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Risk factors for vancomycin-resistant *Enterococcus* colonization in hematologic patients

Faktori rizika od kolonizacije vankomicin-rezistentnog *Enterococcus*-a kod hematoloških bolesnika

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Abstract

Background/Aim. Vancomycin-resistant *Enterococci* (VRE) is one of the most important hospital pathogens. The aim of the study was to evaluate VRE colonization in patients hospitalized at the Hematology Intensive Care Unit, as well as the associated risk factors. **Methods.** A prospective cohort study involved 70 patients hospitalized at the Intensive Care Unit (ICU), Clinic for Hematology, Clinical Center of Serbia, Belgrade, during 3 months. Baseline demographic data, data about antibiotic usage and other risk factors for VRE colonization during the present and previous hospitalizations (within 6 months) were recorded for each patient using the questionnaire. Feces or rectal swab was collected for culture from patients on admission and at discharge in case when VRE was not isolated on admission. *Enterococci* were isolated by standard microbiological methods. Isolate sensitivity was tested by disk-diffusion test using 30 μg/mL (BBL) Vancomycin plates according to the Clinical and Laboratory Standards Institute (CLSI) standard. **Results.** Analysing results showed that 7% of the patients had been already colonized with VRE upon ICU admission. The rate of VRE colonization during present hospitalization was 41.5%. Univariate logistic regression demonstrated the statistically significant differences in diagnosis, length of present stay, use of aminoglycosides and piperacillin/tazobactam in present hospitalization, duration of use of carbapenem and piperacillin/tazobactam in present hospitalization between the VRE-colonized and non-colonized patients. Acute myeloid leukemia (AML), use of carbapenem in previous hospitalization and duration of use of piperacillin/tazobactam in present hospitalization were independent risk factors for VRE-colonized patients according to multivariate logistic regression. **Conclusion.** VRE colonization rate was high among the patients admitted to hematology ICU. Rational use of antibiotics and active surveillance may be helpful preventive measures against the development of bacterial resistance to antimicrobial agents.

Key words:
enterococcus faecium; vancomycin; drug resistance, bacterial; hematologic diseases; risk factors.

Apstrakt

Uvod/Cilj. *Enterococcus* spp. rezistentan na vankomicin (VRE) jedan je od najznačajnih bolničkih patogena. Cilj rada bio je da se utvrde stope rektalne kolonizacije VRE kod bolesnika lečenih u Odeljenju za hematološku intenzivnu negu, i da se sagledaju faktori rizika od kolonizacije. **Metode.** Prospektivnom kohortnom studijom obuhvaćeno je 70 bolesnika lečenih u periodu od tri meseca u Klinici za hematologiju Kliničkog centra Srbije u Beogradu. Podaci o demografskim karatkteristikama bolesnika, upotrebi antibiotika i drugim faktorima rizika od VRE kolonizacije tokom sadašnje i prethodnih hospitalizacija (tokom 6 meseci) prikupljani su za svakog bolesnika uz pomoć upitnika. Bolesnicima je uzmana koprokultura ili rektalni bris na prijemu, a prilikom otpusta onim bolesnicima kod kojih na prijemu nije izolovan VRE. Osetljivost izolata proverena je disk-difuzionim testom sa diskovima vankomicina 30 μg/mL (BBL) u skladu sa *Clinical and Laboratory Standards Institute* (CLSI) standardima. **Rezultati.** Na prijemu je bilo 7% VRE kolonizovnaih bolesnika. Stopa VRE kolonizacije tokom tekuće hospitalizacije iznosila je 41,5%. Univarijantna logistička regresija pokazala je statistički značajne razlike u pogledu dijagnoze, dužine sadašnje hospitalizacije, primeni aminoglikozida i piperacilin/tazobaktama u sadašnjoj hospitalizaciji, dužini primene karbapenema i piperacilin /tazobaktama u sadašnjoj hospitalizaciji između bolesnika kolonizovanih VRE i nekolonizovanih bolesnika. Multivarijantnom logističkom regresijom ustanovljeno je da su akutna mijeloidna leukemija (AML), primena karbapenema u prethodnoj hospitalizaciji i dužina primene piperacilin/tazobactama u sadašnjoj hospitalizaciji bili nezavisni faktori rizika od kolonizacije bolesnika VRE. **Zaključak.** Zabeležena je visoka stopa kolonizacije pacijenata VRE. Racionalna upotreba antibiotika i aktivni nadzor mogu biti korisne mere prevencije nastanka rezistencije bakterija na antibiotike.

Ključne reči:
enterococcus faecium; vankomicin; lekovi, rezistencija mikroorganizama; hematološke bolesti; faktori rizika.

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Introduction

Bacteria of *Enterococcus* genus are a significant cause of hospital-acquired infections (HAI), second among urinary tract infections (UTI) and third among bacteriemias. There are many different species of *Enterococci*. The most prevalent species culturated from humans are *E. faecalis* (the most common) and *E. faecium. Enterococci* have both an intrinsic (nature) and acquired resistance to antibiotics, making them important nosocomial pathogens. They are intrinsically resistant to penicillin (low level), all cephalosporins, aztreonam, macrolides, and low levels of clindamycin. This natural resistance is present in all members of species and is chromosomally mediated. Acquired resistance to antibiotics includes resistance to glycopeptides, β lactamases fluoroquinolones, tetracycline, high aminoglycoside doses and glycopeptides (vancomycin), as a result of mutations in DNA or the acquisition of new gene(s). Glycopeptides (vancomycin) resistance has been seen in around 70–78% of the nosocomial *E. faecium* population [1–3]. The mechanism of vancomycin resistence is due to preventing the synthesis of peptidoglycan precursors of the bacterial cell wall by blocking two steps: the transglycosylation and the transpeptidation [3]. It is mediated by 5 genes referred to as *vanA,* which can induce high level resistance to both vancomycin and teicoplanin; *vanB* which is found intrinsically in non-pathogenic enterococcal species; *vanC; vanD*, and *vanE*. *VanA* is more widely distributed [4].

Vancomycin resistant *Enterococcus* spp. (VRE) was first isolated in England and France in 1986 and later on in other European and countries worldwide [5]. The proportion of enterococcal bacteremia attributable to VRE in the UK in 2007 was 8.5–12.5% for all enterococci [6]. From 2005 to 2008, a significant decrease in vancomycin resistance was observed in France (from 2 to 0.6%), Greece (from 37 to 28%), Israel (from 46 to 20%) and Italy (from 19 to 6%). Ireland, Luxembourg and Greece in 2009 reported resistance proportions above 25%, while the majority of countries (18 of 26 countries) reported resistant proportions below 7%. Several countries reported it even below 1% (Bulgaria, Estonia, Finland, France, Norway and Sweden) [3]. During the past four years, a significant increase was observed only in Austria. In 2010, 28 EU countries reported 5,577 isolates of *E. faecium*, of which 7.4% were resistant to vancomycin. During the four past years, only Latvia reported an increased trend of these enterococci [7].

According to the USA National Nosocomial Infections Surveillance (NNIS), the percentage of enterococcal isolates resistant to vancomycin increased from 12% in the period 1998–2002 to 28.5% of all isolates in 2003 [8].

VRE can cause different types of HAI, like urinary tract, surgical site, bacteriemia, meningitis, endocarditis, most commonly in immunocompromised patients. Enterococci are responsible for high morbidity and mortality rates in these patients [9].

Risk factors for VRE colonization and infections are the following: age, hepatic and renal dysfunction, hematological diseases, chronic diseases, application of the invasive diagnostic and therapeutic procedures, stay in intensive care unit (ICU), abdominal surgery, transplantation, prolonged hospitalization, and broad-spectrum antibiotic use [10, 11].

VRE may survive on dry surfaces several weeks (from 7 days to 4 months). Consequently, VRE is most commonly transmitted in hospitals from person to person by direct contact with personnel and patient's hands, either from feces, urine, or blood of a person carrying the organism. It can also be spread indirectly *via* hand contact with open wounds, or with contaminated environments (some parts of medical equipment and working surfaces contaminated by VRE) [12]. VRE colonization could persist for years. The colonized patients are significant reservoirs and sources of contamination of the environment. VRE is not transmitted through the air [13].

Methods

This prospective cohort study involved 70 patients hospitalized at the Intensive Care Unit (ICU), Clinic for Hematology, Clinical Center of Serbia, Belgrade, in the period from September to December 2011. The coproculture or rectal swab was collected from all the patients on admission and before discharge.

All the patients were daily observed during their hospitalization by an epidemiologist. Clinical charts were systematically reviewed and, when necessary, the medical staff was interviewed. Baseline demographic data, data about antibiotic usage and other risk factors for VRE colonization during present and previous hospitalization were recorded for each patient using the questionnaire. The following characteristics related to the patients, and applied diagnostic and therapeutical procedures were recorded: age, sex, underlying disease, recent prior hospitalization (within 6 months) and recent antimicrobial use, operation, inserted central venous and urinary catheter, mechanical ventilation, antibiotic prophylaxis and therapy (type of antibiotics), and VRE isolated from coproculture on admission and the discharge. Surveillance for VRE infections was carried out during the course of this study.

VRE isolation and identification

Enterococci were isolated by standard microbiological methods. Enterococcal identification was based on cultural characteristics, Gram-stained specimens, mobility in 0.5% agar, capacity of pigment production and biochemical characteristics. Isolate sensitivity was tested by the disk-diffusion test using 30 μg/mL (BBL) Vancomycin plates according to the Clinical and Laboratory Standards Institute (CLSI) standard. The plates were incubated 24 hours at 35°C. The increase in more than one colony or a part of colony was interpreted as resistance to vancomycin [14, 15]. E-test was not used in methodology given than only enterococcal isolates were recovered and identified from rectal swab or feces for the purpose of determination of the carrier state.

The descriptive and analytical methods were used for data processing: χ^2 test (for categorical data) or *t*-test (for continuous variables). The results were expressed as percentages or as mean ± standard deviations. To identify the risk

factors of VRE colonization, the univariate logistic regression and multivariate logistic regression analyses were used. Statistical data processing was carried out by SPSS program (version 10).

Results

Study population and patients characteristics

The study included 70 patients hospitalized at the Clinic for Hematology, Clinical Center of Serbia during the study period. Out of all these patients, 5 (7%) were found to have been already colonized with VRE upon ICU admission. The VRE positive patients on admission had been hospitalized at the Clinic for Hematology within the previous six months. During that hospitalization they received antibiotic therapy.

Out of 65 VRE negative patients on admission, 27 (41.53%) were colonized with VRE strains during current hospitalization. The characteristics of these patients are presented in Table 1. There were 38 (58.4%) males; the mean age of the subjects was 52.7 years (ranged from 23 to 80 years).

found a significant difference (OR: 3.06; 95%CI: 1.09–8.60; $p = 0.033$) in frequency of this diagnosis between the two groups of subjects. Furthermore, there was a significant difference in length of present hospitalization between the group of colonized patients (median days 35 ± 10.23) and non-colonized patients (median days 24.4 ± 10.6) (OR: 1.11; 95%CI: 1.04–1.19; $p = 0.002$).

Univariate logistic regresion analysis failed to find any significant difference in other characteristics between the two study groups of patients: age, sex, prior hospitalizations, antibiotic use, insertion of the urinary and central venous catheter and infection.

Antibiotic use and duration of antibiotic use

During the present hospitalization, only 15.4% patients did not receive antibiotics; 43.1% received one or two antibiotics and 41.5% three or more antibiotics. Tables 2 and 3 summarize the antibiotic use and duration of antibiotic use among the patients with and without VRE colonization. According to univariate logistic regression, the risk factors that were significantly associated with VRE colonization in-

Table 1

Characteristics of the VRE negative patients* on admission and during hospitalization

Patients' variables	n (%) of patients		OR (95%CI)	*p*
	VRE non-colonized (n = 38)	VRE colonized (n = 27)		
Age (years), mean (± SD)	52.3 (13.0)	53.3 (12.6)	1.00 (0.96–1.04)	0.175
Sex (males/females)	21 (55.26)	17 (62.96)	1.37 (0.50–3.77)	0.847
Diagnosis on admission				
acute myeloid leukemia	15 (39.47)	18 (66.66)	3.06 (1.09–8.60)	0.033
non Hodgkin lymphoma	6 (15.78)	1 (3.70)	0.20 (0.02–1.81)	0.121
acute lymphocytic leukemia	9 (23.68)	5 (18.51)	0.73 (0.21–2.49)	0.175
chronic lymphocytic leukemia	4 (10.52)	2 (7.40)	0.68 (0.11–4.00)	0.669
Length of stay (days), median ± SD				
previous hospitalization	8.26 (12.3)	8.56 (13.62)	1.00 (0.96–1.04)	0.927
present hospitalization	24.4 (10.6)	35 (10.23)	1.11 (1.04–1.19)	0.002
previous admission in other hospital	29 (76.31)	18 (66)	1.10 (0.80–1.52)	0.524
Antibiotic use				
previous hospitalization	26 (68.42)	20 (74.07)	1.10 (0.80–1.52)	0.524
present hospitalization	34 (89.47)	26 (96.29)	0.75 (0.25–2.27)	0.622
Central venous catheter			1.79 (1.43–2.24)	0.999
previous hospitalization	30 (78.94)	22 (81.48)	0.85 (0.24–2.98)	0.801
present hospitalization	21 (55.26)	19 (70.31)	1.92 (0.67–5.46)	0.220
Bladder catheter				
previous hospitalization	1 (2.63)	2 (7.40)	2.96 (0.25–34.42)	0.386
present hospitalization	11 (28.94)	8 (29.62)	1.03 (0.35–3.05)	0.952
Hospital infection				
previous hospitalization	5 (13.15)	2 (7.40)	0.52 (0.09–2.94)	0.467
present hospitalization	24 (63.15)	15 (55.55)	0.72 (0.26–1.99)	0.538

VRE – vancomycin resistent enterococci; *total number of VRE negative patients on admission = 65.

In our study groups of colonized and non-colonized patients, 18 (66.67%) and 15 (39.47%) had acute myeloid leukemia (AML), respectively. Univariate logistic regression

cluded aminoglycoside use (OR: 3.88; 95%CI: 1.14–13.1; $p = 0.030$) and piperacillin/tazobactam use (OR: 4.68; 95%CI: 1.57–13.9; $p = 0.005$) during present hospitalization (Table

2). Statistically significant difference (OR: 1.36; 95%CI: 1.94–1.69; $p = 0.006$) was also noted in the length of piperacillin/tazobactam use between colonized (median days 3.22 ± 3.93) and non-colonized patients (median days 0.87 ± 1.84) during the present hospitalization (Table 3).

Univariate logistic regresion analysis failed to show any significant difference in the frequency of use of other antibiotics (cephalosporins, quinolones, glycopeptides, antianaerobic drugs and cotrimoxazole) and the duration of antibiotic therapy between the two studied groups of patients.

Table 2

Antibiotic use during previous and present hospitalization (univariate logistic regression)

Used antibiotics	n (%) of patients		OR (95%CI)	*p*
	VRE non-colonized (n = 38)	VRE colonized (n = 27)		
Cephalosporins				
previous hospitalization	1 (2.63)	1 (3.70)	1.42 (0.08–23.7)	0.806
present hospitalization	8 (21.05)	8 (29.62)	1.57 (0.50–4.91)	0.431
Carbapenems				
previous hospitalization	2 (5.26)	6 (22.22)	5.14 (0.95–27.8)	0.057
present hospitalization	13 (34.21)	15 (55.55)	2.40 (0.87–8.61)	0.090
Quinolone				
previous hospitalization	3 (7.89)	6 (22.22)	3.33 (0.75–14.7)	0.113
present hospitalization	17 (44.73)	12 (44.44)	0.98 (0.36–2.66)	0.988
Glycopeptide				
previous hospitalization	1 (2.63)	3 (11.11)	1.05 (0.93–1.19)	0.393
present hospitalization	3 (7.89)	3 (11.11)	1.01 (0.90–1.15)	0.758
Antianaerobic agents				
previous hospitalization	1 (2.63)	0	0.97 (0.92–1.02)	0.396
present hospitalization	7 (18.42)	5 (18.51)	1.00 (0.28–3.58)	0.992
Cotrimoxazole				
previous hospitalization	1 (2.63)	2 (7.40)	2.96 (0.25–34.4)	0.386
present hospitalization	2 (5.26)	1 (3.70)	0.69 (0.60–8.04)	0.769
Aminoglycosides				
previous hospitalization	5 (13.55)	3 (11.11)	0.80 (0.18–3.79)	0.825
present hospitalization	5 (13.55)	10 (37.03)	3.88 (1.14–13.1)	0.030
Piperacilin/tazobactam				
previous hospitalization	3 (7.89)	3 (11.11)	1.45 (0.27–7.84)	0.660
present hospitalization	8 (21.05)	15 (55.55)	4.68 (1.57–13.9)	0.005

VRE – vancomycin resistent enterococci.

Table 3

Duration of antibiotic use (univariate logistic regression)

Used antibiotics	Duration (days), median ± SD		OR (95%CI)	*p*
	VRE non–colonized (n = 38)	VRE colonized (n = 27)		
Cephalosporins				
previous hospitalization	0.13 ± 8.11	0.30 ± 1.53	1.13 (0.73–1.75)	0.582
present hospitalization	2.29 ± 5.53	1.96 ± 3.70	0.98 (0.88–1.09)	0.787
Carbapenems				
previous hospitalization	0.17 ± 1.13	1.18 ± 2.74	1.39 (0.94–2.05)	0.098
present hospitalization	2.47 ± 4.39	5.04 ± 5.37	1.11 (1.00–1.23)	0.046
Quinolones				
previous hospitalization	1.71 ± 6.05	2.48 ± 5.97	1.02 (0.94–1.10)	0.609
present hospitalization	5.13 ± 7.18	3.93 ± 5.96	0.97 (0.90–1.05)	0.473
Glycopeptides				
previous hospitalization	0.91 ± 4.77	0.51 ± 1.71	1.04 (0.82–1.32)	0.719
present hospitalization	1.32 ± 3.02	1.52 ± 2.75	1.01 (0.85–1.20)	0.835
Antianaerobic agents				
previous hospitalization	0.15 ± 0.82	0.12 ± 0.38	0.70 (0.27–1.83)	0.475
present hospitalization	0.63 ± 1.55	1.07 ± 2.51	1.11 (0.86–1.43)	0.385
Cotrimoxazole				
previous hospitalization	0.15 ± 0.82	0.48 ± 1.74	1.39 (0.79–2.43)	0.243
present hospitalization	0.63 ± 1.55	0.22 ± 1.15	1.08 (0.62–1.86)	0.776
Aminoglycoside				
previous hospitalization	1.36 ± 4.20	0.44 ± 1.76	0.89 (0.73–1.10)	0.319
present hospitalization	1.45 ± 42.4	1.93 ± 2.74	1.03 (0.90–1.18)	0.605
Piperacilin/tazobactam				
previous hospitalization	0.94 ± 4.19	1.25 ± 3.83	1.01 (0.90–1.15)	0.758
present hospitalization	0.87 ± 1.84	3.22 ± 3.93	1.36 (1.94–1.69)	0.006

VRE – vancomycin resistent enterococci.

Multivariate logistic regresion analysis included all the values of $p < 0.1$ (diagnosis, number of hospital days in present hospitalization, carbapenem use in earlier and present hospitalization, use of aminoglycosides and piperacillin/tazobactam use in present hospitalization, duration of carbapenem use in previous and present hospitalization as well as length of piperacillin/tazobactam use in present hospitalization).

The results of multivariate analysis demonstrated that the diagnosis of the disease (AML), carbapenem use in earlier hospitalization and length of piperacillin/tazobactam use in present hospitalization were independent risk factors of colonization of patients with VRE (Table 4).

pneumonia) between the two study groups of patients (Table 5). In the VRE-colonized patients, vancomycin resistant *Enteroccus* spp was a cause of urinary tract infections in 4 patients, while VRE was not isolated as the cause of infection in the non-colonized patients (Table 6).

Discussion

The patients affected by malignant hemopathies are often rehospitalized and, therefore possibilly colonized by hospital pathogens including VRE. Immunosuppressed patients appear to be at special risk for VRE colonization and severe

Table 4

Risk factors for vancomycin-resistant *Enterococci* (VRE) colonization according to multivariate logistic regression analysis

Risk factors	B	SE	OR (95%CI)	*p*
Diagnosis (AML)	0.001	0.001	0.92 (0.99–1.0)	0.048
Antibiotic use				
Carbapenems – previous hospitalization	1.651	2.487	5.21 (0.04–6.81)	0.040
Duration of antibiotic use				
Piperacillin/tazobactam – present hopsitalization	1.918	0.884	6.80 (1.20–3.85)	0.030

AML – acute myeloid leukemia.

Table 5

Infection in vancomycin-resistant *Enterococci* (VRE) – colonized patients in present hospitalization

Hospital–acquired infections (HAI)	n (%) of patients		Total patients n (%)
	VRE non–colonized (n = 38)	VRE colonized (n = 27)	
Urinary tract infections	12 (31.6)	8 (29.6)	20 (30.8)
Bloodstream infections	8 (21.1)	3 (11.1)	11 (16.9)
Pneumonia	0 (0.0)	2 (7.40)	2 (3.1)
Without HAI	18 (47.3)	14 (51.9)	32 (49.2)
Total n (%)	38 (100.0)	27 (100.0)	65 (100.0)

Table 6

Cases of infection in the vancomycin-resistant *Enterococci* (VRE) – colonized and non–colonized patients in present hospitalization

Hospital acquired infection (HAI)	n (%) of patients		Total patients n (%)
	VRE non–colonized (n = 38)	VRE colonized (n = 27)	
Urinary tract infection			
Esherichia coli	5 (41.6)	0 (0.0)	5 (25.0)
Klebsiella spp.	5 (41.6)	1 (12.5)	6 (30.0)
Enterococcus spp.(vancomycin sensitive)	1 (8.3)	3 (37.5)	4 (20.0)
Enterococcus spp.(vancomycin resistent)	0 (0.0)	4 (50.0)	4 (20.0)
Providentia retgery	1 (8.3)	0 (0.0)	1 (5.0)
Total patients, n (%)	12 (60)	8 (40)	20 (100.0)
Bloodstream infection CNS*	4 (50.0)	2 (66.6)	6 (54.5)
Klebsiella spp	1 (12.5)	0 (0.0)	1 (9.09)
Pseudomonas spp.	1 (12.5)	0 (0.0)	1 (9.09)
Stenotrophomonas maltophilia	1 (12.5)	0 (0.0)	1 (9.09)
Acinetobacter spp.	1 (12.5)	0 (0.0)	1 (9.09)
Esherichia coli	0	1 (33.3)	1 (9.09)
Total patients, n (%)	8 (72.72)	3 (27.28)	11 (100.0)

***CNS – coagulasa negative staphylococci.**

Infections and pathogens

Data processing did not reveal any significant difference in the incidence of all HAI ($p > 0.05$), as well as certain HAI (urinary tract infections, bloodstream infections and VRE infections. VRE are a particular problem in the intensive care units of large hospitals where they usually occur. Our study was designed to evaluate the colonization rate during hospitalization at the Hematology ICU, colonization rate on admission, and risk factors of colonization. The re-

sults of this study showed that 7% of the patients were VRE positive on admission (VRE isolated from feces culture or rectal swab). The colonization rate during ICU hospitalization was 41.5%.

A relatively small number of articles described VRE colonization in hematological patients [10, 16–19]. However, *Enteroccoci* have recently emerged as nosocomial agents, especially in patients with hematooncological diseases. The studies from France and the Netherlands showed that 37%, and 49% of hematological patients were colonized by VRE, respectively [16, 17]. Contrary to these results, VRE colonization in the USA immunocompromised patients was reported in considerably lower percentage. It was noted that out of 2,115 hematological patients, 4.7% patients had verified rectal VRE colonization. Among all colonized patients, 5.4% were patients with leukemia, 4.9% with hematopoietic stem cell transplantation recipients, and 2.2% with lymphoma [20]. In other study which was carried out on the hematologyoncology unit, 7.7% of patients, predominantly with hematologic malignancies, were colonized or infected with VRE during the study period [21]. A much higher rate of VRE colonization in our study (41.5%) is probably the result of the lack of contact isolation measures and the increased use of antibiotics. This is supported by the fact that only 15% of patients did not receive antibiotics during present hospitalization.

Many years ago, it was demonstrated that 5–50% of all antibiotic prescriptions are considered inappropriate which can cause the emergence and dissemination of resistant organisms. However, there is not standard treatment protocol for antibiotic prescription in our country. Beside that, the antibiotics were until recently available over-the-counter in the pharmacies. Antibiotic prescription is frequently done without antibiograms or even without bacteriology isolation of pathogens. All of the above mentioned can lead to high rates of bacterial resistance to antibiotics. It is important to emphasize that there is an increasing trend of vancomycin resistance in our country [22].

Our study failed to find any association of sex and the age and development of VRE fecal colonization at discharge. Our findings are consistent with the results of similar studies conducted in Korea [23]. On the contrary, a study carried out at the Thessaloniki University Clinic confirmed that VRE colonization was significantly more frequent in patients older than 60 years of age [24].

Analysis of our results showed that VRE colonization was significantly more frequent in patients with AML. Multivariate regression analysis demonstrated that AML was an independent risk factor for VRE colonization. Similar results were found in other studies as well [21]. It can be explained by the long length of hospitalization. Namely, duration of hospital stay of our patients with AML ranged from 28 to 40 days, in distinction from the patients with acute lymphocytic leukemia (ALL), non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (HLL) who were hospitalized during significantly shorter period. From the aspect of a clinician this may be explained by the length of therapy protocol application. Besides other risk factors, the length of hospital stay is considered as one of most important risk factor [10, 11, 24]. Accordingly, AML patients are at higher risk of colonization than patients with shorter hospitalization. The results of this study are only the introduction to more comprehensive and detailed analysis of the problem not only in hematological, but also in other immunocompromised patients.

Our study failed to find any significant difference in previous hospitalization between the two studied groups of patients, contrary to the results of other studies [20, 25]. However, mean length of present hospitalization in VRE-colonized patients was significantly longer in relation to non-colonized patients, *ie* 35 *vs* 24 days.

The results of several studies showed that the use of glycopeptides, second and third generation cephalosporins and antianaerobic antibiotics are associated with the patients colonized with VRE [25, 26]. Only few studies analyzed the association of quinolone use and VRE colonization. Our study failed to establish any significant difference in the use and length of use of antibiotics from the group of quinolones (ciprofloxacin) between the two groups of subjects what is compatible with the findings obtained in other studies [25].

Several studies on the effect of carbapenem as the risk factor for VRE colonization did not show any significant difference in the use of these antibiotics between VRE-colonized and non-colonized patients [20, 26]. However, in other studies prior carbapenem use was a significant risk factor for VRE colonization [24, 25]. Our study showed that there was a difference in the duration of the use of antibiotics from carbapenem group (imipenem and meropenem) between two groups of subjects in repeated hospitalization. Mean duration of carbapenem use in the VRE-colonized and in non-colonized patients in the repeated hospitalization was 5 and 2.47 days, respectively. Moreover, carbapenem use in previous hospitalization was an independent risk factor of VRE colonization.

Vancomycin is a glycopeptide antibiotic that is used to treat infections caused by Gram-positive bacteria. For many years, it has traditionally been reserved as a drug of "last resort", used to treat severe infections for which other antibiotics had failed. Vancomycin use has increased linearly in the last decades, especially for infections related to the presence of indwelling vascular catheter which is the case with hematology-oncology patients. Vancomycin is not recommended for regular antibioprophylaxis in surgery. However, a growing number of infections caused by *Staphylococcus aureus* resistant to meticillin has led to the widespread use of vancomycin in hospitals. The results of studies on association of vancomycin use and VRE colonization and infection have been controversial. A meta-analysis of 20 studies showed that the use of vancomycin increased the risk of VRE colonization by 4.5 times [27]. Using the multivariate analysis, Ostrowsky et al. [28], in contrast, demonstrated that there was no association between the vancomycin use and VRE colonization. In addition, the results of recent systematic review did not determine a potential role for vancomycin usage reduction in controlling VRE colonization [29]. Our study did not find any significant difference in the frequency

of vancomycin use and length of its use between the two groups of patients.

Analysing a study on the effect of use of antibiotics from the group of aminoglycosides failed to find any significant difference in the frequency of use of these antibiotics between VRE-colonized and non-colonized patients [17]. On the contrary, our results showed a significant difference in the use of aminoglycoside antibiotics between the two groups of subjects during repeated hospitalization. In the VRE-colonized group, 37% of the patients were administered aminoglycoside antibiotics while in the non-colonized group 13% of the patients received these antibiotics.

Analysing of our data showed that the VRE-colonized patients received piperacillin/tazobactam in a significantly higher percentage (55%) then non-colonized patients (21%). An average length of piperacillin/tazobactam use in the VRE-colonized and non-colonized patients during the present hospitalization was 3.22 and 0.87 days, respectively. Moreover, the use of this antibiotic in present hospitalization was an independent risk factor of VRE colonization. The incidence of VRE was positively correlated with the use of piperacillin/tazobactam or beta-lactam agents in other studies as well [30].

As the part of normal fecal flora, *Enteroccoci* were not traditionally considered as important nosocomial pathogens. But, they have emerged as increasingly important pathogens with increased resistance to antibiotics. VRE become one of the leading causes of HAI, especially of urinary tract and bloodstream infections. VRE commonly colonise, but less frequently cause the infections. However, colonization precedes most infections. In our study, VRE was a cause of UTI in the colonized patients but not in the non-colonized patients. VRE infections are not more virulent than other enteroccocal infections. But, VRE infections are very problematic for treatment. *Enterococci* are the most frequent cause of UTI. During one year of surveillance, organized by the National Healthcare Safety Network in the USA there was found that *Enteroccocus* spp was third the most frequent pathogen of HAI participated with 12% in the overall number of pathogenic isolates. Regarding rank-order distribution, it was at second position for bloodstream infections and at third position for UTI. *E. faecium* and *E. faecalis* showed a high proportion of vancomycin resitance: 98.4–99.5% and 91.9–98.4%, respectively [31].

Conclusion

VRE colonization rate was high among patients admitted to hematology ICU. Rational use of antibiotics and active surveillance may be helpful preventive measures against the development of bacterial resistance to antimicrobial agents.

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