Journal of Advances in Medicine and Medical Research

22(8): 1-10, 2017; Article no.JAMMR.34535
Previously known as British Journal of Medicine and Medical Research
ISSN: 2231-0614, NLM ID: 101570965

Nosocomial-acquired Non-fermenting Gram-negative Bacilli: Impact on Morbidity and Mortality in a Brazilian University Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VN and SGS designed the study. Authors GLM and ROF wrote the protocol, managed the experiments and the analyses of the study. Authors CDV and SGS managed the literature searches. Authors VN and CDV performed the statistical analysis. Authors CDV, VN and SGS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/34535
Editor(s):
(1) Zoran Todorovic, Department of Pharmacology, Faculty of Medicine, University of Belgrade, Serbia.
Reviewers:
(1) Wagih Mommtaz Ghannam, Mansoura University, Egypt.
(2) R. Jasmine, Bishop Heber College, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/19872

Original Research Article

Received 31st May 2017
Accepted 26th June 2017
Published 5th July 2017

ABSTRACT

Aims: To investigate non-fermenting Gram-negative bacilli (NFGNB), *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, recovered from clinical isolates and surveillance cultures, accessing their role on morbidity and hospital mortality.
Study Design: This observational case-control study was conducted in the Hospital of the Universidade Federal de Minas Gerais, between March 2012 and September 2013.
Methodology: We included 102 patients categorized by 51 study group (those infected or colonized by multidrug resistant NFGNB, mainly *A. baumannii*) and 51 controls (patients colonized or infected by other multiresistant Gram-negative bacteria). Demographic (gender and age) and

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clinical data (diagnosis of sepsis, antimicrobial intake during hospitalization, among others) were compared between groups. Hospital mortality and length of hospital stay were investigated.
Results: The average age was of 61 years (± 15) in the case group and of 54 (± 18) in the control group (*P*= .004). Male represented 54.9% of the case group and 43.1% of the control (*P*= .32). It was observed higher mortality rates in the case group (33.3% vs. 9.8%; *P*=.008), which also had longer periods of hospital stay (57.5 days vs. 29.9; *P*< .001), longer antimicrobials intake (28.7 days vs. 15; *P*<.001) and more complex treatment regimens than the control group. Multivariate analysis showed that NFGNB colonization was associated with mortality during hospital stay (OR 4.60; CI 95% [1.54 to 13.69]).
Conclusion: Recovery of NFGNB seems to be associated with poor outcomes in hospitalized adult patients, even when the analysis is adjusted for other potential risk factors. Other studies involving larger samples of patients are necessary in an attempt to better understand the meaning of these preliminary findings.

Keywords: Acinetobacter baumannii; Pseudomonas aeruginosa; gram-negative bacteria; hospital mortality; healthcare-associated infections; multidrug resistance.

1. INTRODUCTION

Acinetobacter baumannii and *Pseudomonas aeruginosa* are aerobic non-fermenting Gram-negative bacilli (NFGNB) commonly involved in healthcare-associated infections worldwide; yet, an increasing prevalence of these pathogens has been observed in Brazilian hospitals [1,2]. To make matters worse, *A. baumannii* and *P. aeruginosa* usually presents a multidrug resistant (MDR) profile, making its therapy especially challenging [3]. Despite the high scores of mortality rates observed in *A. baumannii* associated infections, it is uncertain if and how this species could directly contribute to death [4]. *P. aeruginosa* is considered a highly virulent microorganism and the mortality rates associated with its presence ranges from 40% to more than 60%. Isolation of MDR *P. aeruginosa* strains could be associated with higher mortality rates [5]. These two-bacterial species are commonly associated with elderly patients, prolonged periods of hospital stay, presence of comorbidities and invasive procedures [3]. Herein we sought to investigate some key clinical characteristics of a group of patients colonized and/or infected by these NFGNB, comparing them with controls colonized and/or infected by other Gram-negative species.

2. MATERIALS AND METHODS

2.1 Study Design and Hospital Setting

This was an observational, cross-sectional controlled study, conducted in the Hospital das Clínicas of the Universidade Federal de Minas Gerais, in Belo Horizonte, Brazil. This is a 511-bed public hospital including a 51-bed of adult intensive care unit (ICU). It is also a reference center for highly complex diseases.

2.2 Patients Inclusion

We included patients admitted to the hospital between March 2012 and September 2013, since they were aged of 18 years or more and were culture-positive or swab-positive for NFGNB. Exclusion criteria were as follows: a) isolation of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* previously to hospitalization; b) isolation of NFGNB without performing the susceptibility test against antimicrobial agents; c) patients colonized by Gram-negative bacilli coming from other medical institutions; d) patients who refused to sign the informed consent and e) patients who received hospital discharge before the first assessment for the study. Patients were separated in two categories, the case group (cultured positively for MDR *Acinetobacter baumannii* or *Pseudomonas aeruginosa*) and the control group (cultured positively for other MDR Gram-negative bacilli). MDR was defined as bacterial species that were resistant to at least three different classes of antimicrobial agents [6] or to carbapenems. Patients were sequentially selected based on a weekly report sent by the Microbiology Laboratory to the main investigators. We maintained a proportion of 1:1 cases and controls groups, for each case enrolled, one eligible control was selected. Each patient was included only once.

2.3 Definitions and Outcomes

The investigated outcomes were all-cause in-hospital mortality, length of hospital stay and ICU

admission during hospitalization. For the subgroup of patients admitted to the ICU, we further evaluated the following endpoints: ICU length of stay, need of invasive mechanical ventilation, use of vasoactive drugs and renal replacement therapy. All patients admitted to ICU were classified according to Acute Physiology and Chronic Health Evaluation II (APACHE II) scores [7]. "Days under antibiotic therapy" was considered the total number of days in which the patient received antibiotic therapy - consecutively or not - irrespective of number of drugs composing a given schema of treatment [8]. As previously shown, this measure gives a reasonable estimation of the antibiotic usage, but it does not reflect the exact exposure to these drugs [8]. The comorbidities evaluated were heart failure, according to the New York Heart Association, class III or IV [9], liver cirrhosis [10], diabetes mellitus; chronic obstructive pulmonary disease (COPD), previously described on medical records; renal insufficiency, dialytic or non-dialytic, diagnosed during hospital admission or by preliminary blood tests; cerebrovascular disease prior to the index hospitalization; confirmed solid organs and hematological malignancies; drug and/or disease-related immunosuppression, such as use of corticosteroids, human immunodeficiency virus (HIV) infection, among others; presence of bed sores and other skin wounds prior to hospitalization; and chronic bedridden status. We also assessed for the use of invasive medical devices such as central venous catheter and indwelling urethral catheter, among others. Sepsis was defined following the Surviving Sepsis Campaign [11]. The relationship between microbial isolation (NFGNB or other MDR Gram-negative bacilli) and sepsis was established by recovering bacteria from clinical samples during these episodes. The treatment of the infectious episodes presented by the included patients was defined by the treating physicians based on local protocols, with no interference of the investigator team. In general, the main antibiotic used to treat NFGNB infections was polymixin E or polymixin B [12], associated or not to other classes (eg, carbapenems) [1].

2.4 Statistical Analysis

Epi info TM version 7.1.3.3 was used to data entry. Statistical analyzes were performed using the software Statistical Package for Social Sciences (SPSS) 15.0 for windows - SPSS Incorporation, Chicago, Illinois, United States of America. Categorical variables were analyzed by descriptive analysis using a frequency distribution and the measures of central tendency of the continuous variables were obtained (mean and standard deviation or median and 25 percentile or Q1 and 75 percentile or Q3). The non-parametric Kolmogorov-Smirnov test investigated the distribution of continuous variables; Fisher exact or the Chi-square tests compared variables expressed in proportion and Student's t or Mann Whitney tests were used as recommended. Independent variables associated to mortality rates were analyzed by binomial logistic regression. Variables with *P* values <.250 and those clinically relevant (e.g.: age) were included in the models. A backward approach was used to construct the model and its stability was tested by Hosmer-Lemeshow test. Statistical significance was considered when two-tailed *P* values were <.05 for all analyzes.

2.5 Ethical Aspects

The Research Ethics Committee of the Federal University of Minas Gerais approved the project under the protocol number 40736. Written informed consent was obtained from included patients and all data collected was used only for this study.

2.6 Microbial Identification and Antimicrobial Susceptibility Tests

Gram-negative bacteria were identified and had their antimicrobial susceptibility profile determined using the automated system vitek® 2 compact (Biomerieux, Marcy l' Etoile, France).

3. RESULTS

3.1 General Characteristics of the Study Population

During the study period, 477 patients were assessed for potential eligibility and included in the study according to the inclusion and exclusion criteria. From these, 102 patients were included in the study, being 51 in the case group and 51 in the control group (Fig. 1).

The main baseline characteristics of the included patients are presented in Table 1. Considering the whole sample of patients, the mean age was 61(±15) years for the case group and 54 (±18) years for the control group. Graph 1 demonstrate the sites were the microbial samples were recovery from the two groups (P < .001) and

Graph 2 exhibit the presence of comorbidities also for case-control patients (P= .05). We did not observe a significant difference on the proportion of comorbidities between the two groups.

3.2 Outcomes

Regarding the all-cause mortality, five (9.8%) and 17 (33.3%) patients died during hospital stay, in the control group and in the case group, respectively (*P*= .008). Besides, patients belonging to case group had longer length of hospital stay (medium 48 days; P25-P75: 28-87) than those in the control group (18 days: P25-P75: 7-47) (*P*< .001). In order to investigate the independent association of NFGNB colonization and/or infection and hospital mortality we performed a univariate logistic regression analysis including the variables potentially associated with this outcome. Then, all variables with *P* values lower than 0.25 in this model were included in a multivariate model (Table 2). From the five variables with P value < 0.25 in the univariate analysis, only age (older than 57 years) and infection by NFGNB were independently associated with hospital mortality (OR: 4.38 [1.46 – 13.15]; P = 0.008).

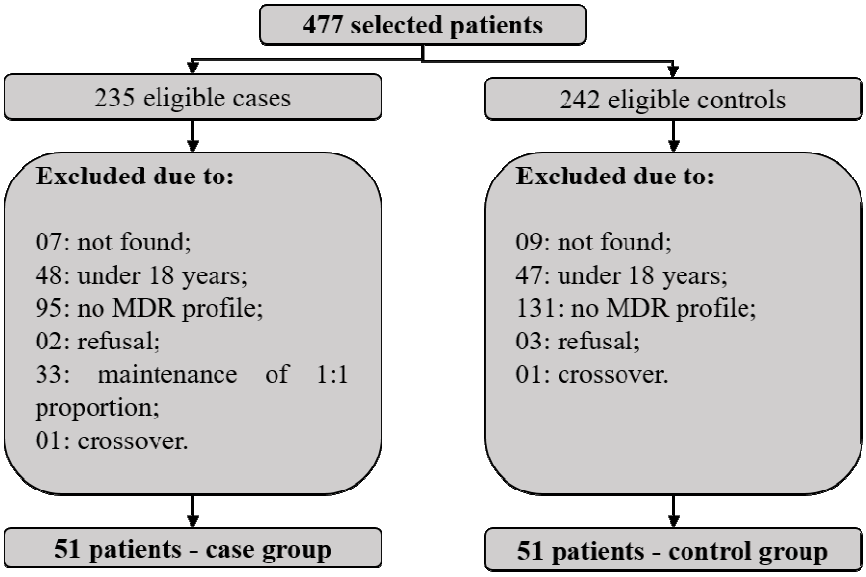
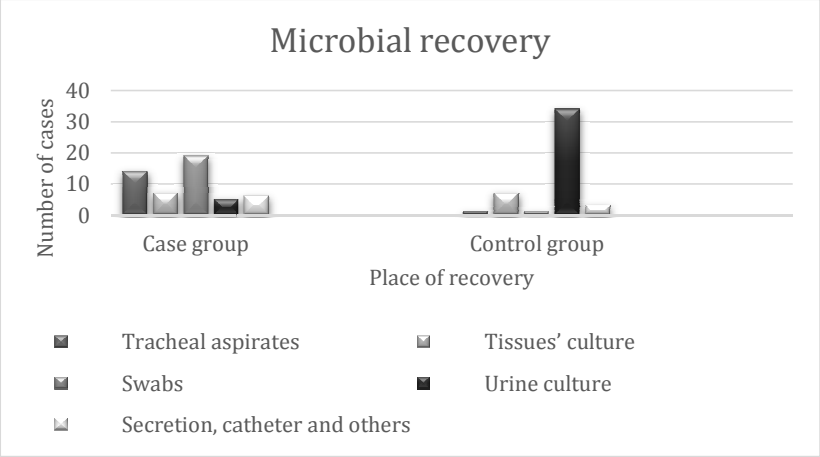


Fig. 1. Flowchart of the patient inclusion process
MDR: Multidrug resistant;

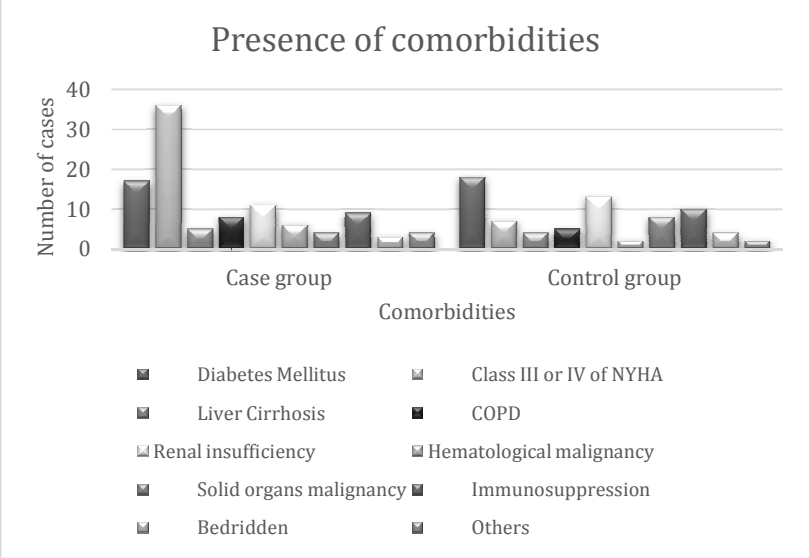
Table 1. Main characteristics of the 102 patients included, according to the group

Variable	Case n (%)	Control n (%)	*P*-value
Age (years)	61 ± 15	54 ± 18	.04[b]
Living in Belo Horizonte city	20 (39.2)	33 (64.7)	.01[a]
Place of hospital admission			
Emergence room	3 (5.9)	20 (39.2)	< .001[a]
Hospital wards	16 (31.4)	27 (52.9)	
Intensive Care Unit	32 (62.7)	4 (7.8)	
Hospitalization in the past 3 months	21 (41.2)	17 (33.3)	.37[b]
Hospitalization in the past 12 months	22 (43.1)	19 (37.2)	.68[b]
Time (days) from hospitalization and inclusion (mean P25%-75%)	17 (11 e 33)	7 (01 e 15)	< .001
Time (days) free of antimicrobial-use (mean P25%-75%)	10 (1 e 24)	23 (7 e 41)	.5

Data are presented as n (%) or mean ± standard deviation. [a]: Chi-square; [b]: t-test



Graph 1. Site of microbial recovery from case-control patients



Graph 2. Presence of comorbidities for case-control patients

Table 2. Univariate logistic regression

Variable	OR (CI 95%)	*P* value
Age > 57 years-old	1.97 (0.69 – 1.56)	.200
Absence of comorbidities	0.34 (0.73 – 1.61)	.176
Mc Cabe scores	1.10 (0.51 – 1.99)	.733
NFGNB presence (case group)	4.60 (1.54 – 13.69)	.006

OR: Odds Ratio; CI: confidence interval

It was observed a statistical significant difference (P < .001) when we compare the groups according to the use of invasive medical devices (49 patients [96.0%] vs 31 [60.7%]), vasoactive drugs (32 patients [62.7%] vs 9 [17.6%]), vasoactive amines (5.8 ±8.0 days vs. 0.6±1.6) and hemodialysis (17 patients [33.3%] vs 6 [11.7%]). When colonized and infected patients where compared, it was also observed a significant difference (P < .001) between the groups (43 patients [84.3%] vs 14 [27.4%]). The length of hospital stay was also significantly different between the two groups (57.5 days ± 35.7 vs. 29.9±32.1; P < .001). Other clinical

characteristics of the study group are demonstrated on Table 3. Graph 3 demonstrate the differences between groups considering their Intensive Care Unity - ICU stay. It is important to highlight that the differences observed were statistically significant (P < .001) for every period (none, one, two or three times). The difference between groups regarding the presence of sepsis and the recovery and identification of bacteria were also significant (P < .001) and are demonstrated on Graph 4.

3.3 Microbiological Data

Most patients in the case group had *A. baumannii* (48 patients, 94.1%), and only three (5.9%) presented *P. aeruginosa.* Among the control group, *Escherichia coli* (n=27; 52.9%) and *Klebsiella pneumoniae* (n=13; 25.5%) were the most frequently recovered bacteria followed by *Enterobacter cloacae*, *Proteus mirabilis* and *Serratia marcescens* (n=3; 5.9% each). The frequency of antimicrobial use during hospital stay was higher among case-group patients (28.7 ± 20.4) than controls (15.0 ± 21.8) (*P*< .001). The first group received more drugs (6.0 ± 3.3 vs 2.8 ± 2.6; *P*< .001) and more complex therapies (3.6 ± 1.7 vs. 3.6 ± 1.7; *P*< .001). The number of patients considered infected - as compared to colonized - was significantly higher (*P*< .001) among case group (n=43 (84.3%) vs control group 14 (27.4%). Possibly the association of these variables could explain that more complex therapies could be associated with infection, being probably a surrogate of illness severity. According to Dani [13] most authors considered the colonization and infection as part of the same physiopathological.

All microorganisms included in this study were resistant to at least three antimicrobial agents of different classes. In *A. baumannii* and *P. aeruginosa*, it was observed especially to the meropenem, while to the Enterobacteriacea this profile was variable. Since its clinical relevance, the antimicrobial profile to *A. baumannii* strains is demonstrated in the Table 4.

4. DISCUSSION

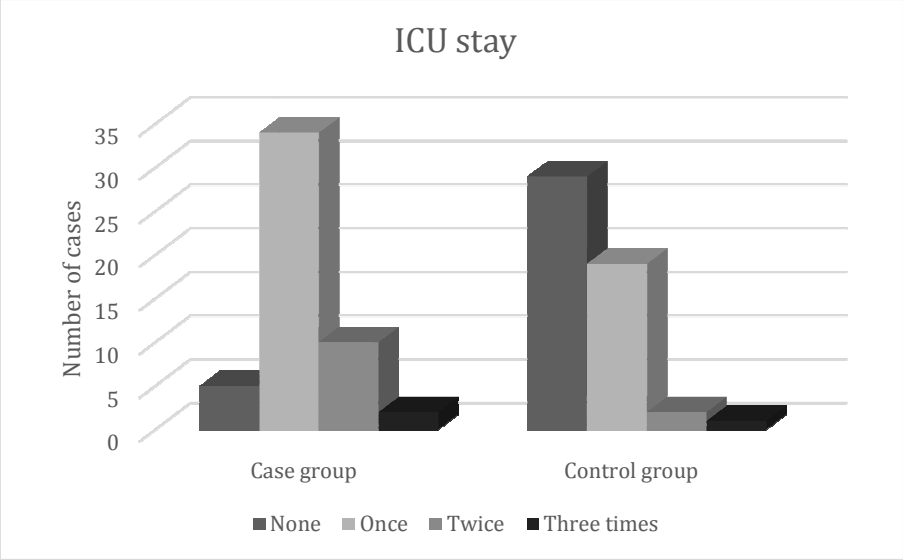
4.1 General Characteristics of the Study Population and Outcomes

As presented in Table 1, the mean age was significantly lower in the control group (*P*= .04) as compared to cases. No significant difference in gender distribution was observed between the two groups. As expected, most cases of NFGNB were identified during ICU stay. According to the literature, higher number of comorbidities could be associated with NFGNB colonization and infection [3,14,15,16,17]. In this study patients belonging to the case group were, in average, seven years older than the controls. A systematic review [18] investigated the relationship between mortality rates and NFGNB colonization and/or infection and found no elucidative answers to this scenario. The authors concluded that the available studies are too heterogeneous to be compared.

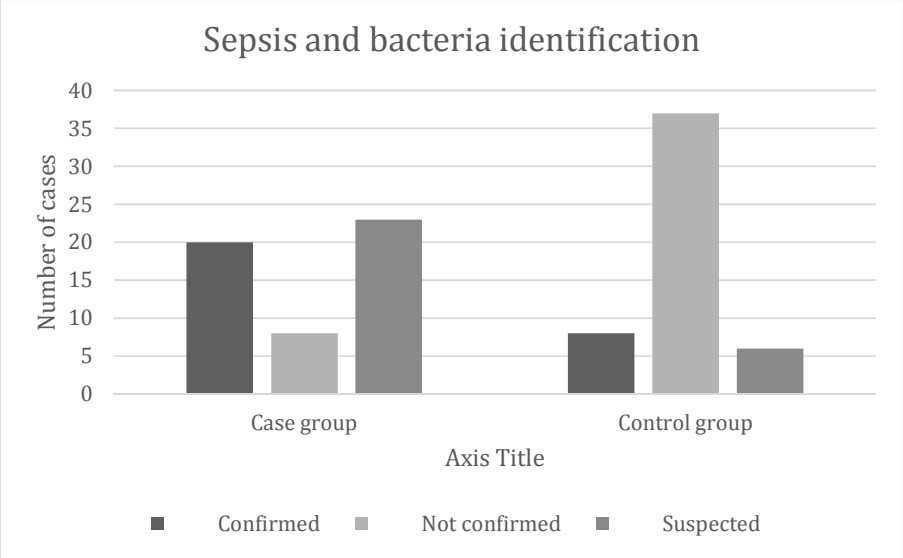
Table 3. Clinical characteristics of the 102 patients included, according to the group

Variable	Case n (%)	Control n (%)	*P* -value
Hospital evolution			
Discharge	34 (66.7)	46 (90.2)	< .008[b]
Death	17 (33.3)	5 (9.8)	
Sepsis during hospitalization	46 (90.2)	15 (29.4)	.01[b]
Hospitalization in ICU (days±SD)	19.4±18.4	3.3±5.4	< .001[b]
Invasive mechanical ventilation	43 (84.3)	14 (72.5)	< .001[b]
Days under invasive mechanical ventilation (±SD)	13.9±19.4	1.7±3.3	< .001[a]
Tracheostomy	15 (29.4)	4 (7.8)	< .001[b]
Hemodialysis (days±SD)	9.5±24.8	4.1±13.0	.01[a]
Antimicrobial use during hospitalization	50 (98.0)	48 (94.1)	.60[b]
Antimicrobial use during hospitalization (days±SD)	28.7±20.4	15±21.8	< .001[a]
Number of therapeutic schemes (mean±SD)	3.6±1.7	2.2 ±1.7	< .001[a]
Number of antimicrobials (mean±SD)	6.0±3.3	2.8±2.6	< .001[b]
APACHE II[c]	13 (9 - 19)	14.5 (7 -17)	.70[a]
Mc Cabe Score 1	10	21	
2	19	17	.04[b]
3	22	13	

[a]: Chi-square; [b]: t-test; [c]: medium, Q1 and Q3



Graph 3. Number of intensive care unit (ICU) stay for case-control patients



Graph 4. Number of patients with sepsis and bacteria identification for case-control groups

Table 4. Antimicrobial susceptibility of 48 *Acinetobacter baumannii* strains, according to their profile: Sensitive (S), intermediate resistance (I) and full resistance (R)

Antimicrobial agents	Susceptibility patterns (%)				
	$MIC_{50\%}$	$MIC_{90\%}$	S	I	R
	Acinetobacter baumannii strains (n=48)				
Ampicillin / Sulbactam	16	32	1,6	37,1	61,3
Ceftazidime	256	256	25,8	3,2	71,0
Gentamicin	2	192	67,7	8,1	24,2
Meropenem	32	32	1,6	0	98,4
Polimixin B	0,5	0,5	98,4	1,6	0
Tigeciclin	0,50	0,75	17,7	33,9	48,4

4.2 Microbiological Data

Memish et al. [19] investigated non-fermenters recovered from hospitals in Saudi Arabia and found high values of resistance rates. The recovered *P. aeruginosa* were resistant to gentamicin, amikacin, ciprofloxacin and imipenem; *A. baumannii* were resistant to gentamicin, amikacin, trimethoprim/ sulfamethoxazole and ciprofloxacin. Another study [20] investigated resistance profile of NFGNB isolated from patients admitted in ICU that had developed infections after 48 hours of admission inside Indian hospitals in Nagpur. The authors observed that *P. aeruginosa* was the commonest isolate obtained followed by *A. baumannii*. Studies in the literature [21,22] emphasized that these species were the most common NFGNB recovered from endotracheal aspirate in a tertiary care hospital in India. In the present study forty-six (90%) patients of the case group were admitted to the ICU during the hospitalization compared to 22 (43.0%) patients of the control group (*P*< .001). In an analysis including only the subgroup of patients that were admitted to the ICU, case group patients had significantly longer stay in this unit than controls (19.4±18.4 vs 3.3±5.4, *P*= <.001). Patients submitted to invasive mechanical ventilation (case 43 (84.3%) vs control group 14 (72.5%); *P* <.001), for longer periods (case 13.9±19.4 vs control group 1.7±3.3; *P* < .001) and using invasive medical devices (case 49 (96.0%) vs control group 31 (60.7%); *P* < .001) exhibited higher chances of being colonized / contaminated by NFGNB. The likelihood was confirmed by the statistical significance observed. Suwantar at and Carroll [6] informed that the recovery of NFGNB is most often related to nosocomial infections such as urinary tract infections, ventilator associated pneumonia, surgical site infections and bacteremia. Results of the present study suggest that more severely ill patients (undergoing tracheostomy, receiving invasive medical devices or vasoactive amines and/or hemodialysis) are more exposed to NFGNB infection during hospital stay (*P*< .001). The present study compared patients colonized and/or infected by MDR NFGNB with those infected by other MDR Gram-negative bacilli glucose-fermenting. The first group was composed of more seriously ill patients and had higher hospital mortality rates. Given the cross-sectional design of this study, we cannot affirm if case group patients remained longer periods hospitalized because of the NFGNB infection / colonization or if they had enhanced chances to acquire these pathogens as a consequence of the prolonged hospital stay. Moreover, according to our results, being infected and/or colonized by NFGNB was independently associated with higher risk of all-cause hospital mortality, even after adjustment for other potential risk factors. Finally, case patients stayed longer in the hospital than controls, needed ICU admission more frequently and were more exposed to antimicrobial therapy than individuals infected by MDR glucose fermenting Gram-negative bacteria.

Case patients were initially admitted to the hospital predominantly in the ICU (62.7%), followed by hospital wards (31.4%) and ER (5.9%). For control patients, it was observed an opposite pattern, with hospital wards being the prevailing unit (52.9%) followed by ER (39.2) and ICU (7.8%). Overall, our finding suggests that fragile patients, receiving more complex life support and requiring ICU hospitalization are the most susceptible group to acquire NFGNB colonization/infection. As previously mentioned, the relationship between hospital units and being infected by NFGNB was also informed by other authors [14,15]. There were also differences between groups regarding the site of microorganism recovery (Table 1). Although age was not directly related to hospital mortality by multivariate analysis, case-patients were a mean of seven years older than control-patients. This could have contributed to the worst prognosis observed in the first group. Higher mortality rates were observed among the case-patients. Even though the presence of NFGNB showed to be independently associated to the all-cause hospital mortality, the cross-sectional design of our study precludes us to affirm it, since we are not able to rule out the presence of non-investigated confounding risk factors. Finally, the length of therapy and the complexity of antimicrobial therapy were superior for patients colonized/infected by NFGNB. The present study design did not allow us to differentiate if the composition of the therapeutic schemes was a cause or a consequence of NFGNB isolation.

5. LIMITATIONS

First, our study was carried out in a single hospital and the results could not be generalized to other healthcare institutions. Besides, it is important to highlight that the obtained data represent the reality of a university hospital in a middle-income country, and that it could be a comparative basis s to other healthcare services

around the world. Second, the sample size of patients studied here was small, limiting some statistical inferences. Lastly, we were not able to follow up the studied patients after hospital discharge. This strategy would allow us to investigate about microbial decolonization and long-term morbidity and mortality rates. It is important to highlight that some of the limitations mentioned in the present study were also mentioned in the literature [13].

6. CONCLUSION

Patients colonized by NFGNB exhibited higher mortality rates, were exposed to longer periods of hospital stay and received longer and more complex antimicrobial therapy. Our findings emphasize the relevance of NFGNB isolation in the outcome of hospitalized patients. Statistical analyzes stablished a positive correlation between the recovery of NFGNB and mortality rates. Prospective studies including larger sample sizes are necessary to clarity the correlation between these groups of bacteria and hospital mortality. The present study shares the epidemiological profile of a Brazilian university hospital expecting that the data could help other healthcare professionals to deal with NFBGN colonization and infection.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

This work was supported by CNPq, FAPEMIG and CAPES. We kindly thank to all involved patients who participated in the present study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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