caused by multiple different VRE isolates. Subsequent infection, or infection without colonization, was an unlikely phenomena except when an outbreak of colonization occurred.

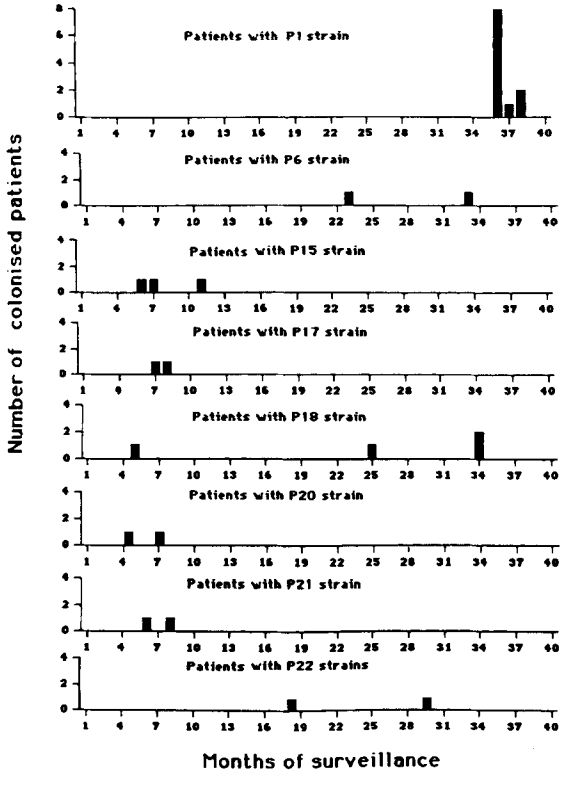

Figure 3 Epicurves of cases of colonization caused by vancomycin-resistant *Enterococcus faecium* isolates with PFGE types found in more than one isolate.

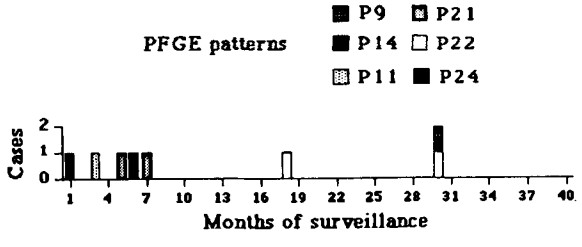

Figure 4 Epicurve of VRE isolates of various PFGE types carrying plasmid pb10.

Table 2 Matched relative risks of VRE colonization in case patients and controls during the endemic period (first case-control study)

Characteristics	Cases (n=45)	Controls (n=70)	Odds ratio	95% CI	p value
Matched characteristics:					
Sex (males)	30	42	–	–	NS
Age (years, mean ± 50)	13 (1–78)	12 (1–71)	–	–	ND
Underlying disease					
Leukemia	8	12	–	–	ND
Solid tumor	37	57	–	–	ND
Length of hospital stay before colonization (median, range)	14 (1–53)	14 (1–45)	–	–	ND
Number of stool cultures performed[a] (median, range)	2 (0–8)	2 (1–7)	–	–	ND
Ward during hospitalization					
Intensive care unit	1	2	–	–	ND
Pediatrics	27	41	–	–	ND
Hemato-oncology	17	27	–	–	ND
Selected risk factors:					
Exposure to cephalosporins[b]	41	42	3.2	1–9.0	0.047
Duration of cephalosporin treatment (days: median, range)	12 (0–38)	5 (0–38)			0.006
Exposure to parenteral glycopeptide[c]	38	49	2.2	0.8–6.7	0.1
Duration of parenteral glycopeptide treatment (days: median, range)	11 (0–39)	7 (0–37)			0.23
Oral vancomycin	2	3	2	0.3–14.2	0.85

CI = confidence limit intervals; NS = not significant; ND = not done.
[a] During the surveillance-for-detection period.
[b] Second- or third-generation cephalosporin.
[c] Intravenous.

In another study in cancer patients a high rate—6.5% of 61 patients—of VRE bloodstream infection was observed. However, the rate of colonization of these patients was very high [27]. Others [11] have shown that VRE infections in non-cancer hospitalized patients, occurred only in a renal unit, where the rate of colonization was three times greater (15% versus 5%) than in patients hospitalized elsewhere in the hospital. In the absence of cases of infections, a 3.5% rate of carriage has been also reported for hospitalized patients [28].

The isolates we obtained were predominantly of the VanA/*vanA* phenotype/genotype. It seems unlikely that the low percentage of *vanB* isolates was due, even in part, to the relatively high concentration (10 mg/L) of vancomycin in our selective medium, since some isolates with a low level of resistance to vancomycin (*vanB* or *vanC*$_1$ genotypes) were isolated, and others [8] have described the same proportion of VanA/*vanA* isolates among VRE isolates.

We also obtained predominantly *E. faecium* isolates, whereas, within the genus *Enterococcus*, *E. faecalis* is usually considered to be responsible for the majority of infections [1,29]. However, a shift towards *E. faecium* [7–9] has been reported recently. This could be explained by the emergence of glycopeptide resistance, predominant in *E. faecium* isolates [3,30]. Indeed, it was shown in one study that the proportion of vancomycin-resistant *E. faecium* increased from 15% to 75% over a 3-year period, when the 3% proportion of vancomycin-resistant *E. faecalis* remained constant [8].

Our results showed that VRE colonization was either endemic or epidemic in our institute. When endemic, it was caused by many different isolates, as determined by PFGE typing. Such an intra-hospital diversity has been reported previously [3,31]. It seems, however, that during this period patient-to-patient transmission occurred in some instances. Such was the case for strains with P17 and P21 PFGE types. Two patients hospitalized in the same ward were colonized with each isolate, respectively, within a limited period of time.

The mechanisms of transmission of VRE isolates have not been investigated in the present study. However, the hands of hospital personnel, as well as contaminated vectors, have been implicated in similar instances [32–34]. Nevertheless, the mechanism of acquisition remains unknown for patients colonized with an isolate not found in others.

Table 3 Clinical features and outcomes of 10 patients infected with VRE (Institut Gustave-Roussy, Villejuif, France

Status	Case no.	Age (years)	Underlying diseases	Source of isolates	Species	PFGE type (site of infection/stool)	Time interval between detection of colonization and infection (days)	Treatment	Outcome	
									Infection	Colonization
Colonized and infected	1	38	Leukemia	Blood culture	*E. faecium*	P19/P19	71	None	Death D2[a]	Still present at death
	2	12	Burkitt lymphoma	Blood culture	*E. faecium*	P1/P1	33	Ampicillin + gentamicin	Survival	VRE long-term[b] carriage
	3	7	Lymphoma	Blood culture	*E. faecium*	P1/P1	18	Ampicillin + gentamicin	Death D20	Still present at death
	4	3	CNS tumor	Blood culture	*E. faecium*	P1/P1	2	Ampicillin + gentamicin	Death D35	Still present at death
	5	6	Burkitt lymphoma	Intra-abdominal abscess	*E. faecium*	P9/P9	3	None	Survival	VRE long-term carriage[c]
	6	52	Intestinal carcinoma	Intra-abdominal abscess	*E. faecium*	P2/P32	3	Ampicillin + gentamicin	Survival	VRE long-term[d] carriage
Infected only	7	5	CNS tumor	Blood culture	*E. faecium*	P1/NA	NA	None	Survival	NA
	8	56	Carcinoma	Blood culture	*E. casseliflavus*	P43/NA	NA	Teicoplanin	Survival	NA
	9	42	Leukemia	Blood culture	*E. faecium*	P18/NA	NA	None	Death D42	NA
	10[e]	72	Carcinoma	Pus	*E. faecium*	P23/NA	NA	None	Survival	NA
				Pus	*E. avium*	P44/NA	NA	None	Survival	NA

[a] Patient died 2 days after the first positive blood culture was performed.
[b] Eight months of follow-up.
[c] Four months of follow-up.
[d] Three months of follow-up.
[e] Patient no. 10 had two episodes of infection 2 months apart caused by *E. faecium* and *E. avium vanA* isolates, respectively.
NA=not applicable.

It has been suggested that the transposable nature of the genetic elements which encode for glycopeptide resistance in enterococci is conducive to dissemination of this resistance [7,25]. In our study the apparent relatedness of plasmids carried by isolates of various PFGE types suggests that a similar phenomenon could be involved. However, here again the precise mode of transmission remains unknown.

Whatever the mechanism of genetic dissemination, we found an association between VRE colonization and previous treatments with second- or third-generation cephalosporins. Such treatments have been previously associated with the occurrence of nosocomial *E. faecalis* bacteremia [8]. However, the precise mechanisms whereby colonization is thus increased remain hypothetical. We did not find a significant association between colonization and parenteral exposure to glycopeptides. This might be explained by the low elimination of glycopeptides by the intestinal tract after parenteral injection. Two reports [33,35] found a significant link between VRE infection and previous glycopeptide treatments, but one [11] found no association with VRE fecal carriage, as in our work.

In all instances, colonization per se did not appear to confer a major risk of infection, since during the first 30 months of the study period only 1/49 (2%) patients became infected with the colonizing VRE isolate.

When the outbreak of colonization occurred, several patients became colonized by the same isolate over a short period of time, and several were infected. Of interest is the fact that some non-colonized patients developed an infection. The simultaneous presence of multiple colonized patients shedding VRE isolates in their feces might have increased the risk of transfer and of direct inoculation of sterile sites, in spite of the fact that the personnel had taken the precautions required for strict hygiene standards. This hypothesis would be consistent with the fact that we found no significant risk factors associated with infection in colonized patients (second case-control study).

Among the 10 infected patients in the present study, only one, a 38-year-old man, died during the septic episode caused by a VRE isolate. Others have reported that the mortality associated with vancomycin-resistant *E. faecium* isolates was lower in adult patients than that related to vancomycin-susceptible *E. faecium* isolates [36]. In another study of VRE infections, an overall mortality of 46.6% has been reported for patients with VRE bacteremia [37]. However, VRE bacteremia cleared respectively in 8/9 (88%), and in 5/6 (83%) patients in two other reports [11,38]. Patients with peritoneal dialysis-associated peritonitis due to VRE also seem to have a fair prognosis [39].

Altogether, the prognosis of VRE infections in our study appeared related to that of the underlying disease. This has also been observed by others [11]. The infected and colonized patients remained colonized even when the infectious episode had cleared. Such prolonged colonization has been reported previously both in oncology [27] and in non-oncology [11] patients. So far, however, no effective decontamination regimen has been reported [27].

Since curative treatment is also difficult, it appears that the prognosis of VRE infections and colonization remains dependent on preventive measures [40].

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