ARTICLE

Multi-drug-resistant gram-negative bacterial infection in surgical patients hospitalized in the ICU: a cohort study

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Abstract We sought to identify risk factors for postoperative infections, caused by multi-drug-resistant gram-negative bacteria (MDR-GNB) in surgical patients. This was a retrospective cohort study among patients hospitalized in the intensive care unit (ICU) for more than 5 days, following general surgical operations. Comparison of patients who developed infection caused by MDR-GNB with the remainder of the cohort showed that every minute of operative time, use of special treatments during hospitalization (antineoplastic, immunosuppressive or immunomodulating therapies), every day of metronidazole, and every day of carbapenems use, increased patients' odds to acquire an infection caused by MDR-GNB by 0.7%, 8.9 times, 9%, and 9%, respectively [OR (95% CI): 1.007 (1.003–1.011), $p=0.001$; 8.9 (1.8–17.3), $p=0.004$; 1.09 (1.04–1.18), $p=0.039$; 1.09 (1.01–1.18), $p=0.023$, respectively]. The above were adjusted in the multivariable analysis for the confounder of time distribution of infections caused by MDR-GNB. Finally, the secondary comparison, with patients that did not develop any infection, showed that patients who had received antibiotics, within 3 months prior to admission, had 3.8 times higher odds to acquire an infection caused by MDR-GNB [OR (95% CI): 3.8 (1.07–13.2), $p=0.002$]. This study depicts certain, potentially modifiable, risk factors for postoperative infections in patients hospitalized in the ICU for more than 5 days.

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Introduction

Mortality and prolonged hospitalization among patients who have been submitted to major surgical operations is often attributed to infections that occur in the early postoperative period [1–3]. During the last decade, there has been considerable change in the epidemiology of hospital acquired infections, with gram-negative surpassing gram-positive bacteria as the leading pathogens [4–6]. Furthermore, the emergence of gram-negative bacteria resistant to most of the commonly used antibiotics makes treatment of such infections a clinical challenge [7, 8]. Identification of the risk factors for the development of infections caused by multi-drug-resistant gram-negative bacteria (MDR-GNB) may help clinicians prevent nosocomial infection. This becomes even more important, if we

consider the slow pace of development of new effective antimicrobial agents [9] and the constantly rising prevalence rates of MDR-GNB, especially in intensive care units (ICUs) [10].

Multi-drug-resistance (MDR) has received various definitions in regard to the number of classes of antimicrobial agents of pathogen's resistance [11]. MDR defined as resistance to all but three classes of antibiotics has not been extensively investigated, especially in surgical patients. Based on the above, the aim of this study was to identify risk factors for the development of infections, caused by MDR-GNB, in a cohort of patients hospitalized in the ICU for more than 5 days following general surgical operations. We sought to investigate numerous factors that have been related, by previous studies [12–25], to the development of resistance to antibiotics, both in vitro and in special patient populations. In addition, this cohort study may permit to attribute mortality and length of hospital stay of patients with such infections to the resistance pattern of the pathogen or to the various other comorbidity conditions and complicating factors.

Methods

Study design

A retrospective cohort study at a general, 450-bed, tertiary-care hospital in Athens, Greece. The study was approved by the hospital's ethics review board.

Cohort description

Patients hospitalized in the ICU for more than 5 days following general surgical operations during a 7-year period from the first day of hospital operation in November 2001 to May 2007 were identified. The cohort included only patients without any infection on ICU admission. All cases of infection included were clinically and microbiologically documented. Patients who had a clinical infection that was not microbiologically documented were eliminated from the study.

Group comparison

Patients who had an infection, caused by MDR-GNB, were assigned to the case group (group A). The rest were included in the comparison group (group B). Moreover, there were three comparison subgroups: patients who did not develop any microbiologically documented infection (sub-group B1), patients who developed infection caused by a gram-positive pathogen during the hospital and ICU stay (sub-group B2), and patients who developed infection

caused by gram-negative bacteria susceptible to more than three of the tested antibiotics (sub-group B3).

It should be noted that only one infection per patient was taken into account for this study. Thus, only the first infection caused by MDR-GNB was studied as case. Respectively, for the comparison group, only the first infection caused by pathogens other than MDR-GNB was included. Furthermore, for cases of multimicrobial infection, patients were assigned to group A if at least one of the isolated microbes was an MDR-GNB.

Antibiotic susceptibility testing

Gram-negative bacteria were tested for susceptibility to amikacin, aztreonam, amoxicillin/clavunate, ampicillin, cefaclor, cefepime, cefotaxime, cefotaxin, ceftazidime, cefuroxim-axetil/sodium, cephalothin, ciprofloxacin, genta-mycin, imipenem, meropenem, netilmycin, peflocacin, norfloxacin, ofloxacin, piperacillin, piperacillin/tazobactam or clavunate, tobramycin, trimethoprim/sulfamethoxazole, cefpodoxime, nitrofurantoin, isepamicin, and colistin. Gram-positive bacteria were tested for susceptibility to amoxicillin/clavulanate, ampicillin, ampicillin/sulbactam, cefaclor, cefotaxime, ceftriaxone, cefuroxime-sodium, ciprofloxacin, clindamycin, erythromycin, fosfomycin, fucidic acid, gentamicin, imipenem, norfloxacin, oxacillin, penicillin-G, rifampin, teicoplanin, tetracycline, tobramycin, trimethoprim/sulfamethoxazole, vancomycin, levoflox-acin, azithromycin, clarythromycin, quinupristin/dalfopristin, nitrofurantoin, linezolid, and moxifloxacin.

Definition of infection and outcomes

Two independent investigators, blinded to each other, differentiated colonization to infection and determined the infection-related outcomes. Specifically, the Center for Disease Control and Prevention definitions [26] were used to determine nosocomial infection. Institution of appropriate antimicrobial treatment and isolation of pathogens from other sites that could better account for the clinical manifestations were also taken into account.

The outcomes of this cohort were all-cause in-hospital mortality, mortality attributed to infection caused by the studied isolates, infection outcome, and total length of hospital and ICU stay. Death was attributed to infection if it resulted in severe sepsis or septic shock or organ dysfunction or failure.

Collection and extraction of data

Four investigators retrieved all available medical charts and microbiological testing and extracted data regarding patients' demographics, medical history and comorbidity.

Furthermore, microbial isolates susceptibility to antimicrobials, date of culture and type of specimen were recorded. Invasive procedures, and outcomes, the type of antimicrobials as well as the time and duration of antimicrobial treatment were extracted from medical records. In addition, transfusion of blood products, and renal replacement therapy, as well as special treatments that included antineoplastic, immunosuppressive or immunomodulating therapies were recorded. Invasive procedures included placement of central venous and bladder catheters, nasogastric, and tracheostomy tubes. Details about surgical interventions were also recorded; type of operation, classification, administration of perioperative antimicrobial prophylaxis, operative times (total operative time was defined as the sum of all operations within the study index), electivity of surgery, haemostatic packing, material placement, and reoperation. Moreover, investigators recorded ICU and hospital stay, as well as the use and the duration of mechanical ventilation, and finally calculated the acute physiology and chronic health evaluation (APACHE) II score on admission to the ICU. Finally, the year of admission to hospital was examined as a potential confounder. The rather short time periods from November to December 2001 and from January to May 2007 were examined as a total with the years 2002 and 2006, respectively.

It should be mentioned that the various clinical and laboratory characteristics that were analyzed referred only to the index of hospitalization up to the time of infection and isolation of the first MDR-GNB for the cases and the first infection caused by any other pathogen for the comparison group. For patients who did not develop any infection the relevant data was extracted for the entire hospital stay.

Data analysis

The chi-square and Fisher's exact tests were used to compare groups for dichotomous variables, as appropriate. The t-test and the Mann-Whitney signed-rank test were used to compare groups for normally and non-normally distributed continuous variables, respectively. Variables found to be significantly associated with the development of infection caused by MDR-GNB, in the bivariable analyses, were entered in a multivariable forward, stepwise, logistic regression model and the adjusted odds ratio (OR) and 95% confidence intervals (CIs) were calculated. The probability for removal in the logistic regression model was set at $p > 0.1$. For all tests, two-tailed p values lower than 0.05 denoted statistical significance. Furthermore, variables' colinearity was tested. Tolerance less than 0.1 indicated that the variable was redundant and highly correlated with other variables that were already in the model. Summary measures

of goodness of fit were performed using the Hosmer-Lemeshow test. Additional checks were performed by entering the same variables in relevant backward, stepwise, logistic regression models. The statistical software SPSS, version 17.0 (SPPS Inc, Chicago, Illinois, USA) was employed for all analyses.

Results

Study population

During the study period, 100 patients (54 males) fulfilled the inclusion criteria. The mean age of the study population was 67 years (range 22–92), the mean length of hospital stay was 29 days (range 9–98), the mean length of ICU stay was 16 days (range 6–55) and the mean APACHE II score at first ICU admission was 16 (range 4–33). Patients were submitted to one or more operations, the mean total operative time was 278 minutes (range 45–665) and 42% of patients were reoperated. The range of operations included colorectal (38%), small bowel (19%), stomach (9%), liver (9%), pancreas (6%), and other general surgical operations (19%). Among the studied patients, 48 had clinically and microbiologically documented infections caused by MDR-GNB (32 cases of *Acinetobacter baumannii*, 8 cases of *Pseudomonas aeruginosa*, and 8 cases of *Klebsiella pneumoniae*) (group A), 14 patients had infections caused by gram-positive bacteria (5 cases of *Streptococcus faecalis*, 3 cases of *Staphylococcus aureus* and 6 cases of other gram-positive pathogens) (sub-group B2), and six had infections caused by gram-negative bacteria susceptible to more than three of the tested antibiotics (5 cases of *Pseudomonas aeruginosa* and 1 case of *Klebsiella pneumoniae*) (sub-group B3). Furthermore, there were two patients who had infection caused by *Candida albicans*. Finally, there were 30 patients that did not have any clinically diagnosed nosocomial infection during hospitalization (sub-group B1). It should be noted that there were 14 cases of multi-microbial infections (23% of infections were mulitmicrobial). In all multi-microbial infections an MDR-GNB was isolated and patients were assigned to group A. Cases included in sub-groups B2 and B3 were relatively low; thus, we did not perform any statistical comparison. Out of 70 cases of infection, 43% were respiratory tract, 24% were abdominal, 17% blood stream, 9% catheter related and 7% in other sites.

Univariate analysis

In Table 1, we present the comparison of various characteristics between the group of patients that developed infections caused by MDR-GNB and other patient sub-

Table 1 Comparison of the group of patients that developed infections caused by multi-drug-resistant gram-negative bacteria (MDR-GNB) with other patient groups

Variable	Group A: Patients with infections caused by MDR-GNB ($n=48$)	Group B: Patients without infection caused by MDR-GNB ($n=52$)	P value (comparison between groups A and B)	Sub-group B1: Patients without any infection ($n=30$)	P value (comparison between group A and sub-group B1)
Demographics [mean ± SD or n (%)]					
Gender male	24 (50)	30 (57.7)	0.54	16 (53.3)	0.82
Age	65.1±13.8	69.3±13.7	0.14	71.4±13.4	0.046
Surgery related data within the study index [mean±SD or n (%)]					
Elective	35 (72.9)	34 (65.1)	0.52	21 (70)	0.8
Reoperation	26 (54.2)	16 (30.8)	0.025	9 (30)	0.06
Total operative time, minutes	333.3±131.4	228.8±123.9	<0.001	221.4±132.8	<0.001
Number of surgeries	1: 22 (45.8) 2: 16 (33.3) 3: 10 (20.8)	1: 36 (69.2) 2: 11 (21.2) 3: 5 (9.6)	0.054	1: 21 (70) 2: 6 (20) 3: 3 (10)	0.11
Contaminated	35 (72.9)	29 (55.8)	0.09	14 (46.7)	0.03
Material placement	2 (4.2)	2 (3.8)	0.93	1 (3.3)	0.85
Haemostatic packing	7 (14.6)	4 (7.7)	0.35	1 (3.3)	0.14
Invasive procedures, hospitalization, and device placement within the study index/duration, days [mean±SD or n (%)]					
Length of hospital stay	20.7±12.9	18.3±11.7	0.52	21.9±11.8	0.26
Length of ICU stay	11.2±8.9	8.8±6.4	0.26	11.9±6.0	0.19
Mechanical ventilation	7.8±7.7	4.5±4.7	0.033	5.2±4.9	0.26
Foley catheter	13.1±8.3	11.3±7.3	0.21	11.8±8.6	0.31
Central venous catheter	11.6±8.6	8.3±6.3	0.053	11.1±6.0	0.71
Peripheral venous catheter	11.6±9.2	10.0±6.4	0.48	10.5±5.1	0.98
Tracheostomy	2.6±6.1	0.6±2.5	0.009	0.7±3.2	0.031
Nasogastric tube	11.1±7.3	9.3±5.5	0.31	9.8±5.9	0.55
Perenteric feeding	6.9±8.1	4.5±4.6	0.18	5.7±4.7	0.96
Renal replacement	16 (33.3)	8 (15.4)	0.06	3 (10)	0.029
Blood transfusion	44 (91.7)	35 (67.3)	0.003	20 (66.7)	0.007
Special treatments	25 (52.1)	15 (28.8)	0.025	10 (33.3)	0.16
APACHE II score at ICU admission	16.6±5.5	15.7±5.0	0.39	14.9±4.8	0.24
Comorbidity [mean±SD or n (%)]					
Prior hospitalization (within 3 months prior to admission)	19 (39.6)	19 (36.5)	0.83	9 (30)	0.47
Prior antibiotics use (within 3 months prior to admission)	21 (43.8)	15 (28.8)	0.14	5 (16.7)	0.015
Placement of prosthetic material in the past	3 (6.3)	4 (7.7)	1	2 (6.7)	1
Diabetes mellitus	12 (25)	10 (19.2)	0.63	6 (20)	0.78
Malignancy	33 (68.8)	30 (57.7)	0.3	16 (53.3)	0.23
Hematological disorders	2 (4.2)	2 (3.8)	1	1 (3.3)	1
Liver disease	8 (16.7)	10 (19.2)	0.79	5 (16.7)	1
Respiratory disease	10 (20.8)	10 (19.2)	1	4 (13.7)	0.54
Heart disease	23 (47.9)	31 (59.6)	0.31	21 (70)	0.07

Table 1 (continued)

Variable	Group A: Patients with infections caused by MDR-GNB (n=48)	Group B: Patients without infection caused by MDR-GNB (n=52)	P value (comparison between groups A and B)	Sub-group B1: Patients without any infection (n=30)	P value (comparison between group A and sub-group B1)
Urinary tract disease	11 (22.9)	6 (11.5)	0.1	6 (20)	1
Neurological disorders	7 (14.6)	7 (13.5)	1	6 (16.7)	1
Acute renal failure	1 (2.1)	3 (5.8)	0.61	2 (6.7)	0.56
Chronic renal failure	4 (8.3)	3 (5.8)	0.7	1 (3.3)	0.64
Comorbidities total	2.3±1.5	2.2±1.3	0.79	2.2±1.1	0.88
Antibiotic use/duration of use within the study index, days [mean ± SD or n (%)]					
Quinolones	9.9±9.6	7.7±8.3	0.13	9.3±6.9	0.83
Cephalosporins	2.2±6.5	2.3±4.1	0.24	2.8±4.6	0.25
Metronidazole	10.1±8	6.5±6.2	0.027	7.9±6.5	0.36
Glycopeptides	5.8±7.8	3.1±4.5	0.13	3.9±5.2	0.38
Anti-pseudomonal penicillins	5.1±4.8	3.0±4.3	0.007	3.4±4.4	0.08
Carbapenems	8.9±11.4	3.5±5.6	<0.001	4.1±6.8	0.003
Linezolid	3.6±8.3	1.1±3.4	<0.001	1.7±4.3	0.031
Aminoglycosides	3.0±6.4	2.2±3.9	0.98	2.8±4.7	0.89
B-lactams	0.5±1.4	2.1±5.5	0.08	2.1±4.0	0.07
Cumulative days of antibiotic treatment	57.1±44.0	34.6±23.1	0.005	41.8±21.7	0.25
Total days of antibiotic treatment	16.6±10.7	11.6±7.8	0.022	13.7±5.4	0.69
Year of admission, n (%)					
2002	6 (12.5)	20 (38.5)	0.006	14 (46.7)	0.001
2003	6 (12.5)	11 (21.2)	0.3	3 (10)	1
2004	16 (33.3)	9 (17.3)	0.1	6 (20)	0.3
2005	11 (22.9)	6 (11.5)	0.18	3 (10)	0.23
2006	9 (18.8)	6 (11.5)	0.4	4 (13.3)	0.7
Other outcomes [mean ± SD or n (%)]					
Survival	18 (37.5)	28 (53.8)	0.11	19 (63.3)	0.036
Infection amelioration	23 (47.9)	n/a	n/a	n/a	n/a
Death attributed to infection, n (% of deaths)	21 (70)	n/a	n/a	n/a	n/a
Total length of hospital stay, days	34.6±20	24±13.1	0.01	21.9±11.8	0.01
Total length of ICU stay, days	19.4±12.4	13.1±8.2	0.04	11.9±6.0	0.03

ICU intensive care unit, APACHE II score acute physiology and chronic health evaluation II score, n/a not applicable

groups. There was not any statistically significant difference in age and sex distributions between groups A and B. However, patients in group A were significantly younger compared to patients in sub-group B1 (mean age 65.1 and 71.4 years, respectively, p=0.046).

Reoperation rates and total operative time were significantly higher in group A compared to group B (54.2% vs 30.8%, p=0.025; mean 333.3 vs 228.8 minutes, p<0.001, respectively). Total operative time and rates of contaminated surgery were significantly higher in group A compared to

sub-group B1 (mean 333.3 vs 221.4 minutes, p<0.001; 72.9% vs 46.7%, p=0.03, respectively).

The study of other clinical predictors such as invasive procedures rates, hospitalization duration, device placement rates, and clinical scores showed that patients who developed infections caused by MDR-GNB had sustained more interventions than other patients (Table 1). However, only certain differences were statistically significant. Specifically, mean mechanical ventilation duration, mean tracheostomy duration, rates of blood transfusion, and

special treatments (antineoplastic, immunosuppressive or immunomodulating therapies) were significantly higher in group A compared to group B (7.8 vs 4.5 days, $p=0.033$; 2.6 vs 0.6 days, $p=0.009$; 91.7% vs 67.3%, $p=0.003$; 52.1% vs 28.8%, $p=0.025$, respectively). Furthermore, mean tracheostomy duration, rates of renal replacement therapy and blood transfusion were significantly higher among patients of group A compared to sub-group B1 (2.6 vs 0.7 days, $p=0.031$; 33.3% vs 10%, $p=0.029$, 91.7% vs 66.7%). Of interest, APACHE II score at ICU admission did not differ significantly among all compared groups. Regarding comorbidity, prior antibiotics use (within last 3 months) differed significantly when comparing group A to sub-group B1 (mean 43.8 vs 16.7 days, $p=0.015$).

The analysis of antibiotics use showed that patients of group A had received significantly longer metronidazole, anti-pseudomonal penicillins, carbapenems, and linezolid therapy (up to the day of infection) compared to patients of group B (mean duration of 10.1 vs 6.5 days, $p=0.027$; 5.1 vs 3 days, $p=0.007$; 8.9 vs 3.5, $p<0.001$; 3.6 vs 1.1, $p<0.001$, respectively). Furthermore, the mean cumulative days of antibiotic treatment and total days of antibiotic treatment were significantly more in group A compared to group B (57.1 vs 34.6 days, $p<0.005$, and 16.6 vs 11.6 days, $p=0.022$, respectively). However, the colinearity analysis showed that the covariate of cumulative days of antibiotic treatment was redundant (probably already expressed through the variable of total days of antibiotic treatment) and thus, not included in the multivariable analysis (tolerance=0.081). The relevant comparison of group A with group B1 showed that patients of group A had received significantly longer carbapenems and linezolid therapy (up to the day of infection) compared to patients of group B1 (mean duration of 8.9 vs 4.1 days, $p<0.003$, and 3.6 vs 1.7 days, $p=0.031$, respectively).

Finally, admission to the hospital in the first year of hospital operation was considerably less frequent among patients of group A (12.5%) compared to group B (38.5%, $p=0.006$) and group B1 (46.7%, $p=0.001$).

Regarding other studied outcomes, group A had lower survival rate (37.5%) compared to group B (53.8%, not statistically significant) and sub-group B1 (63.3%, $p=0.036$). Group A had higher mean total length of hospital and ICU stay (34.6 and 19.4 days, respectively) compared to group B (24 and 13.1 days, $p=0.01$ and 0.04, respectively) and sub-group B1 (21.9 and 11.9 days, $p=0.01$ and 0.03, respectively). Of interest, death was attributed to infection in 70% of group A patients who died.

Multivariable analysis

First, we compared patients that developed infection caused by MDR-GNB ($n=48$) (group A) with patients that did not

($n=52$) (group B). In Table 2, we present the findings of the multivariable logistic regression model for infection caused by MDR-GNB (dependent variable). The adjusted odds ratios provided by the final model equation showed that every minute of operative time, use of special treatments during hospitalization (antineoplastic, immunosuppressive or immunomodulating therapies), every day of metronidazole, and every day of carbapenems use increased patients' odds to acquire an infection caused by MDR-GNB by 0.7%, 8.9 times, 9%, and 9%, respectively [OR (95% CI): 1.007 (1.003–1.011), $p=0.001$; 8.9 (1.8–17.3), $p=0.004$; 1.09 (1.04–1.18), $p=0.039$; 1.09 (1.01–1.18), $p=0.023$, respectively). The above predictors were adjusted for admission in the first year of hospital operation [OR (95% CI): 0.1 (0.03–0.43), $p=0.002$]. The overall test for the model was statistically significant ($p<0.001$) and successfully measured for goodness of fit.

Secondly, we compared patients that developed infection caused by MDR-GNB ($n=48$) (group A) with patients that did not develop any infection ($n=30$) (sub-group B1). In Table 3, we present the findings of the multivariable logistic regression model for infection caused by MDR-GNB (dependent variable). The adjusted odds ratios provided by the final model equation showed that every minute of operative time, and use of antibiotics, within 3 months prior to admission, increased patients' odds to acquire an infection caused by MDR-GNB by 0.7% and 3.8 times, respectively [OR (95% CI): 1.007 (1.003–1.011), $p=0.001$; 3.8 (1.07–13.2), $p=0.002$, respectively]. The above predictors were adjusted for admission in the first year of hospital operation [OR (95% CI): 0.07 (0.01–0.4), $p=0.03$]. The overall test for the model was statistically significant ($p<0.001$) and successfully measured for goodness of fit.

Discussion

The main finding of this retrospective cohort analysis, in patients hospitalized in the ICU for more than 5 days following general surgical operations, is that postoperative infection caused by MDR-GNB is independently associated with the total operative time, special treatments during hospitalization (antineoplastic, immunosuppressive or immunomodulating therapies), carbapenems and metronidazole use duration, and prior antibiotics use (within 3 months prior to admission). Furthermore, the study showed that, during the first year of hospital operation, cases of infection caused by MDR-GNB were considerably lower. The above would have been a significant confounder if not adjusted for. This may be attributed to the fact that the hospital was a newly built facility and hospital microbial ecology changed over the first year of operation, to meet local patterns of resistance, as patients from the national

Table 2 Forward multivariable logistic regression model for infection caused by muti-drug-resistant gram-negative bacteria (MDR-GNB) (dependent variable). Comparison of patients that developed infection caused by MDR-GNB (*n*=48) (group A) with patients that did not (*n*=52) (group B)

Variables in the equation	Adjusted odds ratio (95% CI)	Clinical meaning	*p* value
Total operative time, minutes	1.007 (1.003–1.011)	For every minute of surgery, patients' odds to acquire an infection caused by MDR-GNB increased by 0.7%	0.001
Special treatments during hospitalization (antineoplastic, immunosuppressive or immunomodulating therapies)	8.9 (1.8–17.3)	Special treatments increased patients' odds to acquire an infection by MDR-GNB by 8.9 times	0.004
Metronidazole use duration, days	1.09 (1.04–1.18)	For every day of metronidazole use, patients' odds to acquire an infection caused by MDR-GNB increased by 9%	0.039
Carbapenems use duration, days	1.09 (1.01–1.18)	For every day of carbapenems use, patients' odds to acquire an infection caused by MDR-GNB increased by 9%	0.023
Admission in years 2002 (first year of hospital operation)	0.1 (0.03–0.43)	Patients admitted during the first year of hospital operation had decreased odds to acquire an infection caused by MDR-GNB by ten times	0.002

All variables that were significantly associated with infection caused by MDR-GNB in the univariate analysis (Table 1) were inserted in the forward multivariable logistic regression model. Seven variables were not included in the equation (probability for removal was set at *p*>0.10): renal replacement, linezolid use duration, mechanical ventilation duration, reoperation, antipseudomonal penicillins use duration, total days of antibiotic treatment, and tracheostomy duration

Table 3 Forward multivariable logistic regression model for infection caused by multi-drug-resistant gram-negative bateria (MDR-GNB) (dependent variable). Comparison of patients that developed infection caused by MDR-GNB (*n*=52) (group B) with patients that did not develop any infection (*n*=30) (sub-group B1)

Variables in the equation	Adjusted odds ratio (95% CI)	Clinical meaning	*p* value
Total operative time, minutes	1.007 (1.003–1.011)	For every minute of surgery, patients' odds to acquire an infection caused by MDR-GNB increased by 0.7%	0.001
Prior antibiotics use (within 3 months prior to admission)	3.8 (1.07–13.2)	Prior antibiotics use increased patients' odds to acquire an infection caused by MDR-GNB by 3.8 times	0.002
Admission in years 2002 (first year of hospital operation)	0.07 (0.01–0.4)	Patients admitted during the first year of hospital operation had decreased odds to acquire an infection caused by MDR-GNB by 14 times	0.03

All variables that were significantly associated with infection caused by MDR-GNB in the univariate analysis (Table 1) were inserted in the forward multivariable logistic regression model. Seven variables were not included in the equation (probability for removal was set at *p*>0.10): renal replacement, linezolid use duration, carbapenems use duration, trachiostomy duration, patients' age, contaminated surgery and blood transfusion

health system were also admitted. Finally, patients with infection caused by MDR-GNB were hospitalized and treated in the ICU for considerably longer time and had lower survival rates compared to other patient groups.

It is noteworthy that two of the three independent risk factors that were found in the secondary comparative analysis, between patients that developed infection caused by MDR-GNB and patients that did not develop any infection, corroborated the findings applying to the entire cohort (comparison of patients that developed infection caused by MDR-GNB with patients that did not). The secondary analysis additionally revealed prior antibiotics use (within 3 months prior to admission) as an independent risk factor. Metronidazole and carbapenems use duration were not independent risk factors in the secondary comparison. This may be attributed to the fact that patients' number was smaller in the secondary analysis (72 patients compared to 100 of the entire cohort).

It should be mentioned that the inclusion criteria (patients hospitalized in the ICU for more than 5 days following general surgical operations) defined a population of critically ill patients with considerable comorbidity. The high mean APACHE II score at ICU admission (16), the long mean operative time (278 minutes), and high reoperation rates (42%) confirm the above. Furthermore, a large proportion of patients had malignancy (63%) and in many cases surgery, especially reoperation, was palliative. This is a special population and the extremely high rates of infection (70%) and mortality (54%) may not be compared with outcomes of patients that are admitted to the general surgery ward in everyday clinical practice. However, the fact that APACHE II score at ICU admission did not differ significantly among all compared groups is denoting that morbidity and infection predisposing conditions mainly develop during hospital and especially ICU management.

The findings of this cohort are in keeping with various studies. In this study, patients who did not develop any infection had significantly higher survival rates. Similarly, in a study among patients submitted to surgery for colonic cancer postoperative infectious complications were strong risk factors for early death [27]. Several studies have shown that prolonged operative time [25, 27, 28], wound class [29, 30], emergency surgery [29, 31], and number of surgical interventions [31], correlate with increased infection rate. Among patients of this study, metronidazole use duration was an independent risk factor for the development of infection caused by MDR-GNB. According to other investigators, metronidazole may disrupt intestinal anaerobic flora but is minimally active against other intestinal flora, and thus, may promote the overgrowth of gram-negative bacteria [32]. Furthermore, it may increase the frequency of bacterial translocation [33–37] and has been associated with increased

risk to acquire Vancomycin-resistant *Entorococcus* (VRE) bacteremia [34] and carbapenem-resistant *Escherichia coli* [32]. Shorter antimicrobial regimens have been associated with decreased development of antimicrobial resistance by several studies; a study showed that multiresistance emerged less frequently with short-period antibiotic regimens [38], another analysis of patients with suspected ventilator associated pneumonia (VAP) concluded that a shorter antibiotic course may be associated with less antimicrobial resistance on follow-up cultures [39]. Similarly, other investigators found that prolonged use of antibiotics in the ICU was associated with emergence of multiresistance [40]. However, according to another study a shorter duration of carbapenem therapy was not shown to protect against subsequent development of multiresistant bloodstream infection [41].

It should be mentioned that the findings of our study should not be interpreted without the consideration of potential limitations. First, it should be acknowledged that, due to the innate limitations of retrospective studies, certain cases of colonization may have been treated as clinical infections. Moreover, a significant proportion of the patients had more than one incidence of infection; however, only one incidence per patient was analysed. As mentioned above, the inclusion criteria ruled off patients with benign clinical course, and, thus, conclusions may not apply to the majority of patients treated in the general surgery department. Furthermore, the relatively limited number of cases included in this analysis did not permit following the "rule of thumb" in a backwards stepwise logistic regression model (a minimum of 10 binary events per candidate variable inserted in the multivariable model) [42]. However, both models were overall statistically significant in every step of the regression ($p<0.001$), summary measures of goodness of fit were done, colinearity issues were examined and resolved, and an additional check was performed by running the models both forwards and backwards producing the exact same results. Despite these shortcomings, the findings of the study may be useful to clinicians and researchers since this is the first analysis of risk factors for the development of MDR-GNB infections in surgical patients.

In conclusion, this study describes the magnitude of postoperative infectious complications, that are often devastating for patients' survival, in a cohort of patients hospitalized in the ICU for more than 5 days following general surgical operations. Furthermore, it depicts certain, potentially modifiable, risk factors that may be associated with the development of infections caused by MDR-GNB. Based on evidence, provided by this study, specific actions may be taken to improve nosocomial infection prevention in patients submitted to general surgical operations. The presented data may help surgeons and intensivists, who

treat such patients, decide to what extent they are able to modify their therapeutic strategies to achieve the best possible clinical result.

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