



Gastrointestinal Carriage of Vancomycin-Resistant Enterococci and Carbapenem-Resistant Gram-Negative Bacteria in an Endemic Setting: Prevalence, Risk Factors, and Outcomes

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Background: Gastrointestinal carriage of vancomycin-resistant enterococci (VRE) and carbapenem-resistant Gram-negative bacteria (CRGN) constitutes a major public health concern as it may be followed by clinical infection development or lead to intra-hospital dissemination. Detection of carriers and implementation of infection control measures are essential in every hospital. In this study we determined the point prevalence of VRE and CRGN in the fecal flora of the inpatients of a tertiary university hospital in Greece. We determined risk factors for carriage and examined the impact of carriage on hospital outcomes.

Materials/Methods: A point prevalence study of VRE/CRGN rectal carriage of inpatients was conducted on March 2018. Specimens were selectively cultured for VRE/CRGN, microorganisms were biochemically identified, submitted to antibiotic susceptibility testing, and tested for carbapenemase production. Data on potential risk factors and hospital outcomes were collected at the time of culture and until hospital discharge. Multivariable logistic and linear regression models were used, adjusting for confounders.

Results: Four hundred ninety-one patients were enrolled in the study. Of them, 64 (13.0%) were positive for VRE carriage, 40 (8.2%) for CRGN, and 10 patients (2.1%) for both VRE and CRGN. VRE carriage was independently associated with age over 65 years (adjusted OR: 2.4 [95%CI: 1.3, 4.5]) and length of stay (LOS) before rectal sampling (OR: 1.1 [95%CI: 1.0, 1.1]). Carriage of CRGN was associated with 11 days increase of LOS after rectal sampling (β -coef: 11.4 [95%CI: 1.6, 21.2]), with a 3.5-fold increased risk of acquiring a resistant pathogen after rectal swabbing (RR: 3.5 [95%CI 1.2, 9.9]) and with a 6-fold increased risk of mortality (RR: 6.1 [95%CI: 2.1, 17.9]), after adjusting for sex, age, and comorbidity index.

Conclusions: High prevalence rates were found for VRE and CRGN carriage among the inpatients of our hospital. Prolonged hospitalization and age were independent risk factors for VRE carriage, while CRGN carriage was associated with increased risk of acquiring a resistant pathogen, prolonged hospital stay, and increased mortality.

Keywords: vancomycin-resistant enterococci, carbapenem-resistance, carriage, risk-factors, mortality, length of stay

INTRODUCTION

The wide dissemination of carbapenem-resistant Gram-negative bacteria (CRGN) and vancomycin-resistant enterococci (VRE) limits therapeutic alternatives and represents a global public health threat (1). The consequences of multidrug-resistant (MDR) bacterial infections include high morbidity and mortality and considerable economic loss (2). A recent study estimated the impact of infections caused by antimicrobial-resistant bacteria in countries within the EU and the European Economic Area for 2015. It was estimated that ~670,000 infections with resistant bacteria were documented in EARS-Net data, with these infections accounting for ~33,000 attributable deaths and 870,000 disability-adjusted life-years (DALYs). Notably, Greece and Italy contributed the highest burden among all participating countries and, for Greece, most of the infections were due to carbapenem- or colistin-resistant bacteria (3). The hospital environment seems to serve as the breeding grounds for MDR organisms (MDRO) (4). Asymptomatic rectal carriage of these organisms may precede infection and constitutes a reservoir for transmission that may remain unidentified in hospitals that do not implement active surveillance testing (5).

While Greece is considered one of the most common countries in Europe for antimicrobial resistance (6), only a limited number of studies have focused on MDRO rectal carriage and colonization to assess their prevalence, risk factors, and associated adverse outcomes (7–11). A previous study from our hospital has reported a prevalence of 14.3% of VRE carriage among hospitalized patients, identified invasive devices and duration of antimicrobial treatment as risk factors, and found that VRE carriage was not an independent predictor of mortality (12). Similar studies from other regions have reported VRE carriage rates ranging from 2 to 37% (13–15), while prevalence rates for CRGN rectal carriage ranged from 5.3 to 52% (16–18). At the same time, there is an increased risk of carbapenem-resistant enterobacteriaceae (CRE) infection and mortality in patients who test positive for carriage of CRE (19, 20). To the best of our knowledge, there is no previous study from Greece focusing on both VRE and CRGN rectal carriage, exploring respective risk factors and adverse outcomes.

The primary objective of the present study was: (i) to determine the prevalence of rectal carriage of VRE and CRGN, (ii) to identify risk factors for VRE/CRGN rectal carriage, and (iii) to examine the impact of VRE/CRGN rectal carriage on hospital outcomes in inpatients of a University General Hospital in Greece.

MATERIALS AND METHODS

Subjects

The University Hospital “Attikon” in Athens is a modern tertiary care teaching hospital and is the largest in the West Attica region (2,000,000 population), with 750 beds in total and >71,000 admissions/year. The hospital attends to cases of high complexity in internal medicine and surgery.

The first part of the present project was a point prevalence study of VRE/CRGN rectal carriage of hospital patients that was conducted on March 22nd and 23rd, 2018. Adult patients hospitalized in all surgical and internal medicine departments were surveyed by obtaining rectal swab cultures. In total, 17 medical and surgical wards participated in the study: general internal medicine, cardiology, dermatology, neurology, respiratory medicine, obstetrics/gynecology, cardiothoracic surgery, neurosurgery, urology, otorhinolaryngology, orthopedics, vascular surgery, hematology, oncology, nephrology, gastroenterology, and general surgery. The special and intensive care units were not included in the study, as they were already on active surveillance for VRE/CRGN carriage. The psychiatric ward was also excluded because patients lacked the mental competency necessary to participate. The second part of the project was a cohort study that included all participants from the first part and followed them from the time of rectal swab culture until death or discharge from the hospital. Face-to-face completed questionnaires together with medical records and communication with physicians were used to obtain information on potential risk factors at the time of rectal swabbing, while all examined outcomes were extracted from medical records retrospectively. The study was approved by the institutional review board of the hospital (62,17/10/2017) and all patients provided informed consent after a complete description of the study.

Culture, Identification, and Susceptibility Testing

A rectal swab was obtained from every consenting hospitalized patient. The swabs were transferred by using transport swabs in Amies transport medium (Biomedics, Madrid, Spain) and were transported to the microbiology laboratory for selective culture of VRE and CRGN. Bile-esculin agar with vancomycin (6 mg/L) and MacConkey agar with meropenem (1 mg/L) were used for selective cultivation. Microorganisms were biochemically identified by Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD) and submitted to antibiotic susceptibility testing, according to EUCAST 2018 guidelines and breakpoints. The combination disk test was used for screening

carbapenemase production using meropenem 10 µg disks (BIO-RAD, Marnes-la-Coquette, France) with or without inhibitors [phenyl boronic acid (PBA), ethylenediaminetetraacetic acid (EDTA)] (21). The guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (22). A meropenem disk with PBA and EDTA was also included to detect double carbapenemase producers (KPC and VIM), which have been found in Greek hospitals since 2009 (21, 23). The immunochromatographic assay, NG-test CARBA 5 (NG Biotech, 35480 Guipry, France) that discriminates KPC, IMP, VIM, NDM, and OXA-48-like producers, was also used.

Rectal Carriage of VRE/CRGN

All patients that tested positive for VRE and/or CRGN in the rectal swab culture were defined as carriers. Standard infection control measures to reduce transmission were used in these cases. The health personnel had to implement contact precautions (gloves, gowns) for all encounters with the carriers. The wards focused on thorough cleaning of the environment surrounding the positive patient, especially the patient care equipment. Whenever it was possible, the patient was isolated in a single room (24, 25).

Risk Factors for VRE/CRGN Rectal Carriage

Following informed consent and after obtaining the rectal swab, every hospitalized patient completed a face-to-face questionnaire which was captured in a standardized form. These data, along with parameters retrieved from the patients' records, included information on age, gender, ward, length of stay (LOS) before rectal swabbing, transfer from another hospital, comorbidities, presence of indwelling medical devices, chronic immobilization, last year hospitalization or ICU admission, and specific therapies, such as immunosuppressive therapy, antineoplastic, or antimicrobial chemotherapy.

Comorbidities included: chronic kidney disease, diabetes, dermatologic lesions, hematological malignancy, solid organ malignancy, metastatic disease, neurologic disease, heart failure, coronary artery disease, chronic liver disease, cerebrovascular disease, peripheral vascular disease, and chronic obstructive pulmonary disease. A slightly modified Charlson comorbidity index (CCI) (26) that predicts the 10-years mortality for a patient having a range of 17 comorbid conditions was also calculated. Each condition is assigned with a score of 1, 2, 3, or 6, depending on the risk of death associated with this condition; the scores are then summed up and give a total score which predicts mortality. The clinical conditions and scores are as follows: One for each: myocardial infarct, heart failure, peripheral vascular diseases, dementia, cerebrovascular disease, chronic lung diseases, connective tissue diseases, ulcer, and mild chronic liver diseases. Two for each: hemiplegia, moderate or severe kidney diseases, diabetes with or without complications, tumor, leukemia, lymphoma. Three for each: moderate or severe liver disease. Six for each: metastatic solid tumor, AIDS. In our modified version of CCI (modified CCI), we used chronic

immobilization instead of hemiplegia and we did not use data on mild chronic liver diseases, since they were not present in any patient.

Indwelling medical devices included central lines, urinary catheters, pacemakers, and other devices, such as external wound drains, enteral feeding tubes, endotracheal tubes, and tracheostomies.

Chronic immobilization is defined as loss of anatomical movement due to alteration of physiological function, which in daily practice is commonly defined as more than three-day-bed rest or inability to perform mobile activity on a bed, transfer, or ambulation (27, 28).

Last year, hospitalization or ICU admission included hospitalization in an acute care hospital (ward or ICU, respectively) for two or more days in the past 1 year.

Previous antibiotic therapy was defined as prescription of antibiotics for at least 2 days within the past 1 year. Immunosuppressive therapy included administration of steroids, cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, and calcineurin inhibitors during the last year.

Hospital Outcomes

Hospital outcomes were collected prospectively during hospitalization and included: (i) Mortality: death from any cause during hospitalization, (ii) Hospital LOS, (iii) LOS after rectal swabbing, and (iv) Isolation of a resistant pathogen [VRE, methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant Gram-negative bacteria and Gram-negative bacteria resistant to three or more of: beta-lactams, aminoglycosides, quinolones, co-trimoxazole]. from: (a) any clinical culture, and (b) blood, after rectal swabbing.

Statistical Analysis

Statistical analysis was performed using the statistical package STATA, version 13 (StataCorp, College Station, TX). Univariate associations between background characteristics and VRE/CRGN rectal carriage were studied using Pearson's chi-square test for categorical variables (with Fisher's exact test for groups with <5 subjects expected in a cell) and non-parametric Kruskal-Wallis tests for continuous non-normally distributed variables (tested by the Shapiro-Wilk normality test). Since potential risk factors and VRE/CRGN rectal carriage were measured in a cross-sectional design, associations of potential risk factors with VRE/CRGN rectal carriage were estimated with univariate logistic regression models. Estimated associations were described as odds ratios (OR) with 95% confidence intervals. All variables with a $p \leq 0.050$ in univariate analysis were included in a multiple regression model to examine independent risk factors for VRE/CRGN rectal carriage. In the prospective part of our study and in order to estimate the risk of VRE/CRGN rectal carriage on hospital outcomes, we used multivariable log-binomial or log-Poisson (if convergence failed) regression models to estimate relative risks (RRs) with 95% CIs for categorical outcomes (resistant pathogen in any culture and in blood after rectal swabbing, mortality) and multivariable linear regression models to estimate β coefficients with 95% CIs for continuous outcomes (hospital LOS, LOS after rectal

swab). Modified Charlson Comorbidity Index, age, and sex were included as confounders *a priori* in all analyses. All association testing was conducted assuming a $p \leq 0.050$ significance level.

RESULTS

In total, 507 adult patients hospitalized in medical wards were contacted to participate and 491 provided rectal swabs were included in the analysis (participation rate: 96.6%) (Figure 1). Baseline characteristics of the study population according to rectal carriage of CRGN and VRE are presented in Table 1. Forty patients were identified as CRGN carriers (prevalence: 8.2%) and 64 as VRE carriers (prevalence: 13.0%). Of the carrier patients, 30% were hospitalized in single rooms. All VRE isolates were identified as VanA-phenotype *E. faecium* (high level resistance to vancomycin and teicoplanin). Molecular detection of *van* genes was not performed. The respective prevalence for carbapenem-resistant (CR) enterobacteriaceae (CRE), CR-*Acinetobacter spp.*, and CR-*Pseudomonas spp.* carriers was 5.9, 1.8, and 1.2%, respectively. Four different types of carbapenemases were detected in CRE colonized patients: KPC, 65.5% ($n = 19$); NDM, 24.1% ($n = 7$); VIM, 6.9% ($n = 2$); and OXA-48, 3.5% ($n = 1$). Patients colonized with VRE tended to be older (>65 years) compared to non-colonized patients. Regarding CRGN carriers, they were characterized by a higher modified CCI, were more likely to have a urine catheter, and to have been hospitalized in a medical ward/ICU or to have received antibiotics during the last year, compared

with non-carriers. Moreover, both groups of CRGN and VRE carriers tended to have longer LOS before rectal swabbing. Mortality for non-carriers was 4.8% ($n = 19$), lower than that for CRGN (40%, $p < 0.001$) and VRE carriers (15.9%, $p < 0.001$). Hospital outcomes, such as hospital LOS and subsequent resistant pathogens isolated from blood, were also more frequent in both subgroups of CRGN and VRE carriers, compared to non-colonized patients (Table 1).

Table 2 presents the results of the univariable analysis on the association between potential risk factors and CRGN and VRE carriage. The detection of VRE carriage was associated with age over 65 years (OR: 1.9 [95%CI: 1.1, 3.4]) and LOS before rectal swab (OR: 1.1 [95%CI: 1.0, 1.1]). In a multivariate logistic regression model, these variables were also independently associated with VRE carriage [adjusted OR: 2.4 [95%CI: 1.3, 4.5] and 1.1 [95%CI: 1.0, 1.1], respectively]. CRGN carriage was associated with LOS before rectal swab [OR: 1.1 (95%CI: 1.0, 1.1)], hospitalization [OR: 10.4 (95%CI: 1.3, 82.3)] and ICU admission during the past 1 year [OR: 9.3 (95%CI: 2.4, 36.0)], presence of a urinary catheter [OR: 3.5 (95%CI: 1.0, 12.4)] and pacemaker [OR: 6.0 (95%CI: 1.1, 32.6)], metastatic disease [OR: 4.9 (95%CI: 1.2, 20.4)], and high modified CCI [OR: 1.2 (95%CI: 1.0, 1.3)]. When these variables were included in the multivariable model, none of them were identified as an independently associated risk factor for CRGN carriage.

Table 3 shows the multivariable analysis estimating the effect of CRGN and VRE rectal carriage on hospital outcomes, after

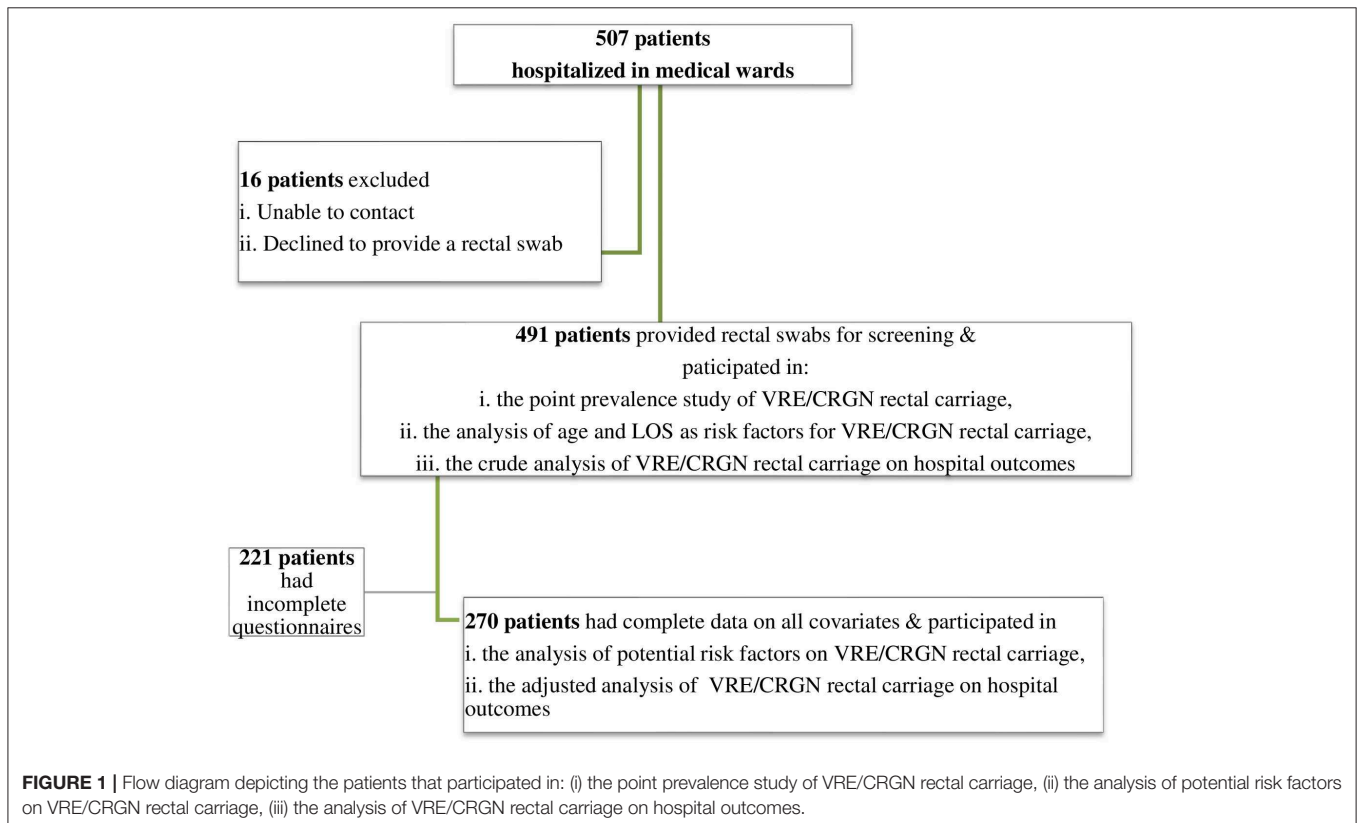


TABLE 1 | Comparison of baseline characteristics between MDRO carriers and non-carriers.

	Non carriers (n = 397)	CRGN carriers (n = 40)	p-value	VRE carriers (n = 64)	p-value
Age (years); median (IQR)	68 (27)	72 (24.5)	0.341	72.5 (20)	0.120
Age ≥65 years; n (%)	220 (55.4)	27 (67.5)	0.142	45 (70.3)	0.025
Males; n (%)	214 (53.9)	19 (47.5)	0.439	35 (54.7)	0.907
LOS before rectal swabbing (days); median (IQR)	6 (8)	12.5 (20)	<0.001	10 (16.5)	<0.001
Ward type; n (%)					
• Internal medicine	230 (57.9)	19 (47.5)	0.204	44 (68.8)	0.102
• Surgery	167 (42.1)	21 (52.5)		20 (31.3)	
• Transfer from other hospital; n (%)	23 (10.1)	3 (27.3)	0.075	5 (17.9)	0.217
Indwelling medical devices; n (%)					
• Central line	16 (7.1)	1 (9.1)	0.808	4 (13.8)	0.212
• Urine catheter	75 (33.2)	7 (63.6)	0.038	9 (31.0)	0.816
• Pacemaker	8 (3.6)	2 (18.2)	0.019	1 (3.5)	0.977
• Other prosthetic material	40 (17.7)	2 (18.2)	0.967	7 (24.1)	0.400
• Chronic immobilization; n (%)	14 (6.2)	2 (18.2)	0.122	1 (3.5)	0.554
Last year; n (%)					
• Hospitalization	109 (48.2)	10 (90.9)	0.007	14 (50.0)	0.930
• ICU admission	13 (5.8)	4 (36.4)	<0.001	0 (0.0)	0.192
• Chemotherapy	32 (14.2)	4 (36.4)	0.045	6 (20.7)	0.353
• Immunosuppressive therapy	22 (9.7)	2 (18.2)	0.364	4 (13.8)	0.497
• Antibiotic treatment	132 (58.7)	11 (100.0)	0.006	15 (53.6)	0.606
Comorbidities; n (%)					
• Chronic kidney disease	23 (10.2)	1 (9.1)	0.907	5 (17.9)	0.221
• Diabetes	57 (25.3)	4 (36.4)	0.415	11 (39.3)	0.116
• Dermatologic lesions	41 (18.1)	3 (27.3)	0.447	1 (3.5)	0.045
• Hematological malignancies	11 (4.9)	1 (9.1)	0.533	1 (3.5)	0.734
• Solid organ malignancy	34 (15.0)	4 (36.4)	0.060	8 (27.6)	0.086
• Metastatic disease	16 (7.1)	3 (27.3)	0.016	5 (17.2)	0.061
• Neurologic disease	35 (15.5)	0 (0.0)	0.157	3 (10.3)	0.464
• Heart failure	30 (13.3)	2 (18.2)	0.642	7 (25.0)	0.097
• Myocardial infarct	26 (11.5)	2 (18.8)	0.503	4 (14.3)	0.667
• Chronic liver disease	8 (3.5)	0 (0.0)	0.526	1 (3.5)	0.980
• Cerebrovascular disease	22 (9.8)	1 (9.1)	0.937	0 (0.0)	0.077
• Peripheral vascular disease	32 (14.2)	2 (18.2)	0.710	5 (17.3)	0.657
• Chronic obstructive pulmonary disease	30 (13.3)	3 (27.3)	0.193	4 (14.3)	0.889
• Modified CCI; median (IQR)	2 (4)	4 (8)	0.049	2 (6)	0.352
Outcomes					
• Resistant pathogen in blood after rectal swabbing; n (%)	8 (2.0)	4 (10.0)	0.003	5 (7.8)	0.009
• Resistant pathogen in clinical culture after rectal swabbing; n (%)	28 (7.1)	12 (30.0)	<0.001	7 (10.9)	0.276
• Hospital LOS (days); median (IQR)	12 (13)	25 (38.5)	<0.001	20.5 (21)	<0.001
• LOS after rectal swabbing (days); median (IQR)	5 (10)	7 (13.5)	0.089	5 (12)	0.436
Mortality; n (%)	19 (4.8)	16 (40.0)	<0.001	10 (15.9)	<0.001

IQR, interquartile range; LOS, length of stay; ICU, intensive care unit; CCI, Charlson comorbidity index; CRGN, carbapenem-resistant Gram-negative bacteria; VRE, vancomycin-resistant enterococci.

Bold indicates significant differences ($p \leq 0.050$) of ANOVA for continuous variables and χ^2 analysis for categorical variables.

Numbers may not correspond to the total due to missing numbers.

(n = 491).

adjusting for sex, age, and modified CCI. Carrying CRGN was associated with 38 days increased hospital LOS (β -coef: 38.0, [95%CI: 22.6, 53.4]) and 11 days increase in LOS after rectal swabbing (β -coef: 11.4, [95%CI: 1.6, 21.2]). More importantly,

it is associated with a 3.5-fold increased risk for acquiring a resistant pathogen after rectal swabbing (RR: 3.5, [95%CI 1.2, 9.9]) and with a 6-fold increase risk for mortality (RR: 6.1, [95%CI: 2.1, 17.9]). We then separately examined different

TABLE 2 | Associations between potential risk factors and carriage of CRGN and VRE.

	CRGN carriers			VRE carriers		
	<i>n</i> *	OR (95% CI)	<i>p</i> -value	<i>n</i> *	Crude OR (95% CI)	<i>p</i> -value
Age ≥65 years	437	1.7 (0.8, 3.3)	0.145	461	1.9 (1.1, 3.4)	0.027
LOS before rectal swabbing	437	1.1 (1.0, 1.1)	<0.001	461	1.1 (1.0, 1.1)	<0.001
Indwelling medical devices						
• Urine catheter	237	3.5 (1.0, 12.4)	0.050	255	0.9 (0.1, 2.1)	0.817
• Pacemaker	236	6.0 (1.1, 32.6)	0.037	254	1.0 (0.1, 8.0)	0.977
Last year						
Hospitalization	237	10.4 (1.3, 82.3)	0.027	254	1.1 (0.5, 2.4)	0.930
• ICU admission	236	9.3 (2.4, 36.0)	0.001	240	NA	NA
• Chemotherapy	237	3.5 (1.0, 12.5)	0.058	255	1.6 (0.6, 4.2)	0.356
• Antibiotic treatment	143	NA	NA	253	0.8 (0.4, 1.8)	0.607
Comorbidities						
• Chronic kidney disease	237	0.9 (0.1, 7.2)	0.907	254	1.9 (0.7, 5.5)	0.228
• Diabetes	236	1.7 (0.5, 6.0)	0.419	253	1.9 (0.8, 4.3)	0.121
• Dermatologic lesions	237	1.7 (0.4, 6.7)	0.452	255	0.2 (0.0, 1.2)	0.077
• Hematological malignancies	237	2.0 (0.1, 16.7)	0.540	255	0.7 (0.1, 5.6)	0.735
• Solid organ malignancy	237	3.2 (0.9, 11.6)	0.073	255	2.2 (0.9, 5.3)	0.092
• Metastatic disease	237	4.9 (1.2, 20.4)	0.028	255	2.7 (0.9, 8.1)	0.070
• Neurologic disease	202	NA	NA	255	0.6 (0.2, 2.2)	0.468
• Heart failure	237	1.5 (0.3, 7.1)	0.644	254	2.2 (0.9, 5.6)	0.104
• Myocardial infarct	237	1.7 (0.4, 8.3)	0.508	254	1.3 (0.4, 4.0)	0.668
• Chronic liver disease	229	NA	NA	255	1.0 (0.1, 8.1)	0.980
• Cerebrovascular disease	235	0.9 (0.1, 7.5)	0.937	231	NA	NA
• Peripheral vascular disease	237	1.4 (0.3, 6.5)	0.711	255	1.3 (0.4, 3.6)	0.658
• Chronic obstructive pulmonary disease	236	2.4 (0.6, 9.7)	0.206	253	1.1 (0.4, 3.3)	0.889
Modified CCI	237	1.2 (1.0, 1.3)	0.037	255	1.1 (1.0, 1.2)	0.069

OR, odds ratio; CI, confidence interval; LOS, length of stay; ICU, intensive care unit; CCI, Charlson comorbidity index; NA, not applicable. Bold indicates significant differences (*p* ≤ 0.050). *Numbers do not correspond to the total in every risk factor, due to missing data.

TABLE 3 | Risk of CRGN and VRE carriage for hospital outcomes.

	CRGN carriers				VRE carriers			
	<i>n</i>	Crude β-coef (95% CI)	<i>n</i>	Adjusted β-coef (95% CI)	<i>n</i>	Crude β-coef (95% CI)	<i>n</i>	Adjusted β-coef (95% CI)
HOSPITAL LOS (DAYS)								
• Total	437	25.0 (17.6, 32.4)	237	38.0 (22.6, 53.4)	437	9.9 (4.8, 15.1)	237	4.7 (-3.2, 12.7)
• After rectal swabbing		7.2 (2.4, 12.1)		11.4 (1.6, 21.2)		1.3 (-2.3, 5.0)		0.4 (-6.1, 5.3)
	<i>n</i>	Crude RR (95% CI)	<i>n</i>	Adjusted RR (95% CI)	<i>n</i>	Crude RR (95% CI)	<i>n</i>	Adjusted RR (95% CI)
RESISTANT PATHOGEN AFTER RECTAL SWABBING								
• In blood	437	5.0 (1.6, 15.8)	237	NA	437	3.9 (1.3, 11.5)	237	1.7 (0.2, 15.1)
• In clinical culture		4.3 (2.4, 7.7)		3.5 (1.2, 9.9)		1.6 (0.7, 3.4)		0.9 (0.2, 3.7)
Mortality		8.4 (4.7, 14.9)		6.1 (2.1, 17.9)		3.3 (1.6, 6.8)		0.5 (0.1, 3.3)

RR, relative risk; β-coef, beta coefficients; CI, confidence interval; LOS, length of stay; CR, carbapenem-resistant; VRE, vancomycin-resistant enterococci; NA, not applicable. Models were adjusted for sex, age and modified Charlson comorbidity index. The values in bold are statistically significant.

CRGN subgroups in order to identify the specific pathogen underlying the observed associations; sample size was marginal for firm conclusions in some cases, but CR-enterobacteriaceae carriage was predictive of all outcomes (**Supplementary Table 1**).

Regarding VRE carriers, although a statistical significant risk was found for hospital LOS, resistant pathogen in blood after rectal swabbing, and mortality in the crude model (RR: 9.9, [95%CI: 4.8, 15.1], RR: 3.9, [95%CI: 1.3, 11.5] and RR: 3.3, [95%CI: 1.6,

6.8] respectively), statistical significance was in all circumstances not shown after adjusting for confounders.

DISCUSSION

In the present study, we calculated simultaneously, for the first time, prevalence rates for VRE and CRGN carriage among inpatients of a Greek tertiary hospital and recognized prolonged hospitalization and age as independent risk factors for VRE carriage. We also showed that CRGN carriage is associated with increased risk of acquiring a resistant pathogen after rectal swabbing, prolonged hospital stays, and increased mortality.

The present study revealed a high prevalence of VRE and CRGN carriage among inpatients of our hospital (13.0 and 8.2% respectively). A previous study from the same hospital had determined a VRE carriage rate of 14.3% (29). Other studies have reported carriage rates that varied with geographic location and the general condition of patients (critically ill or not) (16, 17, 30–33). Greece is regularly regarded as an environment with high-selection pressure for the emergence of extensively drug-resistant Gram-negative bacilli in Europe, due to the over-consumption of antimicrobials both in the community and in the hospitals (34).

The multivariable analysis of potential risk factors showed that prolonged hospitalization and advanced age represent independent risk factors for VRE carriage. VRE can survive on environmental surfaces for a long time, and environmental contamination has been identified as a potential risk factor for VRE transmission to healthcare workers' hands and gloves and, subsequently, to patients (35, 36). Hand hygiene compliance rates in Greek hospitals have been reported to range from 22 to 43% (37–39). Since Greece is currently in the midst of a financial crisis, all Greek hospitals suffer from reductions in nursing and cleaning personnel, which may lead to compromises in infection control practices (40–42). All these factors might play a role in the further spread of VRE, especially in cases of prolonged hospitalization. In addition, long hospital stay may be associated with increased antibiotic consumption, which can also contribute to VRE selection and carriage (43). Moreover, the association of old age with VRE carriage may be attributed to several factors, such as alterations of the immune system, malnutrition, and social and economic factors (32, 44–46).

The univariate analysis revealed several risk factors for CRGN carriage: presence of indwelling devices (urinary catheter, pacemaker), CCI, metastatic disease, prolonged hospitalization before the fecal swab sampling, last year hospitalization, and ICU admission. Although none of these factors were independently associated with CRGN carriage in the multivariable logistic regression model, they all deserve to be considered. Indwelling devices are related to disease severity (47) and are recognized risk factors for healthcare-associated carriage and infection with MDR pathogens (48). Furthermore, serious underlying disease as a risk factor for CRGN carriage has been previously described (49) and can be explained by the patients' exposure to invasive procedures, as well as from impaired host defenses and extensive use of antibiotics. Almost invariably, these patients have longer hospitalization than patients with less severe illnesses (4). Also, previous hospitalization was identified as a risk factor for CRGN carriage. Our hospital is a referral center, where

patients from all over the country are admitted for treatment. The majority of these patients have complex medical diseases, prolonged exposure to a healthcare setting, and an extensive use of antibiotics.

Gut carriage of CRGN was independently associated with increased hospital LOS, risk of acquiring a resistant pathogen after rectal swabbing, and mortality. Several studies have demonstrated that gut carriage and subsequent colonization by *K. pneumoniae* in hospitalized patients is associated with a greater risk of infection by the colonizing strains (50, 51). Little is known, though, about the mechanisms that promote progression from carriage to infection. Increased total LOS among CRE carriers has been previously described (19, 49), as well as the association with increased rate of CRE infections and high mortality (19, 20). Previous research has demonstrated that CRE infections are associated more often with sepsis and increased early mortality rate (52), particularly in vulnerable patients such as pediatric, geriatric, immunosuppressed, hospitalized, and chronically ill (53, 54).

Our study has some limitations. We might have underestimated the prevalence of OXA-48 producers that weakly hydrolyze carbapenems (MIC for meropenem lower than 1 mg/L) because of the selective media we used for the culture (55). Moreover, our study lacks enough power to establish a causal relationship between possible risk factors and MDRO carriage, since the study design was cross-sectional. We were also not able to gather detailed information regarding previous exposure to antibiotics such as carbapenems and vancomycin or to assess their impact on MDRO carriage. In addition, we didn't collect subsequent rectal samples in order to differentiate between transient carriage and colonization. More importantly, we did not collect the required data in order to define specific types of infections from resistant pathogens; instead, we collected data on isolation of a subsequent resistant pathogen from a clinical sample. Our analysis was limited to all-cause, rather than attributable, mortality. Thus, we are unable to determine whether the mortality rates of CRGN carriers were directly attributable to infection or were more likely to occur in patients with other fatal illnesses. Furthermore, although statistically significant conclusions were produced, confidence intervals were wide due to the relatively small sample size. Finally, although we tried to incorporate all known potential risk factors for carriage, infection, and mortality, we acknowledge that residual confounding from unmeasured covariates is still possible.

Nevertheless, the results of this study provide valuable information about the CRGN and VRE carriage burden in our hospital and can be used for improving our infection control strategy. After this study, the infection control team has been reinforced with more personnel. The health care employees of our hospital have been informed about the findings of the study and further educational activities on effective infection control practices have been provided. A new point prevalence study of MDRO carriage is scheduled for the first trimester of 2020 in order to compare results and assess the impact of the intensified infection control effort.

In conclusion, VRE and CRGN represent a serious public health problem and carrier patients represent a silent threat

for hospitals. Efforts to limit the spread of MDRO need to be optimized.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board, University General Hospital Attikon. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

SP, AA, and SV contributed to the study design and reviewed the manuscript. AV and PK contributed to the data analysis and manuscript preparation. SV and AT contributed to the data analysis. PV and MK collected and reviewed patients' data and contributed to the data analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2020.00055/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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