Revista Prevenção de Infecção e Saúde
The Official Journal of the Human Exposome and Infectious Diseases Network

ORIGINAL ARTICLE DOI: https://doi.org/10.26694/repis.v6i0.9732

Biofilm production by resistant non-fermenting gram-negative bacilli from intensive care units

Produção de biofilme por bacilos Gram-negativos não fermentadores resistentes de unidade de terapia intensiva

Producción de biopelículas por bacilos gramnegativos no resistentes a la fermentación en la unidad de cuidados intensivos

Maria Izabely Silva Pimentel[1], Carlos Alberto Medeiros Neto[1], Lamartine Rodrigues Martins[1], Mariana Quitéria de Moraes Silva[1], Igor Vasconcelos Rocha[2], Sibele Ribeiro de Oliveira[1]

How to cite this article:
Pimentel MIS, Medeiros Neto CA, Martins LR, Silva MQM, Rocha IV, de Oliveira SR. Biofilm production by resistant non-fermenting gram-negative bacilli from intensive care units. Rev Pre Infec e Saúde [Internet]. 2020;6:9732. Available from: http://www.ojs.ufpi.br/index.php/nupcis/article/view/9732 DOI: https://doi.org/10.26694/repis.v6i0.9732

[1] Tabosa de Almeida University Center (ASCES-UNITA), Department of Biomedicine, Caruaru, Pernambuco, Brazil.

[2] Aggeu Magalhães Institute (IAM/Fiocruz-PE), Department of Biosciences and Biotechnology in Health, Recife, Pernambuco, Brazil.

ABSTRACT

Introduction: The Intensive Care Unit is one of the hospital departments in which the highest number of bacterial isolates multidrug-resistant to antimicrobials prevails. Infections caused by Non-Fermenting Gram-Negative Bacillus (NFGNB) are of great importance and clinical concern, especially when they are related to multidrug-resistant microorganisms. One of the main mechanisms associated with bacterial resistance is the production of biofilm. **Objective**: To verify the presence, resistance profile as well as bacterial biofilm production of NFGNB isolates from tracheal secretion, blood culture, and surfaces of an Intensive Care Unit. **Outline**: Samples were collected from February to September 2018. MacConkey Agar, Triple Sugar Iron, and oxidase test were used for bacterial identification. Antimicrobial resistance occurred by the Kirby-Bauer disk diffusion method, and the presence of bacterial biofilm was verified by Quantitative Microtiter Dish Biofilm Formation assay. **Results**: Of 30 gram-negative isolates, seven were NFGNB. Four of the genus *Acinetobacter* sp., two of the genus *Pseudomonas* sp., and one of the genus *Burkholderia* sp. were identified. The results showed moderately and strongly biofilm producing isolates. **Implications**: The bacteria found showed high resistance to the antibiotics tested associated with biofilm production. Identifying these pathogens can contribute greatly to more effective hospital antibiotic therapy.

DESCRIPTORS

Biofilms; Drug Resistance, Microbial; Intensive Care Units; Bacteria.

Corresponding author:
Maria Izabely Silva Pimentel
E-mail: izabelypimentel@hotmail.com

Submitted: 2019-11-21
Accepted: 2020-04-23
Published: 2020-04-30

INTRODUCTION

The Intensive Care Unit (ICU) is one of the places where the isolation of pathogenic microorganisms occurs most frequently since the patients present there are more susceptible to the development of infections, especially by resistant microorganisms, with rates of Healthcare-Associated Infections (HAI) ranging from 18 to 54%.[1] HAI are responsible for increasing mortality and morbidity in addition to psychological causes and social consequences to patients.[2] With increased HAI, cross-contamination emerges as an important factor for the patient's condition, as it can happen not only from person to person but also between them and the surrounding ICU surfaces.[3-4]

Studies developed to investigate the most contaminated surfaces inside an ICU reported greater contamination in the right and left bed rails, along with the heart rate monitor shelf, bed height adjusters, infusion pump buttons, among the surfaces near the patient.[5] In general, when evaluating biological samples collected from the ICU for microbiological examinations, tracheal secretion, blood culture, and urine stand out,[6] while other studies have associated a higher prevalence of Non-Fermenting Gram-Negative Bacilli (NFGNB) in tracheal samples.[7] Regarding the blood cultures, considering the sterile physiological property of blood, the finding of microorganisms should be investigated, given the possibility of sepsis.[8] The presence of bacteria in blood cultures suggests a clinical significance because the patient's bacteremia may develop into septicemia.[8-10]

Among the main isolated microorganisms in ICU, the NFGNB are often associated with a high mortality rate, and they can be found in tracheal secretion, blood cultures, and surfaces near the patient. These are non-sporulated aerobic bacteria that do not use carbohydrates as a source of energy through fermentation, degrading them by the oxidative pathway.[11] More than 30 genera were classified as pathogenic, and the following stand out: *Pseudomonas* sp., *Acinetobacter* sp., *Stenotrophomonas* sp., and *Burkholderia* sp.[12]

These microorganisms have been commonly associated with high rates of antimicrobial resistance and also with biofilm production, posing a threat to public health, and it hinders the treatment of infections caused by them. The biofilm production also potentiates the bacterial colonization and is responsible for the selection of resistant bacterial lineages, as the microorganisms present in the core of the biofilm are not affected by the antimicrobials used in patient treatment and remain viable.[8]

Due to the increase in healthcare-associated infections, the identification and knowledge about the etiological agent and the mechanisms related to their resistance to antimicrobials constitutes a fundamental strategy in the control of HAI. Thus, the purpose of this study was to verify the presence, the biofilm formation ability, and the antimicrobial resistance profile of NFGNB isolated from tracheal secretion, blood culture, and hospital surfaces, since such contamination aggravates the clinical profile of patients.

METHOD

This is a descriptive cross-sectional study developed in an ICU of a hospital from Caruaru-PE, Brazil, from January to November 2018. Ethical approval was obtained from the ethical committee of the Centro Universitário Tabosa de Almeida (ASCES-UNITA) (code number 77393617.0.0000.5203/2.348.001 and 77418217.0.0000.5203/2.348.003). Tracheal secretion and blood culture specimens were obtained from the hospital laboratory routine and sent, under

appropriate biosafety conditions, to the ASCES-UNITA Microbiology Laboratory.

Surface samples were collected during the patient's hospitalization, by convenience, from right and left rails of the beds with a sterile swab moistened in Tryptic Soy Broth (TSB) (KASVI® – São José do Pinhais-PR, Brazil).[5] The swabs were deposited again in TSB and incubated at 37°C for 18 to 24 hours. Bacterial growth was evaluated by visual inspection and then seeded on MacConkey Agar plates (KASVI® – São José do Pinhais-PR, Brazil).[9]

Tracheal secretions were recovered from the original collection bottles by using a sterile swab, while for bacterial blood analysis, about 0.25mL of the blood culture was drawn from each vial using a 3 mL syringe. All tracheal secretion and blood culture samples were seeded on MacConkey Agar plates (KASVI® – São José do Pinhais-PR, Brazil) and incubated for 18 to 24 hours to verify bacterial growth.[8]

The identification of the isolates was performed according to macro and microscopic characteristics of the colonies, Gram stain and biochemical test results. The carbohydrate fermentation test was performed in Triple Sugar Iron (TSI) (KASVI® – São José do Pinhais-PR, Brazil), as well as oxidase-based tests (LABTEST® – Lagoa Santa-MG, Brazil) for the identification of non-fermenting gram-negative bacteria and bacterial genus.[9]

Microbial resistance profile analysis was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (KASVI® – São José do Pinhais-PR, Brazil) as proposed by the Clinical and Laboratory Standard Institute (CLSI).[13]

The biofilm production and quantification were determined by O'Toole[14] and Trentin's methodology[15]. Briefly, the isolates were diluted in saline solution until reaching OD625nn density of 0.08 - 0.13 AU (0.5 McFarland scale). 80µL of each bacterial suspension and 120µL of TSB were added in different wells of a microtiter dish. The micro dish was incubated at 37ºC for 18 to 24 hours, and the bacterial suspensions were removed by water washing. Subsequently, 200µL of an aqueous solution of 0.4% crystal violet was added to each well of the microliter dish, which was subsequently incubated for 15 minutes, followed by a new water washing. The production of bacterial biofilm was evaluated by optical microscopy, observing the colonies adhered to the wall and/or bottom of the microtiter dish, like a lumpy. Tests were performed in triplicate, and wells containing sterile TSB were used as negative control for biofilm production.

The biofilm quantification test was performed by adding 200µL of a 30% acetic acid solution in each well to solubilize the biofilm, when it was present. Optical Density (OD) reading from each well was conducted using a microplate reader (Thermo Plate® – China) at 570nm, and the biofilm producers were classified considering the highest OD reading for each isolate (OD_i) and comparing it to the reading of the OD negative control (OD_c)[16] as follows: i) non-adherent ($OD_i \leq OD_c$); ii) weakly adherent ($OD_c < OD_i \leq 2 \times OD_c$); iii) moderately adherent ($2 \times OD_c < OD_i \leq 4 \times OD_c$); and iv) strongly adherent ($4 \times OD_c < OD_i$).

RESULTS

It was observed that from 68 clinical samples used in the study (10 from surface, 49 blood culture, and 9 from tracheal secretion), 64 samples (94%) showed bacterial growth, being 32.81% gram negative. Of these, 33% of NFGNB were identified, being *Acinetobacter* sp. (57%), *Pseudomonas* sp. (28%), and *Burkholderia* sp. (15%) the principal genera of NFGNB, as shown in Table 1. Just one patient had the samples from three different places from the hospital, with positivity for all the samples.

Table 1 – Identification and distribution of bacterial genera of NFGNB.

Microorganisms	Tracheal Secretion No.(%)	Blood Culture No.(%)	Surface No.(%)	Total No.(%)
Acinetobacter sp.	0(0)	2(100)	2(50)	4(57%)
Pseudomonas sp.	1(100)	0(0)	1(25)	2(28%)

Burkholderia sp.	0(0)	0(0)	1(25)	1(15%)
Total of isolates	**1(14)**	**2(29)**	**4(57)**	**7(100)**

Note: No.= number of isolates; %= percentage of isolates.

All *Acinetobacter* sp. isolates showed resistance to ceftriaxone, ciprofloxacin, cefotaxime, ceftazidime, gentamicin, ampicillin associated with sulbactam, and imipenem. 75% of these genera showed resistance to levofloxacin and piperacillin associated with tazobactam, and 50% were resistant to amikacin. 100% were sensitive to minocycline.

The isolates of *Pseudomonas* sp. were resistant to ceftazidime, piperacillin associated with tazobactam, imipenem, and aztreonam, and 50% were resistant to gentamicin.

The isolate of *Burkholderia* sp. showed no resistance to the antimicrobials tested (levofloxacin, ceftazidime, meropenem and minocycline).

The identification of biofilm production was possible by the microscopical observation of the colonies adhered to the wall and/or bottom of the microtiter dish. All the NFGNB isolates were bacterial biofilm producers, quantified as moderately adherent (71% of isolates) and strongly adherent (29%) on biofilm production, and these were identified in *Acinetobacter* sp. (25%) and *Pseudomonas* sp. (50%) according to Table 2.

Table 2 – Resistance profile of clinical isolates, production and quantification of bacterial biofilm.

Isolate	Sample	Bacterial genus NFGNB	Antimicrobial resistance	Production and quantification of bacterial biofilm
1	Tracheal secretion	*Pseudomonas* sp.	Imipenem, aztreonam, ceftazidime, piperacillin-tazobactam, gentamicin	Moderately adherent
2	Left rail	*Acinetobacter* sp.	Ceftriaxone, ciprofloxacin, levofloxacin, ampicillin-sulbactam, imipenem, cefotaxime, ceftazidime, piperacillin-tazobactam	Strongly adherent
3	Right rail	*Pseudomonas* sp.	Imipenem, aztreonam, ceftazidime, piperacillin-tazobactam	Strongly adherent
4	Right rail	*Acinetobacter* sp.	Ceftriaxone, ciprofloxacin, levofloxacin, ampicillin-sulbactam, imipenem, cefotaxime, piperacillin-tazobactam, gentamicin, ceftazidime	Moderately adherent
5	Left rail	*Burkholderia* sp.	No resistance	Moderately adherent
6	Blood culture	*Acinetobacter* sp.	Amikacin, ceftriaxone, ciprofloxacin, levofloxacin, ampicillin-sulbactam, imipenem, cefotaxime, ceftazidime, piperacillin-tazobactam, gentamicin	Moderately adherent
7	Blood culture	*Acinetobacter* sp.	Amikacin, ceftriaxone, ciprofloxacin, ampicillin-sulbactam, imipenem, cefotaxime, gentamicin, ceftazidime	Moderately adherent

DISCUSSION

This study initially aimed to evaluate the hospital surface and tracheal secretion samples and their relationship with *Acinetobacter* sp.; however, due to the limited number of samples, blood cultures were also included, as well as all NFGNB isolates. Due to the study hospital's policy and its Ethics Code restrictions, it was not possible to access the complete patient data, making it difficult to know whether the isolates found were related to their clinical condition.

There are numerous risk factors that lead a patient to be colonized by microorganisms, including prolonged hospitalization, invasive procedures, and underlying diseases such as diabetes mellitus, cardiovascular diseases, malnutrition cases, gastric achlorhydria, among others. Most hospitalizations are not caused by infectious diseases, but these may affect hospitalized patients, thus developing an infectious condition, leading to more extensive treatment and higher risk of acquiring new infections.[17-18]

The most isolated microorganisms in the ICU are gram-negative bacteria of the *Enterobacteriaceae* family.[18-20] Bacteria that do not ferment glucose, most of the time,[8,21-22] are not evidenced, mainly due to the difficulty of their phenotype laboratory identification. Nowadays, these microorganisms have become of greater clinical significance, given its resistance profile and bacterial biofilm production.

In the present study, the NFGNB represented 10.93% of growing samples, with prevalence in surface, blood culture and tracheal secretion samples, the latter with low occurrence of isolates (14.28%) when compared to another study in which of 326 NFGNB isolates from various samples, such as urine, tracheal secretion, sputum, 38.34% were isolated just from tracheal secretion, varying due to study time design and number of samples.[11]

In this study, there was a similar occurrence found in blood culture and surfaces, when compared to studies by Deliberali[11], Oliveira[8] and Rocha[5].

The present work evidenced the genus *Acinetobacter* sp. as the most prevalent on surfaces, and this prevalence was probably related to its nutritional and metabolic versatility since it can survive in low-nutrient environments, using various substrates as carbon source, which increases its colonization time in the hospital environment.[5,23] Because it is a pathogen of great clinical significance, being considered the major cause of infections in the ICU by NFGNB, it was also the most isolated of gram-negative bacteria in the bloodstream in similar studies by Cunha[24] and Pailhoriès[25], that during the period studied by the survey,[24] 5,759 blood culture tests were analyzed, with 1,019 positive results, *Acinetobacter baumannii* was found in 31 samples, it was the sixth most isolated pathogen and corresponded to 3.04% of positive blood cultures. Bacterial studies conducted in 75 different countries showed that hospital ICU patients were infected with *Acinetobacter* sp. in about 9% of participating countries.[26]

Acinetobacter sp. is a pathogen of great importance because it is usually multidrug-resistant to numerous antimicrobials in clinical practice.[24] In this study, isolates of this genus showed sensitivity to the antibiotic minocycline (100%), corroborating other study in this field,[8,27] which presented similar results to this one. Regarding the bacterial resistance profile, other studies[8] showed that *Acinetobacter* sp. isolates showed resistance to amikacin (100%), ciprofloxacin (100%), ceftriaxone (100%), ceftazidime (100%), imipenem (100%) and gentamicin (100%), similar to the present study which highlights what the literature portrays regarding its resistance.

According to a study[28] that evaluated tracheobronchial secretion, in relation to *Pseudomonas* sp., regarding tracheal secretion isolates, there was a single isolate, differing from another similar study[18] in which the prevalence of these bacteria represented 36.43% of the total of 129 tracheal secretion samples, which demonstrates the value of epidemiological analysis of bacterial isolates of clinical importance, as one of the ways to contribute to the control of hospital infection rates.

In this study, biofilm production was observed in all isolates of NFGNB analyzed, being quantified as strongly adherent (28.57%) and moderately adherent (71.43%), which was higher than that reported by similar biofilm production study,[29] which showed 75% of isolates as biofilm producers, with 10% strongly adherent. The same authors also found that 46.7% of the bacteria were multidrug sensitive, and in the present study, it is observed that, in relation to the genus *Pseudomonas* sp., all isolates analyzed were multidrug resistant. This result is similar to those of other studies, in which this bacterial genus was strongly and moderately biofilm producer. The authors also reported the growth capacity of this bacterial genus even in environments with minimal supply of organic matter.[29-30]

NFGNB have acquired importance as causes of HAI, such as the respiratory tract, urinary tract, surgical wounds, among others.[11,31] This has been owing to increased antimicrobial resistance, which is due to the presence of bacterial biofilm that is an

aggravation factor of these infections as it can be formed in some areas of the body and environments, especially the surfaces close to the patients. Bacterial multidrug resistance may persist even after treatment with high doses of antimicrobial drugs.[11,32-33]

Pinheiro[34] found that 70% of all hospital infections are linked to the presence of biofilm in medical devices, also showing that bacteria associated with biofilm production behave differently in their growth rate, resistance to antimicrobial agents, and increased resistance to the host immune response.

Moskowitz[35] reports that the antibiogram may evidence an effective *in vitro* antibiotic therapy, but this method is performed with the bacteria in its planktonic form, which limits the correct evaluation of the antibiotic tested. These bacteria may be protected by the biofilm in the patient, which suggests that the response is not the same as that obtained through the standard antimicrobial resistance test. In the present study, the genus *Burkholderia* was moderately producer of bacterial biofilm, despite its total *in vitro* sensitivity through antibiogram.

The production of bacterial biofilm in hospital samples is associated with public health problems as this mechanism contributes to the decrease in patients' quality of life, increase in length of stay and hospital costs as well as increase in morbimortality.[31,36]

Regarding the measures to contain bacterial biofilm colonization, it is realized that health professionals are among the main vehicles for microbiological dissemination among patients. Early identification of colonized patients can help to control the spread of this kind of infection,[37] and hand hygiene, while simple and affordable, is one of the most effective proven methods to prevent the transmission of resistant isolates and should be constantly stimulated and performed whenever patient contact begins and ends.[38]

CONCLUSION

The NFGNB found in this study proved to be quite resistant to most of the antibiotics tested, which hinders the treatment of infected patients. These microorganisms were moderately and strongly adherent in relation to biofilm production, further aggravating the action of possible antimicrobials used. Strongly adherent bacterial isolates in relation to biofilm production were found on surfaces close to the patient, suggesting greater care for local hygiene in order to prevent possible cross infections. Studies such as this one, in a hospital environment, can help in actions aimed at improving the reduction of resistance rates.

RESUMO

Introdução: A Unidade de Terapia Intensiva é um dos setores no ambiente hospitalar com altas taxas de isolamento de bactérias multirresistentes aos antimicrobianos. Infecções causadas pelos Bacilos Gram-Negativos Não Fermentadores (BGNNF) são de grande importância e preocupação clínica, especialmente quando relacionadas a microrganismos multirresistentes. Um dos principais mecanismos associado com a resistência bacteriana é a produção de biofilme. **Objetivo**: Verificar a presença, o perfil de resistência, bem como a produção de biofilme bacteriano de isolados de BGNNF oriundos de secreção traqueal, hemocultura e superfícies de uma Unidade de Terapia Intensiva. **Delineamento**: As amostras foram coletadas de fevereiro a setembro de 2018. Foi utilizado Ágar MacConkey, *Triple Sugar Iron* e teste de oxidase para a identificação bacteriana. A resistência antimicrobiana ocorreu pelo método de disco-difusão de Kirby-Bauer, e a presença do biofilme bacteriano foi verificada por quantificação evidenciada através de um leitor de placas de microtitulação. **Resultados**: De 30 isolados de Gram-negativos, sete foram BGNNF. Foram identificados quatro do gênero *Acinetobacter* sp., dois do gênero *Pseudomonas* sp., e um do gênero *Burkholderia* sp. Os resultados mostraram isolados moderadamente e fortemente produtores de biofilme. **Implicações**: As bactérias encontradas apresentaram grande resistência associada à produção de biofilme. A identificação desses patógenos pode contribuir sobremaneira numa antibioticoterapia hospitalar mais eficaz.

DESCRITORES

Biofilmes; Resistência Microbiana a Medicamentos; Unidade de Terapia Intensiva; Bactérias.

RESUMEN

Introducción: La Unidad de Cuidados Intensivos es uno de los sectores en el entorno hospitalario con altas tasas de aislamiento de bacterias multirresistentes a los antimicrobianos. Las infecciones causadas por bacilos gram-negativos no fermentadores (BGNNF) son de gran importancia y preocupación clínica, especialmente cuando están relacionadas con microorganismos multirresistentes. Uno de los principales mecanismos asociados con la resistencia bacteriana es la producción de biopelícula. **Objetivo:** Para verificar la presencia, el perfil de resistencia, así como la producción de biopelícula bacteriana, de aislados de BGNNF de la secreción traqueal, hemocultivo y superficies de una Unidad de Cuidados Intensivos. **Delineación:** Las muestras fueron recolectadas de febrero a septiembre de 2018. Se utilizaron el Agar MacConkey, *Triple Sugar Iron* y la prueba de oxidasa para la identificación de bacterias. La resistencia a los antimicrobianos se produjo utilizando el método de difusión en disco de Kirby-Bauer y la presencia de la biopelícula bacteriana se verificó por cuantificación evidenciada a través de un lector de placas de microvaloración. **Resultados:** De los 30 aislamientos gram-negativos, siete eran BGNNF. Fueron identificados cuatro del género *Acinetobacter* sp., dos del género *Pseudomonas* sp., y uno del género *Burkholderia* sp. Los resultados mostraron moderadamente aislado y fuertemente productores de biopelícula. **Implicaciones:** La bacteria encontrada mostró una gran resistencia asociada con la producción de biopelícula. La identificación de estos patógenos puede contribuir en gran medida a una terapia antibiótica hospitalaria más efectiva.

DESCRIPTORES

Biopelículas; Farmacorresistencia Microbiana; Unidades de Cuidados Intensivos; Bacterias.

REFERENCES

1. Oliveira AC, Kovner CT, Silva RS. Nosocomial Infection in an Intensive Care Unit in a Brazilian University Hospital. Rev Latinoam Enferm [Internet]. 2010 Mar–Apr [cited 2020 May 05]; 18(2):233–9. Available from: https://doi.org/10.1590/S0104-11692010000200014

2. Shang J, Needleman J, Liu J, Larson E, Stone PW. Nurse Staffing and Healthcare-Associated Infection, Unit-Level Analysis. JONA: The Journal of Nursing Administration [Internet]. 2019 May [cited 2020 Apr 23]; 49(5):260–5. Available from: https://doi.org/10.1097/NNA.0000000000000748

3. Megeus V, Nilsson K, Karlsson J, Eriksson BI, Andersson AE. Hand contamination, cross-transmission, and risk-associated behaviors: An observational study of team members in ORs. AORN J [Internet]. 2015 Dec [cited 2020 May 05]; 102(6):645e1–e12. Available from: https://doi.org/10.1016/j.aorn.2015.06.018

4. Paz V, Paniagua M, Santillán A, Alaniz M, D'Agostino L, Orellana R, et al. Hospital Environment Hygiene Nurse: A Key Player to Reduce Healthcare Associated Infections by Multi-Resistant Organisms. Infection Prevention in Practice [Internet]. 2020 Mar [cited 2020 May 05]; 2(1). Available from: https://doi.org/10.1016/j.infpip.2019.100030

5. Rocha IV, Ferraz PM, Farias TGS, Oliveira SR. Resistência de bactérias isoladas em equipamentos em unidade de terapia intensiva. Acta Paul Enferm [Internet]. 2015 Sep-Oct [cited 2020 May 05]; 28(5):433–9. Available from: https://doi.org/10.1590/1982-0194201500073

6. Yaghoobi MH, Sabahi M, Ghaderi E, Seifrabiei MA, Bashar FR. Frequency of device-associated infections in intensive care units. Tehran Univ Med J [Internet]. 2020 Feb [cited 2020 May 05]; 77 (11):696–700. Available from: http://tumj.tums.ac.ir/article-1-10201-en.html

7. Cheikh MRE, Barbosa JM, Caixêta JAS, Avelino MAG. Microbiology of Tracheal Secretion: What to Expect with Children and Adolescents with Tracheostomies. Int Arch Otorhinolaryngol [Internet]. 2018 Mar [cited 2020 May 05]; 22(1):50–4. Available from: https://doi.org/10.1055/s-0037-1601403

8. Oliveira MEF, Araújo DG, Oliveira SR. Resistance of non-fermenting Gram-negative bacilli isolated from blood cultures from an emergency hospital. J Bras Patol Med Lab [Internet]. 2017 Apr [cited 2020 May 05]; 53(2):87–91. Available from: https://doi.org/10.5935/1676-2444.20170013

9. Tortora GJ, Funke BR, Case CL. Microbiologia. 12th ed. Porto Alegre: ARTMED; 2017.

10. Hoyos PV, Soto F, Pinzón D, Gonzáles D, Penã C. Caracterización de pacientes pediatricos con hemocultivos positivos del servicio de cuidado intensivo pediatrico del Hospital San José Bogotá, abril 2012 a 2017. Infectio [Internet]. 2019 [cited 2020 May 05]; 23(2):183–8. Available from: https://doi.org/10.22354/in.v23i2.776

11. Deliberali B, Myiamoto KN, Neto CHDPW, Pulcinelli RSP, Aquino ARC, Vizzotto BS, et al. Prevalência de bacilos Gram-negativos não fermentadores de pacientes internados em Porto Alegre-RS. J Bras Patol Med Lab [Internet]. 2011 Oct [cited 2020 May 05]; 47(5):529–34. Available from: https://doi.org/10.1590/S1676-24442011000500006

12. Tanwar J, Sharma M, Parmar A, Tehri N, Verma N, Gahlaut A, et al. Antibacterial potential of silver nanoparticles against multidrug resistant bacterial isolates from blood cultures. Inorganic and Nano-Metal Chemistry [Internet]. 2020 Mar [cited 2020 May 05]. Available from: https://doi.org/10.1080/24701556.2020.1735433

13. CLSI. Performance Standards of Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Watne, PA: Clinical and Laboratory Standards Institute; 2018.

14. O'Toole GA. Microtiter Dish Biofilm Formation Assay. JoVE [Internet]. 2011 [cited 2020 May 05]; 47. Available from: https://doi.org/10.3791/2437

15. Trentin DS, Giordani RB, Zimmer KR, Silva AG, Silva MV, Correia MT, et al. Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against Staphylococcus epidermidis planktonic and biofilm lifestyles. J Ethnopharmacol [Internet]. 2011 Sep [cited 2020 May 05]; 137(1):327–35. Available from: https://doi.org/10.1016/j.jep.2011.05.030

16. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods [Internet]. 2000 Apr [cited 2020 May 05]; 40(2):175–9. Available from: https://doi.org/10.1016/S0167-7012(00)00122-6

17. Sydnor ERM, Perl TM. Hospital Epidemiology and Infection Control in Acute-Care Settings. Clin Microbiol Rev [Internet]. 2011 Jan [cited 2020 May 05]; 24(1): 141–73. Available from: https://doi.org/10.1128/CMR.00027-10

18. Pinheiro RM. Perfil microbiológico de um complexo hospitalar de atenção terciária da rede pública do Ceará: uma ferramenta para promover a aplicação da antibioticoterapia empírica racional. Universidade Federal do Ceará [Monography]. 2018 [cited 2020 May 05]. Available from: http://www.repositorio.ufc.br/handle/riufc/38149

19. Mota FS, Oliveira HA, Souto RCF. Perfil e prevalência de resistência aos antimicrobianos de bactérias Gram-negativas isoladas de pacientes de uma unidade de terapia intensiva. RBAC [Internet]. 2018 [cited 2020 May 05]; 50(3):270–7. Available from: https://doi.org/10.21877/2448-3877.201800740

20. Basso ME, Pulcinelli RSR, Aquino ARC, Santos KF. Prevalência de infecções bacterianas em pacientes internados em uma unidade de terapia intensiva (UTI). RBAC [Internet]. 2016 [cited 2020 May 05]; 48(4):383–8. Available from: https://doi.org/10.21877/2448-3877.201600307

21. Gales AC, Torres PL, Vilarinho DSO, Melo RS, Silva CFL, Cereda RF. Carbapenem-resistant Pseudomonas aeruginosa outbreak in an intensive care unit of a teaching hospital. Braz J Infect Dis [Internet]. 2004 Aug [cited 2020 May 05]; 8(4):267–71. Available from: https://doi.org/10.1590/S1413-86702004000400001

22. Dettori M, Piana A, Deriu MG, Lo Curto P, Cossu A, Musumeci R, et al. Outbreak of multidrug-resistant Acinetobacter baumannii in an intensive care unit. New Microbiol [Internet]. 2014 Apr [cited 2020 May 05], 37(2):185–91. Available from: https://www.researchgate.net/publication/262609935_Outbreak_of_multidrug-resistant_Acinetobacter_baumannii_in_an_intensive_care_unit

23. Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. J Hosp Infect [Internet]. 2012 Jan [cited 2020 May 05]; 80(1):56–60. Available from: https://doi.org/10.1016/j.jhin.2011.08.013

24. Cunha VWS, Canettieri ACV, Bernardes RC. Epidemiological study of Acinetobacter spp. infection in blood cultures of a laboratory in São José dos Campos. Revista Univap [Internet]. 2014 Dec [cited 2020 May 05]; 20(36):66–72. Available from: https://doi.org/10.18066/revunivap.v20i36.226

25. Pailhoriès H, Tiry C, Eveillard M, Kempf M. Acinetobacter pittii more frequently isolated than Acinetobacter baumannii in bloodcultures: the experience of a French hospital. J Hosp Infect [Internet]. 2018 Jul [cited 2020 May 05]; 99(3):1–15. Available from: https://doi.org/10.1016/j.jhin.2018.03.019

26. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA Netw Open [Internet]. 2009 Dec [cited 2020 May 05]; 302(21):2323–29. Available from: https://doi.org/10.1001/jama.2009.1754

27. Bordignon JC, Lima LR. Etiologia de infecções hospitalares e perfil de sensibilidade aos antimicrobianos em um hospital do sudoeste do Paraná, Brasil. RBAC [Internet]. 2017 [cited 2020 May 05]; 49(3):283–8. Available from: https://doi.org/10.21877/2448-3877.201700566

28. Silva JA, Moreira VT, Abreu RNDC, Cavalcante TMC, Filho RNV, Studart RMB. Análise dos registros de enfermagem após coleta de secreção traqueobrônquica para cultura: importância ética e legal. Enferm Foco [Internet]. 2018 [cited 2020 May 05]; 1(9):61–5. Available from http://revista.cofen.gov.br/index.php/enfermagem/article/view/988

29. Lima JLC, Alves LR, Paz JNP, Rabelo MA, Maciel MAV, Morais MMC. Análise da produção de biofilme por isolados clínicos de Pseudomonas aeruginosa de pacientes com pneumonia associada à ventilação mecânica. Rev Bras Ter Intensiva [Internet]. 2017 Jul–Sep [cited 2020 May 05]; 29(3):310–6. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-507X2017000300310&lng=en.

30. Costa KAD, Ferenz M, Silveira SM, Millezi AF. Formação de biofilmes bacteriano em diferentes superfícies de indústrias de alimentos. Rev Inst Laticínios Cândido Tostes [Internet]. 2016 [cited 2020 May 05]; 71(2):75–82. Available from: https://doi.org/10.14295/2238-6416.v71i2.512

31. Nascimento IR, Sena TL. Biofilmes bacterianos: colonização e identificação de micro-organismos causadores de infecção em cateter venoso central. Centro Universitário de Brasília [Internet]. 2017 [cited 2020 May 05]. Available from: https://www.publicacoes.uniceub.br/pic/article/view/5586/3930

32. Laranjeira V dos S, Marchetti DP, Steyer JR, Corção G, Picoli SU. Investigation of metallo-beta-lactamase-producing Acinetobacter sp and Pseudomonas aeruginosa at an emergency hospital in Porto Alegre, State of Rio Grande do Sul, Brazil. Rev Soc Bras Med Trop [Internet]. 2010 Jul–Aug [cited 2020 May 05]; 43(4): 462–4. Available from: https://doi.org/10.1590/S0037-86822010000400026

33. Duarte HA, Lourenço EA, Souza NCP, Silva LF, Ribeiro GJ, Medeiros JRC, et al. Pesquisa de bacilos Gram negativos não fermentadores no interior do corpo de torneiras de um hospital público de Volta Redonda, RJ. RBAC [Internet]. 2016 [cited 2020 May 05]; 48(3): 284–9. Available from: http://www.rbac.org.br/wp-content/uploads/2016/11/ARTIGO-17_RBAC-48-3-2016-ref.-224-maior.pdf

34. Pinheiro S.; Formação de biofilmes: um breve ensaio. Intravenous [Internet]. 2006 May [cited 2020 May 05]. Ano VI, 16:2–3. Available from: http://www.bd.com/Documents/bd-legacy/journal-article/Infusion/brazil-periodicals/IF_Intravenous-Edition-16_JA_PT.pdf

35. Moskowitz SM, Foster JM, Emerson J, Burns JL. Clinically feasible biofilm susceptibility assay for isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. J Clin Microbiol [Internet]. 2004 [cited 2020 May 05]; 42: 1915–22. Available from: https://doi.org/10.1128/JCM.42.5.1915-1922.2004

36. Diriba K, Kassa T, Alemu Y, Bekele S. In Vitro Biofilm Formation and Antibiotic Susceptibility Patterns of Bacteria from Suspected External Eye Infected Patients Attending Ophthalmology Clinic, Southwest Ethiopia Silva. International Journal of Microbiology [Internet]. 2020 [cited 2020 May 05] 2020:1–12. Available from: https://doi.org/10.1155/2020/8472395

37. Almeida KRH, Silva NS, Rocha IV, Araújo AA, Silva LM, Oliveira SR. Surveillance cultures: screening of carbapenemase producing microorganisms and patient safety. Rev Pre Infec e Saúde [Internet]. 2018 [cited 2020 May 05]; 4: 69–77. Available from: https://doi.org/10.26694/repis.v4i0.6977

38. Ridley N, Lecturer S. Effective hand hygiene—wash your hands and reduce the risk. Br J nurs [Internet]. 2020 [cited 2020 May 05], 29(1):10. Available from: https://doi.org/10.12968/bjon.2020.29.1.10

COLLABORATIONS

MISP and CAMN have contributed entirely to the data collection, analysis, interpretation, and design of this manuscript of the project. LRM and MQMS have significantly contributed to the drafting, design, and critical review of this manuscript. IVR and SRO have supervised the final version of this manuscript. All the authors discussed the results, commented on the article, and approved the final manuscript.

ACKNOWLEDGMENTS

Not applicable.

AVAILABILITY OF DATA

Not applicable.

FUDING SOURCE

Not applicable.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.