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Christina Routsi
Maria Pratikaki
Evangelia Platsouka
Christina Sotiropoulou
Vasileios Papas
Theodoros Pitsiolis
Athanassios Tsakris
Serafeim Nanas
Charis Roussos

Risk factors for carbapenem-resistant Gram-negative bacteremia in intensive care unit patients

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C. Routsi (✉) · M. Pratikaki · C. Sotiropoulou · V. Papas · T. Pitsiolis · S. Nanas · C. Roussos
First Department of Critical Care, Medical School, University of Athens, Evangelismos Hospital, 45-47 Ipsilantou Str., 106 76 Athens, Greece
e-mail: chroutsi@hotmail.com; chroutsi@med.uoa.gr
Tel.: +30-210-7201910
Fax: +30-213-2041389

M. Pratikaki · E. Platsouka
Department of Microbiology, Evangelismos Hospital, Athens, Greece

A. Tsakris
Department of Microbiology, Medical School, University of Athens, Athens, Greece

Present Address:
E. Platsouka
Department of Microbiology, Konstantopouleio General Hospital, Athens, Greece

Abstract *Purpose:* Carbapenem-resistant (CR) Gram-negative pathogens have increased substantially. This study was performed to identify the risk factors for development of CR Gram-negative bacteremia (GNB) in intensive care unit (ICU) patients. *Methods:* Prospective study; risk factors for development of CR-GNB were investigated using two groups of case patients: the first group consisted of patients who acquired carbapenem susceptible (CS) GNB and the second group included patients with CR-GNB. Both case groups were compared to a shared control group defined as patients without bacteremia, hospitalized in the ICU during the same period. *Results:* Eighty-five patients with CR- and 84 patients with CS-GNB were compared to 630 control patients, without bacteremia. Presence of VAP (OR 7.59, 95 % CI 4.54–12.69, $p < 0.001$) and additional intravascular devices (OR 3.69, 95 % CI 2.20–6.20, $p < 0.001$) were independently associated with CR-GNB. Presence of VAP (OR 2.93, 95 % CI 1.74–4.93, $p < 0.001$), presence of additional intravascular devices (OR 2.10, 95 % CI 1.23–3.60, $p = 0.007$) and SOFA score on ICU admission (OR 1.11, 95 % CI 1.03–1.20, $p = 0.006$) were independently associated with CS-GNB. The duration of exposure to carbapenems (OR 1.079, 95 % CI 1.022–1.139, $p = 0.006$) and colistin (OR 1.113, 95 % CI 1.046–1.184, $p = 0.001$) were independent risk factors for acquisition of CR-GNB. When the source of bacteremia was other than VAP, previous administration of carbapenems was the only factor related with the development of CR-GNB (OR 1.086, 95 % CI 1.003–1.177, $p = 0.042$). *Conclusions:* Among ICU patients, VAP development and the presence of additional intravascular devices were the major risk factors for CR-GNB. In the absence of VAP, prior use of carbapenems was the only factor independently related to carbapenem resistance.

Keywords Carbapenem-resistance · Gram- negative bacilli · Bacteremia · Blood stream infections · Critically ill patients

Introduction

The prevalence of Gram-negative bacterial pathogens resistant to multiple antimicrobial agents is increasing in hospitals, and particularly in intensive care unit (ICU) settings [1–7]. Carbapenems, such as meropenem and imipenem, are currently considered to be the preferred agents for the treatment of serious bacterial infections caused by multidrug-resistant Gram-negative pathogens, mainly Enterobacteriaceae, *Pseudomonas aeruginosa*, and nonfermenters, i.e., *Acinetobacter baumannii* [2, 3]. However, the emergence of carbapenem resistance among Gram-negative pathogens has been increasingly reported worldwide and is a matter of great concern, since it complicates both empirical and guided treatment. Moreover, carbapenem resistance is also associated with additional mechanisms of resistance to other antibiotic classes [8]. Local knowledge of the microbial etiologies and susceptibility patterns of isolates appears to be important due to geographical variation of occurrence of bacterial pathogens and antimicrobial drug resistance.

A predominance of multidrug-resistant Gram-negative pathogens has previously been observed in our ICU [9]. Subsequently, an increased rate of carbapenem resistant (CR) *A. baumannii* isolates was observed [10]. We conducted the present study to identify risk factors for acquisition of CR Gram-negative bacteremia (GNB) in the ICU. Such knowledge should be useful to identify patients at risk, so that they receive in time a targeted antimicrobial therapy. Unlike previous studies that have focused on risk factors for antibiotic-resistant organisms comparing case patients with resistant pathogens only to those with susceptible, as controls, we used two groups of case patients as proposed by Kaye et al. [11] with two separate case–control analyses to overcome limitations of the usual case–control studies.

Patients and methods

Setting

This prospective, observational study was conducted from January 2006 through August 2007 in the ICU of Evangelismos Hospital in Athens, Greece. This is a 25-bed university ICU in a 1,000-bed tertiary-care hospital for adults. It admits critically ill medical, surgical and trauma patients. Patients with acute coronary syndromes, cardiac surgery and transplantation are managed in special units and are admitted to the general ICU if they have a complicated course and multiple organ failure.

Study design and data collection

All patients consecutively admitted to the ICU for more than 48 h during the study period, were eligible for inclusion in the study. The data were prospectively collected and included demographics, diagnostic category, comorbidities, illness severity, use of mechanical ventilation, development of acute lung injury (ALI), acute respiratory distress syndrome (ARDS), length of ICU stay, laboratory examinations and antibiotic therapy regimen. The illness severity was evaluated by APACHE II and SOFA scoring systems [12, 13], calculated during the first 24 h of ICU admission. All patients had at least one central venous catheter, a peripheral arterial catheter, and a urinary catheter. Additional exposure to intravascular devices (i.e., pulmonary artery catheter, continuous veno-venous hemofiltration catheter, intraaortic balloon catheter or temporary pacemakers), was defined as the "presence of additional intravascular devices". For patients who had more than one episode of GNB, only the first episode was considered. Twelve patients included in this study have also been included in a previous study comparing characteristics and outcome between patients with CR and CS *A. baumannii* bacteremia [10].

Selection of case and control patients

A nested case–case–control design was followed according to that previously proposed [11, 14–16] as an effective method for identifying risk factors for antimicrobial resistant pathogens: the first group consisted of patients who acquired carbapenem-susceptible (CS) GNB and the second group included patients who acquired CR-GNB. Both case groups were compared to a shared control group defined as patients without bacteremia, hospitalized in the ICU during the same period as the case patients.

Definitions

ICU-acquired GNB was defined as the isolation of Gram-negative bacilli in a blood culture specimen obtained more than 48 h after admission to the ICU. The onset of bacteremia was defined as the date of the blood sampling. Blood culture specimens were ordered by the attending physicians in the presence of clinical features compatible with systemic inflammatory response syndrome (SIRS) [17] or when infection was suspected. Blood cultures were obtained via peripheral venous puncture using a standard sterile technique or from a new central venous catheter immediately after placement and prior to breaking the sterile field that was used for the catheterization. Sources of bacteremia were defined according to the criteria proposed by the Centers for Disease Control and Prevention [18]. Documentation of more than one source was defined as multiple-source bacteremia.

For the risk factor analysis, antibiotic exposure was analyzed by classes of antibiotics given prior to bacteremia development in patients with GNB, and for the whole length of ICU stay in patients without bacteremia.

Microbiological methods

Blood cultures were performed using the BACTEC 9240 system (Becton–Dickinson Sparks, MD, USA). Identification and susceptibility of the blood isolates by determining the minimum inhibitory concentrations (MICs) to different antimicrobial agents was performed by the VITEK2 system (bioMERIEUX, Marcy l'Etoile, France). Carbapenem resistance was verified by determination of minimum inhibitory concentrations (MICs) using E-test (AB Biodisk, Solna, Sweden) strips. Interpretation breakpoints were used as follows: *A. baumannii* $\leq$ 4 mg/L as susceptible, $\geq$16 mg/L as resistant; *P. aeruginosa* $\leq$ 2 mg/L as susceptible, $\geq$8 mg/L as resistant; *Enterobacteriaceae* $\leq$ 1 mg/L as susceptible, $\geq$4 mg/L as resistant, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [19]. Intermediate susceptibility was considered as resistance.

Statistical analysis

Continuous variables were expressed as mean value $\pm$ standard deviation or as median and inter-quartile range when were not normally distributed. Groups' comparisons were made by the analysis of variance (ANOVA) method followed by the Tykey-multiple comparisons test. For not-normally distributed data, the Kruskal–Wallis test was used and the Mann–Whitney test for two groups' comparison. Associations between categorical variables were examined by the χ^2 test or the Fisher exact test, when appropriate. In identifying the independent risk factors for development of carbapenem resistance, a (backward stepwise logistic regression) multiple analysis was performed to control for the effects of confounding factors. The variables initially entered into the analysis were those that were statistically significant in the univariate analysis. A p value of <0.05 was considered statistically significant. All independent variables in this study were tested for multicollinearity. The interaction between risk factor variables was investigated. All statistical analyses were performed using the SPSS version 11.5 for Windows (SPSS Inc. Chicago, IL, USA).

Results

During the study period, 1,096 patients were admitted to the ICU. Of these patients, 241 stayed for <48 h and 13 patients were admitted with bacteremia, so they were excluded from further analysis. Of the remaining 842, 43 patients developed only Gram-positive bacteremia and/or candidemia, so they were also excluded. Finally, among the remaining 799 patients, 169 developed GNB giving an incidence of 16.3 per 1,000 patient-ICU days; 84 patients had bacteremia due to CS and 85 patients had bacteremia due to CR isolates (Fig. 1).

CS-GNBs were most common due to *A. baumannii* (48 patients, 57.2 %) and *Klebsiella pneumoniae* (21 patients, 25 %). CR-GNBs were most common due to *A. baumannii* (32 patients, 37.6 %) and *P. aeruginosa* (31 patients, 36.5 %), (Fig. 2).

Demographic and clinical characteristics of all ICU patients included in the study and results of the univariate analysis are shown in Table 1. Exposure to antimicrobial agents is shown in Table 2 and Fig. 3. Results of the multiple analysis are shown in Table 3.

Respiratory tract infection was the most common source in both CS- and CR- GNB, observed in 38 (45 %) and in 40 (47 %) patients respectively, followed by multiple sources in 16 (19 %) and in 11 (13 %) patients respectively. A focus of infection was not identified in 22 (26 %) patients with CS- GNB and in 24 (28 %) patients with CR- GNB, Table 1.

Patients with CS-GNB versus control patients without bacteremia

According to univariate analysis (Table 1), patients with CS-GNB had a longer length of ICU stay and a higher severity of illness on admission than patients without bacteremia. They presented more often history of diabetes mellitus and renal failure. Also, compared to control patients, they were more likely to have additional intravascular devices, ARDS and VAP development. As shown in Table 2, aminoglycosides, carbapenems, quinolones, monobactams, metronidazole, oxazolidinones and antifungals were administrated for significantly longer period in patients with CS-GNB than in patients without bacteremia. By multiple analysis, independent risk factors for CS-GNB acquisition were the presence of VAP (OR 2.93, 95 % CI 1.74–4.93, $p < 0.001$), the presence of additional intravascular devices (OR 2.10, 95 % CI 1.23–3.60, $p = 0.007$) and the SOFA score on ICU admission (OR 1.11, 95 % CI 1.03–1.20, $p = 0.006$), Table 3.

Patients with CR-GNB versus control patients without bacteremia

According to univariate analysis, (Table 1), patients with CR-GNB had a longer length of ICU stay and a higher severity of illness on admission than those without bacteremia. Also, they were more likely to have additional intravascular devices, to developed VAP and ARDS, and they presented more often history of diabetes mellitus and renal failure. As shown on Table 2, compared to control patients, patients with CR-GNB had exposure for longer period to almost all classes of antimicrobial agents, until the

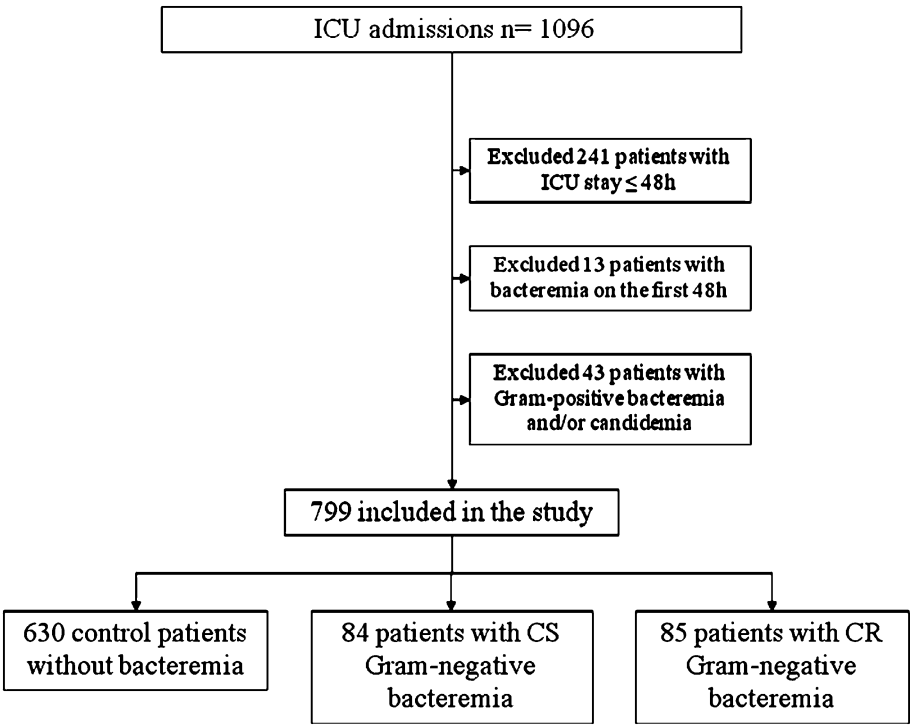

Fig. 1 Schediagram of patients admitted to the ICU between January 2006 and August 2007

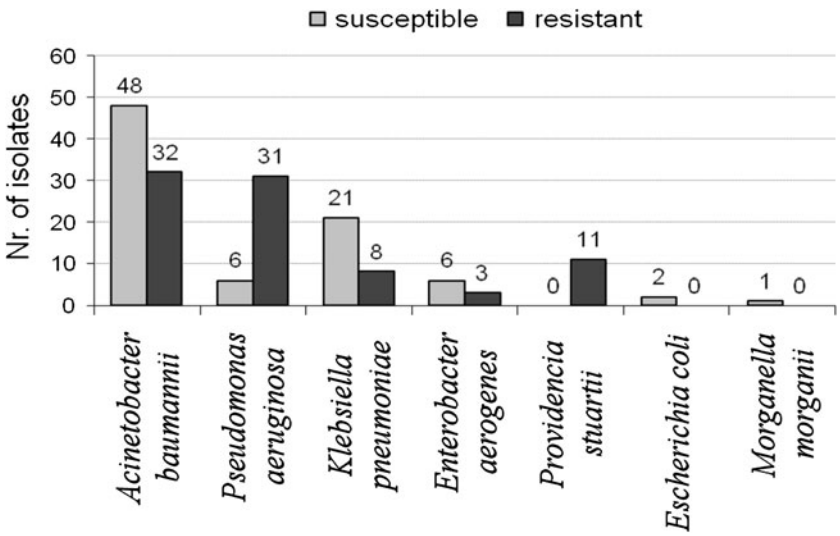

Fig. 2 Frequency of susceptible and resistant to carbapenems Gram-negative pathogens

first episode of bacteremia. Multiple analysis revealed that independent risk factors for CR-GNB acquisition were the development of VAP (OR 7.59, 95 % CI 4.54–12.69, $p < 0.001$) and the presence of additional intravascular devices (OR 3.69, 95 % CI 2.20–6.20, $p < 0.001$), Table 3.

Patients with CS- versus those with CR-GNB

As shown on Table 1, patients with CR-, as compared to those with CS-GNB, had longer hospitalization and longer length of ICU stay prior to bacteremia a significantly longer duration of mechanical ventilation and a longer total length of ICU stay. In addition, patients with CR-GNB were more likely to have acute lung injury and VAP, than were patients with CS-GNB. Finally, patients with CR-GNB had a significantly longer prior exposure to carbapenems than did patients with CS-GNB, to colistin, to glycopeptides, and to antifungals (Table 2; Fig. 3).

Since the length of ICU stay influences the antibiotic regimen administration, the influence of antibiotics was tested after correction for the exposure time. A significant

Table 1 Comparisons of characteristics between control patients, without bacteremia and case patients with Gram-negative bacteremia, due to carbapenem-susceptible and carbapenem-resistant pathogens, univariate analysis

Characteristics	Patients without bacteremia $n = 630$	Patients with GNB, $n = 169$		p value
		Carbapenem-susceptible GNB $n = 84$	Carbapenem-resistant GNB $n = 85$	
Age, year, mean ± SD	56 ± 19	57 ± 20	58 ± 17	0.474
No. of males (%)	420 (67)	59 (70)	53 (62)	0.552
Diagnostic category				
Medical, *n* (%)	246 (39)	40 (48)	17 (20)	
Surgical, *n* (%)	384 (61)	44 (52)	47 (55)	0.082
Hospital LOS, days, median (IQR)				
Before ICU	4 (1–12)	3 (0.3–16)	5 (1–19)	0.298
Before the onset of GNB	–	21 (8–36)	27 (14–41)†	–
ICU LOS, days, median (IQR)				
Before the onset of GNB	–	11 (4–19)	4 (8–24)†	–
Following GNB	–	11 (4–23)	12 (6–29)	–
Total	6 (3–15)	23 (14–44)***	30 (19–52)***,†	<0.001
Duration of mechanical ventilation,				
Days, median (IQR)	7 (3–15)	22 (11–44)***	29 (17–50)***,†	<0.001
APACHE II score on ICU admission, mean ± SD	15 ± 7	19 ± 7***	18 ± 6*	<0.001
SOFA score on ICU admission, mean ± SD	7 ± 3	9 ± 3***	8 ± 3***	<0.001
Co-morbidities, *n* (%)				
COPD	61 (10)	7 (8)	9 (11)	0.881
Diabetes mellitus	59 (9)	15 (18)*	20 (24)***	<0.001
Malignancy	86 (14)	5 (6)	7 (8)	0.063
Neutropenia	11 (2)	2 (2)	3 (4)	0.527
Corticosteroids	27 (4)	2 (2)	4 (5)	0.684
Liver failure	25 (4)	7 (8)	5 (6)	0.171
Renal failure	79 (13)	20 (24)**	19 (22)*	0.003
Source of bacteremia, *n* (%)				
Respiratory tract	–	38 (45)	40 (47)	
Catheter	–	7 (8)	7 (8)	
Unknown	–	22 (26)	24 (28)	0.725
Multiple sources	–	16 (19)	11 (13)	
Other	–	1 (1)	3 (4)	
Patients with additional intravascular devices, *n* (%)	108 (17)	35 (42)***	46 (54)***	<0.001
ALI, *n* (%)	158 (25)	36 (43)**	58 (68)***,††	<0.001
ARDS, *n* (%)	33 (5)	16 (19)***	14 (17)***	<0.001
VAP, *n* (%)	81 (13)	31 (37)***	51 (60)***,††	<0.001
Development of fungemia before GNB, no. of patients (%)	–	6 (7)***	8 (9)***	<0.001
Development of other BSIs	–	14 (17)	36 (42)	<0.001
Temperature, °C on ICU admission	37.1 ± 1.3	37.5 ± 1.3	37.6 ± 1.3†	0.003
Laboratory data, on ICU admission				
Blood glucose, mg/dL	152 ± 66	170 ± 86	160 ± 72	0.056
Serum albumin, gr/dL	3 ± 0.7	2.9 ± 0.7	2.9 ± 0.7	0.124
Hemoglobin, gr/dL	11 ± 2	10.5 ± 2.6*	10.7 ± 2.3*	0.049

GNB Gram negative bacteremia, *ICU* intensive care unit, *LOS* length of stay, *APACHE* acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment, *ALI* acute lung injury, *ARDS* acute respiratory distress syndrome, *VAP* ventilator associated pneumonia, *IQR* interquartile range

* $p < 0.05$ vs. patients without bacteremia

** $p < 0.01$ vs. patients without bacteremia

*** $p < 0.001$ vs. patients without bacteremia

† $p < 0.05$ vs. patients with carbapenem-susceptible Gram-negative bacteremia

†† $p < 0.001$ vs. patients with carbapenem-susceptible Gram-negative bacteremia

relation was found between duration of exposure to carbapenems (OR 1.079, 95 % CI 1.022–1.139, $p = 0.006$) and colistin (OR 1.113, 95 % CI 1.046–1.184, $p = 0.001$), with the acquisition of CR Gram-negative isolates, whereas the length of ICU stay before the bacteremia, was not found significant.

During the process of analysis, a statistically significant interaction was noted between carbapenem and

Table 2 Duration of antibiotic exposure of ICU patients with Gram-negative bacteremia due to carbapenem-susceptible or carbapenem-resistant isolates and patients without bacteremia, days, median (range), univariate analysis

Antibiotic or/ antibiotic classes	Patients without bacteremia, $n = 630$	Patients with Gram-negative bacteremia, $n = 169$		p value
		Carbapenem-susceptible, $n = 84$	Carbapenem-resistant, $n = 85$	
β-Lactams/β-lactamase inhibitors	3 (0–34)	2 (0–27)	0 (0–20)	0.202
2nd generation cephalosporins	0 (0–16)	0 (0–6)	0 (0–7)	0.388
3rd generation cephalosporins	0 (0–28)	0 (0–18)	0 (0–18)*	0.037
Aminoglycosides	0 (0–28)	2 (0–25)***	0 (0–25)**	<0.001
Quinolones	0 (0–31)	0 (0–21)*	0 (0–18)***	<0.001
Carbapenems	0 (0–62)	3 (0–39)**	10 (0–34)***,††	<0.001
Glycopeptides	0 (0–34)	0 (0–30)	2 (0–32)***,†	<0.001
Oxazolidinones	0 (0–33)	0 (0–30)***	1 (0–27)***	<0.001
Metronidazole	0 (0–39)	0 (0–26)**	0 (0–30)*	0.001
Colistin	0 (0–92)	0 (0–39)	1 (0–48)***,††	<0.001
Monobactams	0 (0–28)	0 (0–10)*	0 (0–20)**	0.005
Antifungals	0 (0–45)	0 (0–30)	0 (0–34)***,†	<0.001
Macrolides	0 (0–18)	0 (0–6)	0 (0–10)*	0.03

* $p < 0.05$ vs. patients without bacteremia
** $p < 0.01$ vs. patients without bacteremia
*** $p < 0.001$ vs. patients without bacteremia
† $p < 0.05$ vs. patients with carbapenem-susceptible Gram-negative bacteremia
†† $p < 0.001$ vs. patients with carbapenem-susceptible Gram-negative bacteremia

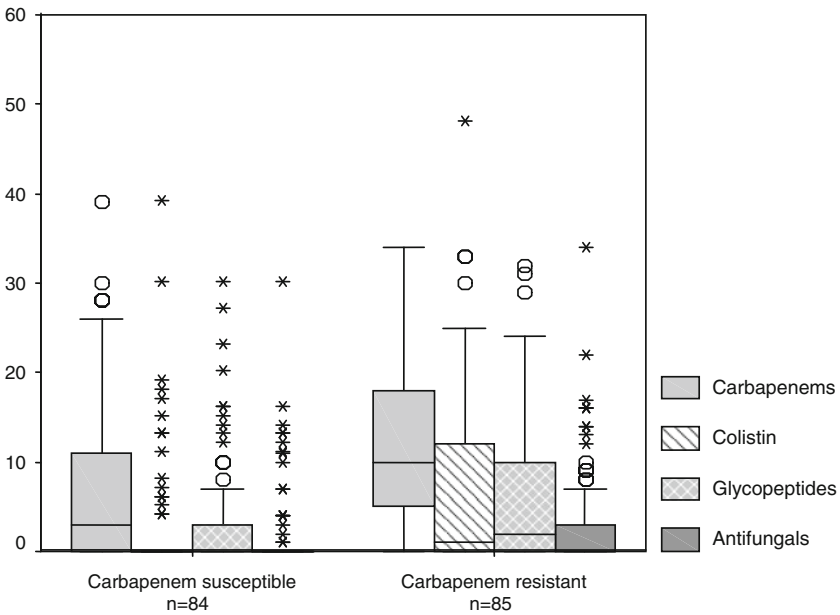

Fig. 3 *Box* plots for the duration (days) of antimicrobial agents administered to patients with carbapenem-resistant ($n = 85$) and to patients with carbapenem-susceptible ($n = 84$) Gram-negative bacteremia: *horizontal bars* represent median values, *boxes* represent interquartile ranges, *whiskers* show ranges, *circles* outliers, and *asterisks* extreme values. The differences were statistically significant for carbapenems ($p < 0.001$), colistin ($p < 0.001$), glycopeptides ($p < 0.05$), and antifungals ($p < 0.05$), Kruskal–Wallis test

colistin administration, and VAP: by applying multiple logistic regression analysis including VAP, carbapenems, colistin administration, and their interaction on CR-GNB development, a statistically significant interaction was detected between carbapenems and VAP: (OR 0.904, 95 % CI 0.82–0.99, $p = 0.048$), Table 4. To further examine the above interaction, an additional analysis was undertaken within patients with GNB by using two statistical models of multiple analysis. The first model included the patients with both VAP and GNB ($n = 82$), and the second, the patients with GNB in the absence of VAP as a source ($n = 87$). In the first subgroup, none of the variables was significantly associated with the acquisition of CR-GNB whereas within the second subgroup previous treatment with carbapenems was the only independent risk factor for the development of CR-GNB (OR 1.09, 95 % CI 1.003–1.177, $p = 0.042$).

Table 3 Differences in independent risk factors, among ICU patients, for development of Gram-negative bacteremia (GNB) susceptible to carbapenems (CS) and resistant to carbapenems (CR), multiple analysis

Patient group, risk factors	Adjusted odds ratio (95 % confidence interval)	*p* value
Patients with CS-GNB		
Presence of VAP	2.93 (1.74–4.93)	<0.001
Additional intravascular devices	2.10 (1.23–3.60)	0.007
SOFA score on admission	1.11 (1.03–1.20)	0.006
Patients with CR-GNB		
Presence of VAP	7.59 (4.54–12.69)	<0.001
Additional intravascular devices	3.69 (2.20–6.20)	<0.001

Table 4 Independent risk factors for development of carbapenem resistant Gram-negative bacteremia among patients with Gram-negative bacteremia, multiple analysis

Risk factor	Adjusted odds ratio (95 % confidence interval)	*p* value
Prior receipt of colistin	1.07 (1.01–1.14)	0.033
Prior receipt of carbapenems	1.08 (0.99–1.17)	0.075
VAP	2.93 (1.05–8.21)	0.041
Carbapenems × VAP	0.90 (0.82–0.99)	0.048

Discussion

This study assessed the risk factors for CR-GNB among patients in a multidisciplinary ICU. The main findings are: (i) among ICU patients, the development of CR-GNB, was independently associated with the presence of VAP as a source of bacteremia and excess use of intravascular devices, whereas the development of CS-GNB was associated with the same risk factors plus the severity of organ failure on ICU admission; (ii) among patients with GNB, the presence of VAP and prior use of carbapenems and colistin were independently associated with carbapenem resistance. In the absence of VAP as a source of bacteremia, the only independent risk factor for CR isolate development was the prior carbapenem use.

In the past decade publications, risk factors specific methodological issues were raised. The importance of control group selection on the results of risk factor analysis for antibiotic-resistant isolates has been shown [11, 14–16]. Accordingly, in the present study two groups of case patients, those with CS- and those with CR-GNB, were compared with the same control group, i.e. ICU patients without bacteremia development, admitted to the ICU during the study period. This double case design was used to avoid overestimation of the risk factors found in studies comparing patients with resistant bacteria only to those with susceptible, as it has been pointed out. To our knowledge, only few studies have included double case patients in similar studies [20, 21].

There is little information about the direct effect of patient associated factors on resistance acquisition. In contrast to a traditional thinking, increased severity of illness may not necessarily be a predisposing factor of infection with antibiotic resistant organisms [14]. Indeed, the findings of the present study clearly demonstrate the absence of an independent relationship between illness severity at ICU admission and subsequent acquisition of CR-GNB, confirming that the resistance development does not obligatorily occur to the more severely ill patients. However, the severity of illness was independently associated with CS-GNB development.

Apart from the illness severity, antimicrobial resistance is thought to be more common in patients who have had a prolonged ICU stay, advanced age, prior therapy with antibiotics and therapy with invasive devices [22]. Indeed, in the present study, patients with CR- as compared to those with CS-GNB did have a longer length of hospital and ICU stay before the development of bacteremia in the univariate analysis. However, this variable did not remain significant in the multiple analysis. The incidence of documented catheter related bacteremia was low during the study period. However, the presence of additional intravascular devices was an independent risk factor for both CS- and CR-GNB development, probably reflecting the illness severity and the more often health care personnel contacts. Regarding the patients' age, we did not find any relation between it and the acquisition of either CS- or CR-GNB.

The increase in resistance among Gram-negative bacteria is frequently related to the high selective pressure of antimicrobials commonly used in hospitalized patients [2, 23–26]. In accordance, in the present study, extensive use of carbapenems, colistin, glycopeptides and antifungals seemed related to the carbapenem resistance in the univariate analysis. However, after controlling for confounding factors and interaction, prior prolonged exposure to carbapenems was the only independent risk factor for ICU acquired CR-GNB. Carbapenems have been identified as a risk factor for CR Gram-negative isolates in previous studies [27–30]: carbapenem use was independently associated with imipenem-resistance [27]; imipenem exposure was the major risk factor for imipenem-resistant *P. aeruginosa* [21, 28], and also for CR *K. pneumoniae* isolation [29]. However, there are exceptions to these findings: fluoroquinolones and antipseudomonal penicillins (and not carbapenems) were independent risk factors for CR *K. pneumoniae* infection elsewhere [31]. Notably, none of these studies, except one [27], have focused on ICU patients and none of them on bacteremia exclusively; therefore, data on the risk factors for CR-GNB in the ICU are still limited.

Apart from antibiotic pressure, the acquisition of antibiotic-resistant bacteria within the ICU also represents

the result of the horizontal transfer, usually via the hands of personnel and inanimate objects. Therefore, any antibiotic use may play a minor role in those patients who have acquired the organism by horizontal transfer [14]. This study, as other clinical studies [28, 31, 32], cannot separate these two modes of CR Gram-negative pathogens acquisition because clonality of strains was not evaluated. During two earlier epidemic outbreaks of multidrug resistant *A. baumannii* [33] and pandrug-resistant *P. aeruginosa* [34] in our unit, cross-transmission had been confirmed. However, regardless of any mode of transmission, this study clearly shows the impact of carbapenem exposure on carbapenem resistance acquisition in ICU and confirms the common knowledge in an unquestionable infection, such as bacteremia.

In the present study, among the entire number of ICU patients, the presence of VAP was found to be the most important risk factor for both CS- and CR-GNB development. Interestingly, among the patients with GNB, those with VAP had an increased likelihood of having a CR isolate (Table 1). This variation emphasize the importance of defining the control group before we proceed to interpretation of the results in risk factor analysis studies, according to the above mentioned epidemiological principles [11, 14–16]. In accordance, previous studies have shown the respiratory system to be the most frequent site of infections due to CR isolates: Raymond et al. [35] found that, the risk of CR bacteremia was about eight times higher for patients with recent prior VAP, as compared to other sites of infection. Similarly, in a previous study from our ICU, comparing CS versus CR *A. baumannii* bacteremia, the presence of VAP was the most important risk factor for CR *A. baumannii* acquisition [10].

There are certain limitations to our study. First, since the clonality of the isolates was not investigated, possible cross-transmission events could not been distinguished from within-host resistance development. Second, previous colonization that might have influenced the antibiotic prescription, was not recorded because active surveillance of patient's floras was not performed in our unit as a routine. Finally, the single center design of the present study and the high proportion of multidrug resistant pathogens in our unit probably limit the generalizability of our findings.

In summary, our results suggest that VAP development and the presence of additional intravascular devices promote the acquisition of CR-GNB. When bacteremia occurred in the absence of VAP, prior use of carbapenems was the only factor independently related to carbapenem resistance, indicating the need for prudent and rational use of carbapenems, along with compliance with basic control measures for nosocomial infections.

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Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Gaynes R, Edwards JR, The National Nosocomial Infections Surveillance System (2005) Overview of Nosocomial Infections caused by Gram-negative bacilli. Clin Infect Dis 41:848–854
2. Clark NM, Patterson J, Lynch JP (2003) Antimicrobial resistance among gram negative organisms in the intensive care unit. Curr Opin Crit Care 9:413–423
3. Paterson DL (2006) Resistance in gram-negative bacteria: enterobacteriaceae. Am J Infect Control 34:S20–S28
4. Flournoy DJ, Reinert RL, Bell-Dixon C et al (2000) Increasing antimicrobial resistance in Gram-negative bacilli isolated from patients in intensive care units. Am J Infect Control 28:244–250
5. Kollef MH, Fraser VJ (2001) Antibiotic resistance in the intensive care unit. Ann Intern Med 134:298–314
6. Jones RN, Phaller MA (1998) Bacterial resistance: a worldwide problem. Diagn Microbiol Infect Dis 31:379–388
7. Fridkin SK (2001) Increasing prevalence of antimicrobial resistance in intensive care units. Crit Care Med 29:N64–N68
8. Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos G, Vatopoulos A, Gniadkowski M, Toth A, Pfeifer Y, Jarlier V, Carmeli Y; CNSE Working Group 2010) Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euroserveill 15(46). pii 19711
9. Pratikaki M, Platsouka E, Sotiropoulou C, Vassilakopoulos T, Paniara O, Roussos C, Routsi C (2009) Risk factors for and influence of bloodstream infections on mortality: a 1-year prospective study in a Greek intensive care unit. Epidemiol Infect 137:727–735
10. Routsi C, Pratikaki M, Platsouka E, Sotiropoulou C, Nanas S, Markaki V, Vrettou C, Paniara O, Giamarellou H, Roussos C (2010) Carbapenem-resistant versus carbapenem-susceptible *Acinetobacter baumannii* bacteremia in a Greek intensive care unit: risk factors, clinical features and outcomes. Infection 38:173–180
11. Kaye KS, Harris AD, Samore M, Carmeli Y (2005) The case–case–control study design: addressing the limitations of risk factor studies for antimicrobial resistance. Infect Control Hosp Epidemiol 26:346–351
12. Knaus WA, Draper EA, Wagner DP, Zimmerman JA (1985) APACHE II: a severity of disease classification system. Crit Care Med 13:818–829

13. Vincent JL, De Mendoka A, Cantraine F et al (1998) Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units. Results of a multicenter, prospective study. Crit Care Med 26:1793–1800
14. Paterson DL (2002) Looking for risk factors for the acquisition of antibiotic resistance: a 21st-century approach. Clin Infect Dis 34:1564–1567
15. Harris AD, Samore M, Lipsitch Kaye KS, Perencevich E, Carmeli Y (2002) Control-group selection importance in studies with antimicrobial resistance: examples applied to *Pseudomonas aeruginosa*, enterococci, and *Escherichia coli*. Clin Infect Dis 34:1558–1563
16. Harris AD, Karchmer TB, Carmeli Y, Samore MH (2001) Methodological principles of case-control studies that analyzed risk factors for antibiotic resistance: a systematic review. Clin Infect Dis 32:1055–1061
17. Levy MM, Fink MP, Marshall JC et al (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 29:530–538
18. Garner JS, Jarvis WR, Emori TG et al (1998) CDC definitions for nosocomial infections, 1988. Am J Infect Control 16:128–140
19. Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing. Twenty-second Informational Supplement M100—S22. 32(3)
20. Kritsotakis EI, Tsioutis C, Roumbelaki M, Christidou A, Gikas A (2011) Antibiotic use and the risk of carbapenem-resistant extended-spectrum-β lactamase-producing *Klebsiella pneumoniae* infection in hospitalized patients: results of a double case–control study. J Antimicrob Chemother 66:1383–1391
21. Harris AD, Smith D, Johnson JA, Bradham DD, Roghmann M-C (2002) Risk factors for imipenem-resistant *Pseudomonas aeruginosa* among hospitalized patients. Clin Infect Dis 34:340–345
22. Niedreman MS (2001) Impact of antibiotic resistance on clinical outcomes and the cost of care. Crit Care Med 29(Suppl):N114–N120
23. Gaynes R (1997) The impact of antimicrobial use on the emergence of antimicrobial-resistant bacteria in hospitals. Infect Dis Clin North Am 11:757–765
24. Loeffler JM, Garbino J, Lew D et al (2003) Antibiotic consumption, bacterial resistance and their correlations in a Swiss university hospital and its adult intensive care units. Scand J Infect Dis 35:843–850
25. Friedrich LV, White RL, Bosso JA (1999) Impact of use of multiple antimicrobials on changes in susceptibility of gram-negative aerobes. Clin Infect Dis 28:1017–1024
26. Hsueh PR, Chen WH, Luh KT (2005) Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991–2003 at a university hospital in Taiwan. Int J Antimicrob Agents 26:463–472
27. Akinci E, Colpan A, Bodur H, Balaban N, Erbay A (2005) Risk factors for ICU-acquired imipenem-resistant Gram-negative bacterial infections. J Hosp Infect 59:317–323
28. Troillet N, Samore MH, Carmeli Y (1997) Imipenem-resistant *Pseudomonas aeruginosa*: risk factors and antibiotic susceptibility patterns. Clin Infect Dis 25:1094–1098
29. Kwak YG, Choi SH, Choo EJ, Chung JW, Jeong JW, Kim NJ, Woo JH, Ryu J, Kim YS (2005) Risk factors for the acquisition of carbapenem-resistant *Klebsiella pneumoniae* among hospitalized patients. Microb Drug Resist 11:165–169
30. Ong DSY, Jongerden IP, Buiting AG, Leverstein-van Hall MA, Speelberg B, Kesecioglu J, Bonten MJM (2011) Antibiotic exposure and resistance development in *Pseudomonas aeruginosa* and Enterobacter species in intensive care units. Crit Care Med 39:2458–2463
31. Falagas ME, Rafailidis PI, Kofteridis D, Virtzili S, Chelvatzoglou FC, Papaioannou V, Maraki S, Samonis G, Michalopoulos A (2007) Risk factors of carbapenem-resistant *Klebsiella pneumoniae* infections: a matched case-control study. J Antimicrobial Chemother 60:1124–1130
32. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y (2008) Predictors of carbapenem-resistant *Klebsiella pneumonia* acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 52:1028. doi: 10.1128/AAC01020-07
33. Kraniotaki E, Manganelli R, Platsouka E, Grossato A, Paniara O, Palu G (2006) Molecular investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii*, with characterization of class 1 integrons. Int J Antimicrob Agents 28:193–199
34. Mentzelopoulos S, Pratikaki M, Platsouka E, Kraniotaki H, Zervakis D et al (2007) Prolonged use of carbapenems and collistin predisposes to ventilator-associated pneumonia by pandrug-resistant *Pseudomonas aeruginosa*. Intensive Care Med 33:1524–1532
35. Raymond DP, Pelletier SJ, Crabtree TD, Evans HL, Pruett TL, Sawyer RG (2003) Impact of antibiotic-resistant Gram-negative bacilli infections on outcome in hospitalized patients. Crit Care Med 31:1035–1104