Vancomycin-Resistant Enterococci, Colonizing the Intestinal Tract of Patients in a University Hospital in Greece

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Objectives. Determine the prevalence of Vancomycin-resistant enterococci (VRE) colonizing the intestinal tract of hospitalized patients and define risk factors. Material and Methods. A point prevalence survey of VRE fecal carriage was carried out among patients who stayed at a 600-bed teaching hospital for at least two days. Resistance to vancomycin was detected by the E-test method. Epidemiological data was recorded for all patients included in the study and was used for the risk factor analysis. Results. A total of 128 patients hospitalized for at least two days were enrolled in this investigation. Thirty-nine patients (30.5%) were colonized with vancomycin-resistant enterococci. Twenty-three of the 39 strains were identified as *Enterococcus faecium*, 13 were identified as *Enterococcus gallinarum* and three strains as *Enterococcus casseliflavus*. The risk factors that were significantly associated with VRE colonization included length of hospital stay (13.2 days *vs*. 8.6 days), age (60.7 years *vs*. 47.7 years) and the presence of underlying malignancies (28.2% *vs*. 11.2%). An association was found between VRE colonization and the use of antimicrobials with anaerobic activity, such as metronidazole, piperacillin/tazobactam and imipenem. The use of vancomycin was associated with VRE colonization in the intensive care unit. Conclusions. VRE colonization must be monitored, and risk factors should be determined, because they are useful for screening hospitalized patients for VRE colonization in order to establish prevention and control measures.

Key Words: Vancomycin-resistant enterococci, inestinal tract, hospitalized patients.

Enterococcus species have recently emerged as important nosocomial pathogens [1]. According to data from the National Nosocomial Infections Surveillance System (NNIS), enterococci are the fourth-leading cause of nosocomial infections (third among bacteremias and second among urinary tract infections) in the United States [2-4]. Their resistance to several antimicrobial agents, whether intrinsic (low-level resistance to penicillin, cephalosporins, and aminoglycocides), or acquired (glycopeptides, high concentrations of aminglycosides), is of great concern. Vancomycin-resistant enterococci (VRE) were first isolated in 1986 in Europe [5] and in 1987 in the United States [6]; since then, their presence has increasingly been detected throughout the world. In the United States, the Centers for Disease Control and Prevention recorded a 20-fold increase in the incidence of vancomycin-resistant enterococci (VRE) associated with nosocomial infections between 1989 and 1993 [7]. Since the VanA and VanB vancomycin resistance determinants are transferable, glycopeptide resistance could be passed on to other pathogens, such as methicillin-resistant *Staphylococcus aureus*, thus creating a highly dangerous pathogen difficult to treat with currently-available antibiotics.

There had been only one report of VRE infection in our hospital [8], until recently, when three patients in ICU were identified as having a bloodstream infection with VRE. We conducted a point prevalence culture survey to investigate intestinal colonization with VRE among hospitalized patients. We also recorded epidemiological data in order to determine which patients are at high risk for VRE colonization.

Material and Methods

Prevalence study. In 2005, 22,378 patients were admitted to the AHEPA university hospital, a 426-bed acute and chronic-care facility for an average of seven days per admission. The AHEPA hospital's Ethics Committee approved this study April 10, 2005, prior to the initiation of sample collection. On April 29, 2005, a rectal swab or fecal sample was taken from all patients hospitalized in ICU's for at least two days and from 25% sample of the patients hospitalized for more than two days in all the other departments of the hospital (they where chosen randomly in alphabetical order). Renal and oncology patients that did not for meet the inclusion criterion of hospitalization for at least two days were not included. The specimens were transferred to the microbiology laboratory for selective culture of glycopeptide-resistant enterococci.

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Epidemiological investigation. Epidemiological data was recorded for each patient, including age, sex, prior hospitalization during the last month, prior hospitalization in other departments of our hospital, antibiotic treatment, length of hospital stay, primary diagnosis, surgery, invasive procedures, endoscopies, antibiotic treatment during the last 30 days, use of steroids and medical history.

Selective culture and biochemical identification of vancomycin-resistant enterococci. All samples were plated onto selective D-Enterococcosel agar plates (bioMe´rieux, Marcy l'Etoile, France). They were supplemented with 8 mg of vancomycin per mL agar. All plates were incubated aerobically at 35.8°C for 48 h. From each plate, one or more colonies morphologically resembling enterococci (i.e., dark brown halo) were initially identified by Gram staining, growth in 6.5% NaCl broth, and bile esculin hydrolysis. All presumed enterococci were further identified, as described by Facklam and Collins [9], by using the following physiological tests: lack of pigmentation and motility, pyrrolidonyl arylamidase; leucine aminopeptidase; acid formation from mannitol, sorbitol, sorbose, arabinose, raffinose, and sucrose; arginine hydrolysis; and tolerance to tellurite. All isolates were also identified in parallel with the API-20 S Streptococcus system (bioMe´rieux).

Susceptibility testing. Resistance to vancomycin was detected by the E-test (AB Biodisk, Solna, Sweden). An inoculum with turbidity equivalent to that of a 0.5 McFarland standard and Mueller-Hinton agar were used. Plates were read after incubation at 37°C for 24 h, and the minimum inhibitory concentrations (MICs) obtained by the E-test were rounded to the nearest higher doubling dilution. All enterococci with decreased susceptibility to vancomycin (MICs>4 mg/liter) were subjected to further susceptibility tests by standard agar dilution and broth dilution methods, according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [10]. *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as reference strains. The glycopeptide agents, vancomycin and teicoplanin, were tested. Results were interpreted according to the standards of the NCCLS: for vancomycin, resistance was defined as an MIC of >32 μg/mL and susceptibility was an MIC of <4 μg/mL; for teicoplanin, resistance was an MIC of >32 μg/mL and susceptibility was an MIC of <8 μg/mL [11].

Statistical analysis. Statistical analysis was preformed using the SPSS software. Categorical variables were compared using either Fisher's exact or the χ^2 test. Continuous variables were compared using the Kruskal-Wallis nonparametric test. The standard significance level, $p<0.05$, was used, and all tests of statistical significance were two-tailed.

Results

One-hundred-twenty-eight patients hospitalized for at least two days were enrolled in this investigation. The mean age of the study participants was 51.6 (SD=24.2) years; 72 of them (56.3%) were male. Thirty-nine patients (30.5%) were colonized with VRE strains: 10 (7.8%) in the ICU, 14 (10.9%) in a surgical ward and 15 (11.7%) in an internal medicine ward. No VRE strain was isolated in the pediatric ward.

Twenty-three of the 39 strains were identified as *Enterococcus faecium*, 13 were identified as *Enterococcus gallinarum* and three strains as *Enterococcus casseliflavus*. Table 1 provides the results of the *in vitro* susceptibility tests. Four *E. faecium* strains were resistant to vancomycin and teicoplanin (MICs > 32 μg/mL, VanA phenotype). Nineteen *E. faecium* strains had intermediate resistance to vancomycin (MICs from 6-12 μg/mL) and were sensitive to teicoplanin (MICs from 0.25 to 1 μg/mL, VanB phenotype). Thirteen *E. gallinarum* and three *E. casseliflavus* strains had intermediate resistance to vancomycin (MICs from 6-12 μg/mL) and were sensitive to teicoplanin (MICs from 0.25 to 1 μg/mL, VanC phenotype).

The risk factors that were significantly associated with VRE colonization included length of hospital stay (13.2 days *vs*. 8.6 days, p=0.001), age (60.7 years *vs*. 47.7 years, p=0.002) and the presence of underlying malignancies (28.2% *vs*. 11.2%). Table 2 summarizes the characteristics and risk factors of patients with and without VRE. Isolation of VRE was not significantly associated with stay in the intensive care unit (ICU), surgical ward or internal medicine ward. Prior admission to other medical departments (23.1% *vs*. 10.1%) was associated with VRE colonization, but no specific correlation was identified with a specific department.

The use of antimicrobial agents is summarized in Table 3. The only association found was between VRE colonization and use of antimicrobials with anaerobic activity, such as metronidazole, piperacillin/tazobactam and imipenem. No single antimicrobial agent was associated with VRE acquisition, although prior carbapenem and ureidopenicillin use approached significance (p=0.06). Thus, we explored the possibility of relationships within the different departments. In ICU's, vancomycin use was associated with VRE colonization (40% of patients with VRE in ICU had been treated with vancomycin *vs*. 8.7% of patients not colonized, p=0.03). Surgical procedures, use of central venous catheters, corticosteroid use, diabetes mellitus, mechanical ventilation and gastrointestinal endoscopies were not associated with an increased risk of VRE colonization.

Discussion

In 2003, 15 years after the first published report of clinical strains of vancomycin-resistant enterococci, VRE have become a major threat to hospitalized patients [12]. According to the

Table 1. Antimicrobial susceptibilities of 39 vancomycin-resistant entercoccus strains

Enterococcuss species	Ward	LOS Days	Age Years	Vancomycin		Teicoplanin		Phenotype
				MIC (µg/mL)	S/I/R	MIC (µg/mL)	S/I/R	
E. faecium	ICU	7	27	256	R	256	R	VanA
E. faecium	Int. medicine	12	72	256	R	32	R	VanA
E. faecium	Surgical	11	70	256	R	32	R	VanA
E. faecium	Int. medicine	20	55	64	R	256	R	VanA
E. faecium	ICU	2	70	8	I	0.75	S	VanB
E. faecium	Surgical	27	72	8	I	0.19	S	VanB
E. faecium	ICU	7	41	6	I	0.5	S	VanB
E. faecium	ICU	3	76	6	I	0.19	S	VanB
E. faecium	ICU	19	31	8	I	0.25	S	VanB
E. faecium	Int. medicine	14	41	12	I	1	S	VanB
E. faecium	Int. medicine	23	19	8	I	0.75	S	VanB
E. faecium	Int. medicine	13	67	8	I	1	S	VanB
E. faecium	Int. medicine	11	75	8	I	0.75	S	VanB
E. faecium	Int. medicine	20	54	8	I	0.75	S	VanB
E. faecium	Surgical	25	35	8	I	0.25	S	VanB
E. faecium	Surgical	29	65	8	I	0.5	S	VanB
E. faecium	Surgical	14	51	8	I	0.75	S	VanB
E. faecium	Surgical	26	68	8	I	1	S	VanB
E. faecium	Surgical	15	52	8	I	0.5	S	VanB
E. faecium	Int. medicine	11	72	8	I	0.5	S	VanB
E. faecium	Int. medicine	17	76	8	I	0.75	S	VanB
E. faecium	Int. medicine	6	57	6	I	0.5	S	VanB
E. faecium	Surgical	14	72	8	I	0.38	S	VanB
E. gallinarum	Surgical	10	67	12	I	0.5	S	VanC
E. gallinarum	ICU	32	70	8	I	0.5	S	VanC
E. gallinarum	ICU	16	70	8	I	0.38	S	VanC
E. gallinarum	ICU	30	55	8	I	0.5	S	VanC
E. gallinarum	Int. medicine	4	92	8	I	0.5	S	VanC
E. gallinarum	Surgical	4	75	6	I	0.19	S	VanC
E. gallinarum	Int. medicine	8	46	8	I	0.38	S	VanC
E. gallinarum	Int. medicine	8	71	8	I	0.38	S	VanC
E. gallinarum	Surgical	8	58	8	I	0.75	S	VanC
E. gallinarum	Surgical	6	66	6	I	1	S	VanC
E. gallinarum	Int. medicine	11	73	8	I	0.75	S	VanC
E. gallinarum	Int. medicine	11	70	8	I	1	S	VanC
E. gallinarum	ICU	6	70	8	I	0.5	S	VanC
E. casseliflavus	ICU	6	68	8	I	0.19	S	VanC
E. casseliflavus	Surgical	8	70	8	I	1	S	VanC
E. casseliflavus	Int. medicine	2	31	8	I	0.75	S	VanC

LOS = Length of Stay; ICU = intensive care unit; S = susceptible, intermediate, resistant.

National Nosocomial Infections Surveillance System (NNIS), the percentage of enterococcal isolates exhibiting vancomycin resistance in ICUs in the USA reached 25.2% of 1,579 isolates tested by the year 1999, a 43% increase in the mean rate of resistance compared with the years 1994-1998 [13]. By the year 2000, the percentage reached 26.3% out of 2,575 isolates tested [14]. The prevalence of VRE in Europe is still very low. VRE can cause important nosocomial epidemics and can increase morbidity, mortality and costs [15,16].

Intermediate resistance to vancomycin in *Staphylococcus aureus* (VISA) was first described in 1996 [17]. It is possible that VanA resistance genes could be transferred from enterococci to *S. aureus* via plasmid-mediated conjugation. In June 2002, the first documented case of infection caused by vancomycin-resistant *S. aureus* (VRSA) was reported in a patient from Michigan, USA [18]. Cultures also identified concomitant infection due to VRE. This VRSA isolate contained the VanA gene, which suggests that the resistance determinant

Table 2. Epidemiological data of patients with and without vancomycin-resistant entercoccus (VRE) strain colonization

Characteristic	VRE-positive No. (%)	VRE-negative No. (%)	P
Age (years ± SD)	60.7 (16.2)	47.7 (26.1)	0.002
Sex			
Male	24 (61.5)	48 (53.9)	NS
Female	15 (38.5)	41 (46.1)	NS
Hospitalized in:			
ICU	10 (7.8)	23 (18)	NS
Surgical ward	14 (10.9)	23 (18)	NS
Internal medicine ward	15 (11.7)	25 (19.5)	NS
Length of stay (days)	13.2 (8.2)	8.6 (7.2)	0.001
Previous hospitalization	7 (17.9)	21 (23.6)	NS
Previous admission in other department:	9 (23.1)	9 (10.1)	0.05
ICU	4 (10.3)	3 (3.4)	NS
Surgical ward	4 (10.3)	6 (6.7)	NS
Malignancies	11 (28.2)	10 (11.2)	0.019
Diabetes mellitus	4 (10.3)	10 (11.2)	NS
Mechanical ventilation	7 (18.4)	19 (21.6)	NS
Catheters:			
Central venous	12 (30.8)	36 (40.4)	NS
Bladder	14 (35.9)	34 (38.2)	NS
Antibiotic use	23 (59)	54 (60.7)	NS
Surgical procedures	15 (38.5)	24 (27)	NS
Upper GI endoscopies	1 (2.6)	5 (5.6)	NS
Lower GI endoscopies	1 (2.6)	2 (2.2)	NS

GI = gastrointestinal tract.

Table 3. Antimicrobial agents administrated to patients within the previous 30 days

Antimicrobials	VRE-positive	VRE-negative	P
Antianaerobic agents*	10 (25.6%)	10 (11.2%)	0.03
Aminoglycosides	6 (15.4%)	14 (15.7)	NS
Quinolones	4 (10.3%)	5 (5.6%)	NS
Carbapenems	3 (7.7%)	1 (1.1%)	0.06
Ureidopenicillins	8 (20.5%)	8 (9%)	0.06
Vancomycin	4 (10.3%)	6 (6.7%)	NS
Cephalosporins	6 (16.4%)	15 (16.9%)	NS

* = metronidazole, imipenem, piperacillin/tazobactam; NS = not significant.

might have been acquired from VRE. The potential for VRE to pass genes conferring vanvomycin-resistance to *S. aureus* provoked a concern for understanding VRE epidemiology.

VRE infections are very low in Greece, as has been sporadically reported [19], in spite of the fact that it is one of the countries with the highest prevalence of high-level gentamycin-resistant enterococci, as reported recently in a European prevalence study [20]. Little is known about VRE colonization in Greece and no study has ever been contacted. In our study, 39 patients (30.5%) were found to have VRE: 10 (7.8%) in the ICU, 14 (10.9%) in surgical wards and 15 (11.7%) in internal medicine wards. The prevalence of fecal colonization by VRE reported in other European studies was: 2% in the Netherlands [21], 4.9% in ICUs of French general hospitals [22] and 3.5% in Belgium [23].

Most clinical isolates were *Enterococcus faecium* (58.9%), while *E. gallinarum* accounted for 33.3% and *E. casseliflavus* accounted for 7.6% of the isolates. No *E. faecalis* strains were detected. In the USA, the number of *E. gallinarum* and *E. casseliflavus* strains among VRE strains is very low, from 0.5-1% [24]. These species are not always taken into account, because their resistance to glycopeptides is intrinsic and their pathogenicities are very low. Four *E. faecium* strains had a VanA phenotype and the rest had a VanB phenotype.

The mean length of stay in our hospital is seven days. Colonized patients in our study had been hospitalized longer (13.2 days) than the average patient. Patients with VRE were older, 60.7 years *vs*. 47.7 years for VRE-negative patients. Prior antimicrobial therapy has been known to be a risk factor for VRE acquisition. The only association found in our study

was between antimicrobials with anaerobic activity (such as metronidazole, piperacillin/tazobactam, imipenem) and VRE colonization. The use of carbapenems and ureidopenicillins as risk factors approached significance. In the subpopulation of ICU patients, vancomycin use was related with VRE colonization. In studies during VRE epidemics, a clear relationship between antibiotic use, including especially vancomycin, with VRE colonization has been reported [25,26]. Patients with malignancies were more often colonized with VRE (28.2% *vs.* 11.2%). Malignancies are a risk factor for VRE colonization and infection, as reported by other authors, especially because of the prolonged hospitalization and the extensive use of antibiotics by these patients [27,28]. Many authors have reported that prior ICU admission is a risk factor for VRE colonization, but we were unable to identify such a relationship in our study.

Most VRE isolates were Van B (there where only four van A and 16 van C isolates), thus stratification for risk factors was not possible.

Whereas VRE in the USA seems to be a hospital problem, probably caused by the extensive use of vancomycin and other antimicrobial agents, such as cephalosporins [29,30], the occurrence of VRE in Europe is possibly boosted by the use of glycopeptide analogs as growth factors in bioindustry and the consequent transmission of VRE via the food chain [31-33]. Torres et al. have suggested that VRE can be part of the intestinal microflora of patients inside and outside the hospital [34].

All patients in our survey were hospitalized for at least two days. Our study was designed as a one-day prevalence study, so we were not able to acquire any data about the previous status of the patients. We assumed that the patients were colonized during their hospital stay, but the fact that two patients with VRE were hospitalized for only two days and one patient with VRE for only three days (none of these patients had any previous hospitalization) means that these particular patients might have been colonized in the community. VRE colonization is an important factor leading to nosocomial dissemination of the organism. Also, VRE colonization independently increases a patient's risk of developing infections, such as bloodstream infections [35,36].

The Centers for Disease Control and Prevention recommends that hospitals develop a comprehensive plan to prevent and control infection and colonization of patients with VRE [37]. This plan should include prompt identification of VRE-colonized/infected patients, initiation of isolation precautions to prevent patient-to-patient transmission of VRE and prudent use of antimicrobials, especially vancomycin. Isolation of colonized patients is very difficult, because of the large number of patients, as seen in our study; thus great emphasis must be given to hygiene measures. The Infectious Diseases Control Committee of our hospital is taking into account the risk factors found in our study in order to identify patients who may be colonized with VRE. Patients who are hospitalized for a long time, patients with malignancies, and those who have extensively used vancomycin or antimicrobials with anaerobic activity are now routinely tested for VRE colonization.

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