

# Resistance of non-fermenting Gram-negative bacilli isolated from blood cultures from an emergency hospital

## *Resistência de bacilos Gram-negativos não fermentadores isolados de hemoculturas de um hospital de emergência*

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### ABSTRACT

**Introduction:** Non-fermenting Gram-negative bacilli (NFGNB) are a heterogeneous group of microorganisms that do not have the ability to ferment carbohydrates as a way to obtain energy. There are more than 120 species classified as pathogenic, among them, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. Infections caused by these microorganisms are mostly acquired in the hospital environment, since they are opportunistic pathogens and are among the most important bacteria of greater clinical and epidemiological relevance. **Objective:** This study evaluated the resistance profile of NFGNB isolated from blood cultures at an emergency hospital in the city of Caruaru, Agreste Pernambuco (PE), Brazil. **Methods:** The strains present in the blood cultures were isolated on culture media MacConkey and Triple Sugar Iron (TSI) agar. The samples were also submitted to the oxidase test and the polymyxin resistance test, in addition to the Gram staining, to better identify NFGNB bacterial genera. An antibiogram test was carried out to verify the resistance profile. **Results:** It was found that from 87 (100%) isolated and analyzed strains, 11 (13%) were classified as NFGNB. The genus *Acinetobacter* sp. was the most frequently found (55%). The *Acinetobacter* sp. strains were resistant to gentamicin, meropenem, imipenem, amikacin, ciprofloxacin, ceftazidime and ceftriaxone. **Conclusion:** Screening of resistant NFGNB isolated, as well as greater attention to hospital-acquired infection control practices and epidemiological surveillance systems, in addition to continued care with regard to the targeted use of antibiotics can contribute to successful this battle against infections by these microorganisms.

**Key words:** hospital-acquired infection; Gram-negative bacteria; antibacterial agents.

### INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are a heterogeneous group of microorganisms that do not have the ability to ferment carbohydrates as a way of obtaining energy. They are widely distributed in nature, and can be found in water, soil, and plants. More than 120 species of NFGNB were classified as pathogenic, highlighting the following as etiological agents involved in most hospital-acquired infections: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. The pathologies caused by these microorganisms are mostly acquired in hospital environments, since they are opportunistic pathogens,

and the importance of these infections has been increasing since the 1970s<sup>(1-4)</sup>. NFGNB are among the most clinically and epidemiologically relevant bacteria, however the phenotypic diagnose is not easy in clinical microbiology laboratories, requiring more attention regarding the practice used, and there may be difficulties with the sensitivity and resistance tests of these pathogens<sup>(2, 3, 5, 6)</sup>.

Hospital-acquired infections are those acquired after patient admission, and may occur during hospitalization or after discharge<sup>(7)</sup>. NFGNB is found with a high frequency in the bronchial tree, especially in immunosuppressed and cystic fibrosis patients, which has been shown to be a problem because these bacteria acquire high resistance to a wide variety of drugs, including penicillins, cephalosporins, aminoglycosides, tetracyclines,

fluoroquinolones, trimetropim-sulfamethoxazole, carbapenems and polymyxins, the latter is widely used in the treatment of *P. aeruginosa* and *Acinetobacter* spp. infections<sup>(1, 3, 4, 6, 8, 9)</sup>. The current limitation in the use of antibiotics against NFGNB is due, among other reasons, to its abusive use, often incorrectly applied by some health professionals, which causes a selection of resistant bacteria, making its treatment difficult. The analysis of the degree of sensitivity and resistance of NFGNB is essential for a better understanding of the epidemiology of infections caused by such microorganisms, besides contributing with the proposition of new therapeutic schemes to battle them more efficiently<sup>(3, 7, 10)</sup>.

One of the most important laboratory tests in the diagnosis of sepsis, very common in intensive care units (ICUs) of emergency hospitals, is the blood culture used to isolate and identify pathogenic microorganisms in the blood of a patient who is thought to have a systemic infection, which represents one of the most severe complications of the infectious process, making it significant in relation to its predictive value, since its result will directly reflect on the therapeutics to be adopted by health professionals<sup>(5)</sup>.

Therefore, this study aimed to determine the resistance profile of NFGNB, isolated and phenotypically identified from blood cultures of hospitalized patients in a public emergency hospital in the city of Caruaru, in the Agreste Pernambuco, Brazil.

## METHODS

This is a descriptive and cross-sectional laboratory study, which analyzed the blood cultures obtained from the clinical pathology laboratory of an emergency hospital in the city of Caruaru, Agreste Pernambucano, from April to October, 2015. The beginning of the study occurred after the approval by the ethics committee (CAAE: 36690214.5.0000.5203) of the Asces College.

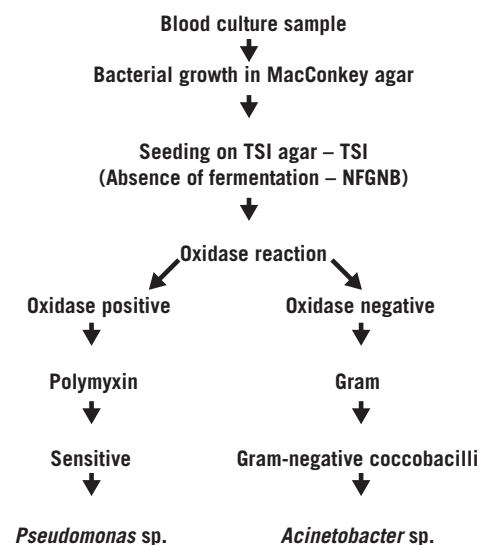
The samples were sent, under ideal conditions of transport, to the Laboratory of Microbiology of the Asces College for the necessary microbiological procedures. Using a 3 ml syringe, about 0.25 ml of the blood sample from the blood cultures was aspirated. After aspiration, for macroscopic visualization, the material was seeded by depletion in Sheep Blood Agar and MacConkey Agar media, the plates were incubated in an oven at 35°C-37°C for 18 to 24 hours.

The Gram-negative bacteria which have grown on MacConkey agar were seeded on Triplice Sugar Iron (TSI) Agar in order to visualize the absence of sugar fermentation. After this proof, they were submitted to the oxidase test, and then, to the polymyxin-B resistance test and the Gram staining for initial phenotypic

laboratory identification of the main bacterial genera belonging to the NFGNB group (**Figure 1**).

The NFGNB, once identified, were submitted to the antibiogram by the Bauer-Kirby disk diffusion method<sup>(11)</sup> to analyze the sensitivity profile to the main antibiotics used against NFGNB and to verify the presence of bacterial resistance. To perform the assay, the suspension of the test bacterium, adjusted to the standard of 0.5 on the McFarland scale, was inoculated onto the surface of the Mueller-Hinton agar plate. Subsequently, antimicrobial discs were placed and the plates were incubated in a bacteriological oven at a temperature of 35°C-37°C for 18 to 24 hours, the whole procedure was performed in a sterile way.

The method was performed following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2015, as well as the choice of the antimicrobial discs that were used<sup>(12)</sup>.



**FIGURE 1** – Simplified flowchart of isolation and identification of the main NFGNB in hospital blood cultures

NFGNB: non-fermenting Gram-negative bacilli; TSI: triple sugar iron.

## RESULTS

From the total of 95 blood cultures analyzed, 87 (92%) were positive, showing bacterial growth, while eight (8%) were negative, with no growth of microorganism. Among the positive blood cultures, 57 (66%) were from male individuals and 30 (34%) from female.

Among the identified bacteria, 11% were classified as belonging to the NFGNB group. *Acinetobacter* sp. was the genus

most frequently found (55%), followed by *Pseudomonas* sp. (18%), *Stenotrophomonas* sp. (18%) and *Burkholderia* sp. (9%) (Figure 2).

In general, regarding the profile of sensitivity and resistance, the *Acinetobacter* sp. strains were resistant to gentamicin, meropenem, imipenem, amikacin, ciprofloxacin, ceftazidime and ceftriaxone. The *Pseudomonas* sp. strains showed sensitivity to all tested antimicrobials: gentamicin, meropenem, imipenem, amikacin, levofloxacin, norfloxacin, ceftazidime and cefepime. All of *Stenotrophomonas* sp. strains presented resistance to ceftriaxone and 50% of them showed resistance to both levofloxacin and chloramphenicol. The *Burkholderia* sp. strains were sensitive to the antibiotics tested (Table).

Carbapenems, despite being a class of antibiotics highly recommended against resistant hospital bacteria, showed reduced effectiveness against the NFGNB strains analyzed in this study, showing, in general, 71% resistance.

## DISCUSSION

NFGNB represented about 13% of the microorganisms found in the positive blood cultures in this study, data lower than that demonstrated by Grillo *et al.* (2013)<sup>(6)</sup> with 28.3% of isolates, and greater than those of Deliberali *et al.* (2011)<sup>(1)</sup> with 2.18%.

*Acinetobacter* sp. has been considered the second NFGNB most frequently found in the laboratory studies of this genus, behind only the *Pseudomonas* sp., however the present study showed a prevalence of 55% for *Acinetobacter* sp. and only 18% for *Pseudomonas* sp., in contrast to Grillo *et al.* (2013)<sup>(6)</sup> study, in which *Pseudomonas* sp. reached a prevalence of 47% and to Deliberali *et al.* (2011)<sup>(1)</sup>, who showed 65% in their study. The increased frequency of hospital-acquired infections associated with *Acinetobacter* sp. and the rapid development of resistance of these microorganisms have become a serious public health problem<sup>(13,14)</sup>.

The *Acinetobacter* sp. strains demonstrated sensitivity exclusively to minocycline (40%), an antibiotic belonging to the tetracycline group. In a previous study, carried out by Deliberali *et al.* (2011)<sup>(1)</sup> there was less sensitivity to tetracycline (10.4%). A study by Pontes *et al.* (2006)<sup>(7)</sup> demonstrated sensitivity of *Acinetobacter* sp. isolates to quinolones and imipenem.

Results from Grillo *et al.* (2013)<sup>(6)</sup> presented significant resistance (> 90%) of *Pseudomonas* sp. in relation to the third generation cephalosporins, such as ceftriaxone. In this study, ceftriaxone was 100% effective against the *Pseudomonas* sp. strains. This bacterium has great clinical relevance, especially when associated with patients who are intubated or using venous catheters, as is the case of the majority of ICU patients<sup>(6, 8, 15, 16)</sup>.

The *Stenotrophomonas* sp., often isolated from clinical specimens, represented 18% of the strains found in the study, presenting total resistance to ceftazidime, and 50% resistance to both levofloxacin and chloramphenicol. It is a NFGNB of

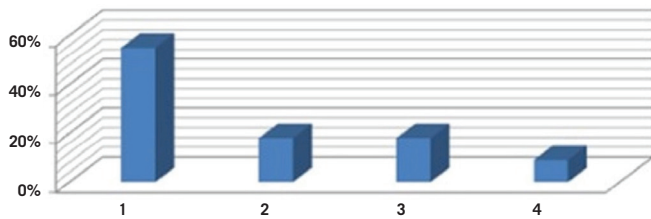


FIGURE 2 – NFGNB genera isolated from blood cultures analysis

NFGNB: non-fermenting Gram-negative bacilli.

1. *Acinetobacter* sp.; 2. *Pseudomonas* sp.; 3. *Stenotrophomonas* sp.; 4. *Burkholderia* sp.

TABLE – Sensitivity and resistance profile of NFGNB isolated from blood cultures

Antibiotics	<i>Acinetobacter</i> sp.		<i>Pseudomonas</i> sp.		<i>Stenotrophomonas</i> sp.		<i>Burkholderia</i> sp.	
	R	S	R	S	R	S	R	S
Minocycline	60%	40%	-	-	-	-	-	-
Ciprofloxacin	100%	0%	-	-	-	-	-	-
Meropenem	100%	0%	0%	100%	-	-	-	-
Imipenem	100%	0%	0%	100%	-	-	-	-
Ceftriaxone	100%	0%	0%	100%	100%	0%	0%	100%
Ceftazidime	100%	0%	-	-	-	-	-	-
Amikacin	100%	0%	0%	100%	-	-	-	-
Gentamicin	100%	0%	0%	100%	-	-	-	-
Levofloxacin	-	-	0%	100%	50%	50%	0%	100%
Norfloxacin	-	-	0%	100%	-	-	-	-
Cefepime	-	-	0%	100%	-	-	-	-
Chloramphenicol	-	-	-	-	50%	50%	0%	100%

NFGNB: non-fermenting Gram-negative bacilli; R: resistance; S: sensitivity.

great importance, intrinsically resistant to a wide variety of antimicrobials, due to the presence of enzymes that inactivate various antibiotics, the impermeability of the outer membrane and the presence of efflux pumps to a multiplicity of drugs. Therefore, the treatment of infections caused by *Stenotrophomonas* sp. requires great attention<sup>(4,17)</sup>.

The *Burkholderia* sp., known as an important opportunistic pathogen, especially in hospital-acquired infections in immunocompromised patients or with cystic fibrosis<sup>(4)</sup>, was the NFGNB less prevalent in this study, representing 9% of the isolates, which differs from the percentage found in the Magalhães *et al.* (2004)<sup>(8)</sup> study, which presented a prevalence of 29.2% of these strains. There was no significant resistance of the *Burkholderia* sp. strains evidenced in this study with sensitivity to the antibiotics tested: ceftriaxone, levofloxacin and chloramphenicol.

Carbapenems are known to be potent agents for the treatment of NFGNB infections, especially in hospitals, but studies have already shown a reduction in its efficacy<sup>(1, 13-15)</sup>. The study by Deliberalli *et al.* (2011)<sup>(1)</sup> presented 32.8% and 37.1% of NFGNB isolates resistant to imipenem and meropenem, respectively. This study detected significant resistance to carbapenems with regard to *Acinetobacter* sp. isolates (100%), showing greater resistance to imipenem and meropenem when compared to the aforementioned study.

No carbapenem has been shown to be more effective than the other, unlike the study by Marques *et al.* (2007)<sup>(15)</sup>, in which meropenem showed resistance greater (52.1%) than imipenem (32.9%). Inappropriate use of this class of drugs may exert selective pressure on nosocomial pathogens, leading to increased bacterial resistance to them due to the selection of less sensitive subpopulations<sup>(16)</sup>.

## CONCLUSION

The study highlights the importance of monitoring the resistance profile of NFGNB in blood cultures of hospitalized patients. This practice can contribute to the implementation of practices in partnership with the Commission for Control of Hospital-Acquired Infections [Comissão de Controle de Infecção Hospitalar (CCIH)] and with the epidemiological surveillance, in order to promote improvements in the key measures for the management of hospital-acquired infections, also contributing to a better targeting in the use of inpatient antibiotics.

Despite their low prevalence in routine in relation to other etiological agents, NFGNB are of significant importance, since they constitute an imminent risk to the hospitalized patients, also by the fact that these bacteria present decreased sensitivity to a large number of drugs. Studies like this, carried out periodically in the hospital environment, may help to control resistance rates, considering that microbiological data contribute to the choice of the most appropriate therapy for each patient.

## ACKNOWLEDGES

To the Laboratory of Microbiology of the Hospital Regional do Agreste, due to the availability of the blood cultures analyzed, and to the Asces College, for the financial support and availability of the infrastructure of the institution's school laboratory.

## RESUMO

**Introdução:** Os bacilos Gram-negativos não fermentadores (BGNNF) são um grupo heterogêneo de microrganismos que não possuem a capacidade de fermentar carboidratos como forma de obtenção de energia. Possuem mais de 120 espécies classificadas como patogênicas, destacando-se entre elas *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* e *Burkholderia cepacia*. As infecções causadas por esses microrganismos são, em sua maioria, adquiridas nos ambientes hospitalares, já que se tratam de patógenos oportunistas, estando entre as bactérias de maior relevância clínica e epidemiológica. **Objetivo:** Este trabalho avaliou o perfil de resistência dos BGNNF isolados de hemoculturas em um hospital de emergência na cidade de Caruaru, no Agreste Pernambuco (PE), Brasil. **Métodos:** As cepas presentes nas hemoculturas foram isoladas nos meios de cultura ágar MacConkey e ágar Triple Sugar Iron (TSI). As amostras também foram submetidas ao teste de oxidase e ao teste de resistência a polimixina, além da coloração de Gram, para melhor identificação dos gêneros bacterianos de BGNNF. Foi realizado o antibiograma para verificação do perfil de resistência. **Resultados:** Verificou-se que das 87 (100%) cepas isoladas e analisadas, 11 (13%) foram classificadas como BGNNF. O gênero *Acinetobacter* sp. foi o mais frequente (55%). As cepas de *Acinetobacter* sp. apresentaram-se resistentes a gentamicina, meropenem, imipenem, amicacina, ciprofloxacina, ceftazidima e ceftriaxona. **Conclusão:** O rastreamento de isolados de BGNNF resistentes, bem como uma maior atenção às práticas de controle de infecção hospitalar e sistemas de vigilância epidemiológica, além do cuidado contínuo em relação ao uso direcionado de antibióticos podem contribuir no combate a infecções por esses microrganismos.

**Unitermos:** infecção hospitalar; bactérias Gram-negativas; antibacterianos.

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