BIOSCIENCE JOURNAL

MOLECULAR ANALYSIS OF THE PREVALENCE OF *Acinetobacter baumannii* IN HOSPITALS AND THE SURROUNDING ENVIRONMENTS: A CROSS-SECTIONAL STUDY

Kerollyn Fernandes Bernardes SILVA[1], Camila Botelho MIGUEL[2], Rony Rocha DE OLIVEIRA JÚNIOR[3], Marcelo Costa ARAUJO[4], Ferdinando AGOSTINHO[5], Carlos UEIRA-VIEIRA[6], Javier Emilio LAZO-CHICA[7], Wellington Francisco RODRIGUES[2,8]

[1] Nursing Course, Universidade Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil.
[2] Medicine course, Centro Universitário de Mineiros, Mineiros, Goiás, Brazil.
[3] Medical Course, Centro Universitário Atenas, Paracatu, Minas Gerais, Brazil.
[4] Clinical Pathology Laboratory - Microbiology Sector of Clinical Hospital, Universidade Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil.
[5] Postgraduate Course in Physiological Sciences, Universidade Federal do Triângulo Mineiro, UFTM, Uberaba, Minas Gerais, Brazil.
[6] Postgraduate Program in Genetics and Biochemistry, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.
[7] Cell Biology Laboratory, Institute of Biological and Natural Sciences, Universidade Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil.
[8] Postgraduate Course in Tropical Medicine and Infectious Diseases, Universidade Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brazil.

Corresponding author:
Wellington Francisco Rodrigues
wellington.frodrigues@hotmail.com

How to cite: SILVA, K.F.B., et al. Molecular analysis of the prevalence of *Acinetobacter baumannii* in hospitals and the surrounding environments: A cross-sectional study. *Bioscience Journal*. 2023, **39**, e39019. https://doi.org/10.14393/BJ-v39n0a2023-63071

Abstract

Acinetobacter baumannii is widely recognized in clinical environments due to its infectious capacity, antimicrobial adaptability, and lethality. Analyzing the prevalence of this agent in intra- and extra-hospital environments may reveal target indicators for appropriate management interventions. In this observational cross-sectional study, we evaluated the prevalence of *A. baumannii* within hospitals with intensive care units and in their external surroundings in a macro-health region of Brazil. Samples of *Columba livia* (pigeon) droppings from the external environment of four hospitals (n = 40), from floor surfaces (n = 20), and door handles (n = 20) of different hospital wards were collected based on random sampling, all of which were evaluated for the presence of *A. baumannii* using polymerase chain reactions (PCR). The sensitivity and specificity of the technique was verified after the collected samples were contaminated with clinical samples positive for *A. baumannii*. We detected a significantly higher *A. baumannii* prevalence (87.50%, CI = 71.29–100.00) in samples collected within the hospital environment compared with those obtained from the external environment (12.50%, CI = 0.00–28. 71) (p = 0.003). In addition, samples collected from floor surfaces contained bacterial densities (181.3 ± 11.58) that exceeded those in environmental (93.32 ± 1.56) and door handle (142.70 ± 17.14) samples by 94% and 78.71%, respectively. The findings of this study will enhance our understanding of the spatial distribution of *A. baumannii* and additionally, validate the efficiency of PCR for diagnosis of this infectious agent.

Keywords: *Acinetobacter baumannii*. Environment. Hospital Infection. Polymerase Chain Reaction

1. Introduction

Acinetobacter baumannii is a common bacterium in hospital environments, which given its infectious capacity, antimicrobial variability, and lethality, is a particular cause of concern worldwide,

notably with respect to developing antibiotic resistance (Peleg et al. 2008; Tada et al. 2020). The emergence of resistant strains, as well as tolerance to disinfectants used for cleaning in hospital environments, predictably pose increased risks to human health (Chin et al. 2018). The spread of *A. baumannii* with multi-drug-resistant antimicrobial profiles has been reported to be rapid (Ribeiro et al. 2020), and this bacterium contributes to increased morbidity and mortality in patients (Espinal et al. 2011; Hou and Yang 2015), which accordingly warrants effective surveillance and antimicrobial treatment.

In addition to hospital environments, pathogenic and multi-drug-resistant *A. baumannii* strains has been shown to be disseminated into the wider environment and initiate infections in the general community (Anane et al. 2020). Indeed, the presence of *A. baumannii* has been widely detected in different environmental samples, including those collected from abattoirs, aquatic matrices (Anane et al. 2020), and pigeon droppings (Morakchi et al. 2017).

The detection of genes encoding carbapenemase in urban pigeon feces has raised concerns regarding the likelihood of birds serving as reservoirs for the spread of these resistant determinants in both animals and humans (Morakchi et al. 2017). Indeed, the dissemination of infectious agents through pigeon feces has been widely demonstrated for several other potentially pathogenic microorganisms, including *Escherichia coli* (Chidamba et al. 2015), *Klebsiella pneumoniae, Aspergillus spp*., and *Cryptococcus neoformans* (De Oliveira Xavier et al. 2019; Ghaderi et al. 2019). In addition, the relationship between hospital contamination and pathogens present in bird feces has previously been established (Afunwa et al. 2020), thereby highlighting the need to monitor the prevalence of these agents and their transmission within and between hospital settings.

In addition to enhancing epidemiological surveillance, rapid differential diagnosis with high sensitivity and specificity is of particular importance with respect to a more efficient and agent-directed drug intervention (Liu et al. 2020). In this regard, culture-dependent techniques for monitoring the prevalence of *A. baumannii* in environmental samples and within-hospital surfaces tend to be limited by the amount of this bacterium in samples, as well as by analyst-dependent variability in test sensitivity (Andini et al. 2018). In contrast, the use of molecular techniques that can be used to detect specific genes associated with infectious agents tends to ensure greater sensitivity and specificity in tests intended for epidemiological surveillance (Bonnin et al. 2012; Leung et al. 2019).

Given the aforementioned considerations, the primary objective of this study was to determine the prevalence of *A. baumannii* within pigeon feces and the intensive care units of hospitals in a city located in the Southeast region of Brazil. In addition, we sought to establish the sensitivity and specificity of a molecular test used for the detection of this bacterium.

2. Material and Methods

Ethical aspects

The procedures and lineage assessments of *Acinetobacter baumannii* isolated from different biological samples collected from hospitalized patients were performed after having initially obtained consent from the responsible sectors and were in accordance with the ethical principles of the Declaration of Helsinki, as well as those set out in Resolution 466/12 of the National Health Council. The study was registered at Platform Brazil and was approved by the Research Ethics Committee of the Clinic Hospital of the Federal University of Triângulo Mineiro (approval number: 3,050,072).

Experimental design and sample collection

This was an observational, cross-sectional study. Samples of *Columba livia* (pigeon) feces were obtained from the surrounding environment of four hospitals with intensive care units located in the macro-region of Triângulo Mineiro, (Uberaba, Minas Gerais State, Brazil) between July and August 2019 (N = 40). In addition, samples from hospital environments were obtained from the corridor surfaces (N = 20) and door handles (N = 20) of different hospital wards. Samples were collected after having mapped and randomized collection points using an electronic draw. Power (greater than 80%) and sampling

determinations were performed using GPower software, version 3.03 (Kiel, Germany). *Columba livia* feces were collected using sterile disposable spatulas and placed in tubes with a maximum capacity of 2 mL (Eppendorf, Fremont, CA), labeled, and taken to the laboratory. Collected intra-hospital samples were mixed with Stuart® transport medium (SPLABOR, Sao Paulo, Brazil). In the laboratory, samples were transferred to cryovials containing trypticase soy broth medium supplemented with 15% glycerol, and subsequently stored at -80^{o}C until used for analysis.

Detection of A. baumannii by polymerase chain reaction (PCR)

After being thawed, samples were cultured on triple-sugar iron agar and isolates thus obtained were processed for PCR. Bacterial DNA was extracted using the ReliaPrep gDNA Tissue Miniprep System (Promega Inc., United States) according to the manufacturer's instructions. The crude extract was homogenized using glass spheres with Tissue Lyzer II (Qiagen, Germany) at a frequency of 30 rotations/s for 5 min. Amplification of the extracted DNA was performed using OXA51 and 16S primer pairs for detection of the oxacillinase and 16S RNA genes, respectively. The PCR reaction mixtures contained 10 µL of MasterMix Green (iQ™ SYBR Green Supermix; Bio-Rad, USA), 1.2 µL of $MgCl_2$, 0.6 µL of DMSO, 0.6 µL of each forward and reverse primer, 1.0 µL of template DNA, and 6.0 µL of nuclease-free water. The PCR conditions were as follows: pre-denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 51/47°C for 30 s (OXA51/16S), and extension at 72°C for 45 s; with a final extension at 72°C for 10 min with holding at 4°C. The annealing temperatures were determined using the "in silico" tool of the Primer BLAST website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

After categorically characterizing samples (positive or negative amplification), images of the bands were captured using a digital video camera (Evolution MP 5.0 - color - Media Cibernetic). Using these images, densities of the positive bands were quantitatively estimated by determining the intensity of each band based on threshold color adjustment using ImageJ software (http://rsb.info.nih.gov/ij/) (Rodrigues et al. 2012), and values of the pixels per evaluated area were obtained using a dedicated NVIDIA® GeForce® RTX 2070 SUPER™ graphics card with 8GB of GDDR6 (https://www.dell.com).

Sensitivity and specificity

Initially, among those samples obtained from both the external environment (feces) and from within the hospitals, 40 negative samples, as determined using the aforementioned procedure, were selected and aliquoted in duplicate (a total of 80 negative samples). Half of the samples collected from each environment were contaminated with clinical samples known to be positive for *A. baumannii,* which were isolated from biological fluids (urine, blood, liquids, and secretions) by the Clinical Pathology Service of the Clinic Hospital of the Federal University of Triângulo. Sensitivity was estimated using the probability of positive results for *A. baumannii* (true positives), calculated as the ratio of true positives to the sum of true and false positives. Similarly, specificity was calculated as the ratio of true negatives to the sum of true and false negatives (Guimarães and Carolina 1985).

Quality control

Internal quality control procedures were adopted to reduce the effects of confounding factors, for which we initially defined and monitored clear objectives, procedures, standards, and criteria for tolerance limits, corrective actions, and recording of activities, as well as the use of controls to assess analytical accuracy (Rodrigues et al. 2014). As a positive control, we used *A. baumannii* strain ATCC 19606. Assessments were performed at all stages of execution, with techniques (extraction, amplification, identification, and analysis of data) being evaluated blindly and in duplicate to ensure reproducibility.

Statistical analysis

The data obtained were tabulated in Microsoft Excel and analyzed using IBM SPSS Statistics 21.0 statistical software. The dependent variables (densities of positive samples in PCR) were evaluated for distribution and variance (the D'Agostino and Pearson normality test and Levene's test for comparison of variances). One-way ANOVA in conjunction with a Holm–Sidak's multiple comparison test was used to determine quantitative differences in the positive frequencies for *A. baumannii*, with the data being expressed as means and standard deviations. Associations (dependencies of the positive and/or negative outcomes of the samples regarding the detection of *A. baumannii*) were assessed using Chi-square or Fisher's exact tests. Odds ratios (Baptista–Pike) with the respective confidence intervals were determined to assess the measure of association between intra- and extra-hospital prevalence. The level of significance for analyses was set at 5% (Arango 2001).

3. Results

We initially assessed the prevalence of *A. baumannii* at selected sites within the four targeted hospitals and in the surrounding external environments, the latter of which included the area in the immediate vicinity of the hospitals and public places (Table 1). Among the samples evaluated, those collected in the intra-hospital environment contained a higher prevalence of *A. baumannii* (87.50%, CI = 71.29–100) than those obtained at sites in the wider environment (12.50%, CI = 0–28.71), with calculated odds ratios indicating an approximately 10-fold greater probability of detecting *A. baumannii* in the hospital environment ($p < 0.05$). Among samples collected from hospital sources, floor samples showed a higher prevalence (56.25%, CI = 31.94–80.56) than those collected from the external environment (16.21%, CI = -3.71–28.71), $p < 0.05$.

Table 1. Prevalence of *Acinetobacter baumannii* in samples collected in selected hospitals and from *Columba livia* (Pigeon) droppings in the environments surrounding these hospitals in a region of southeastern Brazil.

Sample location	Positive - N (%)	Negative - N (%)	Total - N	*p*-value	OR	CI (95%)
Pigeon droppings	2 (5)	38 (95)	40			
Handles	5 (25)	15 (75)	20	0.001*	-----	-----
Floor	9 (45)	11 (55)	20			
Intra-hospital	14 (35)	26 (65)	40	0.001*	10.23	2.24–47.14
Extra-hospital	2 (5)	38 (95)	40			

N = number, OD = Odds ratio, CI = Confidence interval, (*) = significant difference.

Having verified qualitative differences in the frequencies of positivity for *A. baumannii* in the evaluated samples, quantitative difference were determined based on the intensities of PCR-amplified DNA bands (pixel density) resolved on agarose gels (Figure 1). Samples were assessed relative to the respective negative (16S- and OXA-), positive (16S + and OXA +), extra-hospital (16SE and OXAE), and intra-hospital (16SI and 16SE) controls (Figures 1a and b). Comparisons of the average densities of bands for each population enabled us to detect significant differences among the sample groups ($p < 0.05$). Specifically, compared with the pigeon feces samples (93.32 ± 1.56), we detected 94% and 52.91% higher densities in samples collected from hospital floors (181.30 ± 11.58) and door handles (142.70 ± 17.14), respectively (Figure 1c).

Finally, we evaluated the sensitivity and specificity of the test applied using the methodology established for the present study (Table 2). For this purpose, samples obtained from environmental and intra-hospital sources were experimentally contaminated with *A. baumannii* isolated from the hospital environment (samples known to be positive) and were compared with negative control samples. This enabled us to determine that both the sensitivity and specificity of the applied method were 99.99%.

Table 2. Description of the sensitivity and specificity of the PCR technique used for the determination of *Acinetobacter baumannii* in extra- and intra-hospital environmental samples.

Variable	Sampling location	
	Environmental (feces)	Hospital sample
Sample size - N	40	40
Sensitivity - %	99.99	99.99
Specificity - %	99.99	99.99

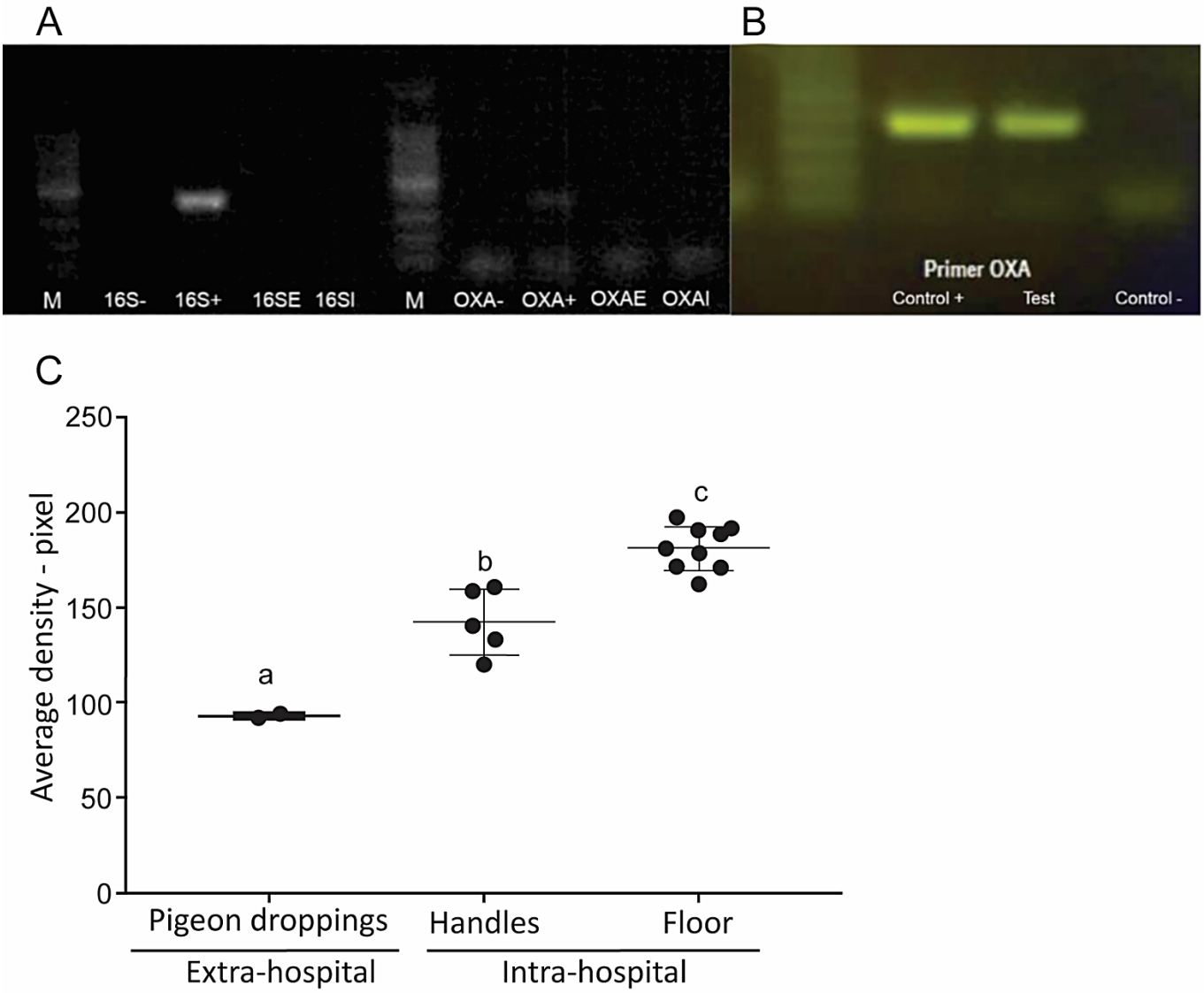

Figure 1. The average densities of *Acinetobacter baumannii* DNA detected in samples collected from different sites. Having established the positive samples among those collected from different sources [pigeon droppings (extra-hospital environment), door handles, or floor (intra-hospital environment)] based on PCR amplification of 16S RNA and oxacillinase genes, the mean density of the bands was determined. A – Representative results for negative (16S- and OXA-), positive (16S+ and OXA+), extra-hospital (16SE and OXAE), and intra-hospital (16SI and 16SE) negative controls; B – Representative results for the positive control (Control +), negative control (control-), and tests (test) for the OXA primer; C – Evaluation of band intensity distribution between the different positive samples. The letters represent significant differences between the groups (a ≠ b ≠ c ≠ a).

4. Discussion

The spread of drug-resistant pathogens via infection is estimated to contribute to approximately 700,000 deaths worldwide each year (Dadgostar 2019). Among these infectious agents, *A. baumannii* is of particular interest, given its propensity to spread, evade available antimicrobial treatments, and cause serious infections in hospitalized patients. In this study, we sought to determine the prevalence of *A. baumannii* in the internal and external settings of hospitals with intensive care units in a region of southeastern Brazil and verified the sensitivity and specificity of the test used to detect the presence of selected marker genes.

Among the samples collected from external environmental sources, we detected a relatively low frequency (N = 2, 5%) of positive rate for *A. baumannii* in pigeon feces. These findings were consistent with those obtained in a study conducted in Poland and Slovenia involving 1,051 bird samples, including cloacal swab samples, where the authors reported only two positive cases of *A. baumannii* (Łopińska et al. 2020), thereby corroborating that *A. baumannii* shows no obvious preference for avian hosts. In this study, oxacillinase gene variants *OXA-71* and *OXA-208* were detected in the two positive samples, both of which are similar to the *blaOXA-51* variant previously described in clinical isolates of *A. baumannii*, thereby highlighting the versatility of this bacterium with regards to the acquisition of resistance and environmental dissemination.

The frequency of *A. baumannii* contamination detected in the external environment is dependent on the material evaluated, as demonstrated by the findings of a study conducted by Anane et al. (2020) in Mthatha, South Africa, where the authors evaluated 598 abattoirs and 689 aquatic samples. Among these, 19.30% were found to be positive for *Acinetobacter* species (N = 248), of which 73.80% were *A. baumannii* (46.40% and 53.60% detected in the abattoir and aquatic samples, respectively). Notably, more than 50% of the isolates exhibited multi-drug resistance

It is thus evident that certain environmental sources have higher frequencies of antimicrobial-resistant *A. baumannii*. In an evaluation of 73 samples of pigeon droppings obtained from two Mediterranean cities [Annaba (Algeria) and Marseille (France)], Morakchi et al. (2017) detected a higher frequency of *A. guillouiae* than that of *A. baumannii*, and among the resistance genes identified in pigeons, 21.90% of the samples were shown to be positive for carbapenem resistance, 10.96% for *blaOXA-23*, 16.44% for *blaOXA-51*-like, and 17.81% for *blaOXA-58.* In this regard, the propagation of resistance genes in the environment is speculated to not only be a consequence of the indiscriminate use of antibiotics, but also be attributable to the activities of environmental vectors such as ants (Moreira et al. 2005; Srivastava et al. 2016; Argudín et al. 2017).

Although in the present study, we detected a high frequency of *A. baumannii* in the hospital environment, the extent of contamination was found to vary depending on the source of the collected sample, with higher frequencies being detected on the surfaces of hospital floors. In this respect, shoes can be identified one of the factors associated with the spread of resistant strains of microorganisms within the environment, along with insufficient hospital disinfection and cleaning. In a systematic review that assessed the findings of 30 studies, the authors surveyed the spread of soil microorganisms via soles of shoes, either by direct contact and aerosolization, and concluded that appropriate hygiene measures are important factors in infection control (Rashid et al. 2017), which can contribute to minimizing the spread of agents and resistance genes within the environment.

Furthermore, Donskey (2019) noted that efforts to improve environmental cleaning and disinfection are generally limited to high-contact surfaces in patient rooms, and emphasized that portable equipment, other devices, and shared floors have largely been overlooked as sources of the spread of pathogens associated with disease development. We additionally believe that implementing efficient techniques with high sensitivity, specificity, and low cost can contribute significantly to the more effective epidemiological monitoring of potentially virulent bacteria such as *A. baumannii*. In the present study, we thus sought to evaluate the specificity and sensitivity of a PCR protocol designed to detect *A. baumannii* in both environmental and hospital samples, using samples previously determined to be positive or negative, and accordingly demonstrated sensitivities and specificities of 99.99% in detection of *OXA51*. Although conventional PCR has previously been demonstrated to have 100% sensitivity and specificity with respect

to the detection of *A. baumannii*, the use of single molecular markers is considered insufficient for precisely distinguishing among members of the *Acinetobacter* sp. complex, whereas combined analysis of *blaOXA-51* and *rpoB* has greater discriminatory power (Vijayakumar et al. 2019). Nevertheless, despite the aforementioned limitations regarding the use of conventional PCR, the technique still has greater sensitivity and specificity (99%–100%) than those of more sophisticated techniques for determining species based on phenotypic characteristics that are commonly used in clinical laboratories (91%–98%) (Vijayakumar et al. 2019). Currently, although there are prospects for the introduction of techniques based on mapping of nucleic acids of isolated agents, which may contribute to gaining a broader understanding of host–pathogen relationships, developing and implementing simple low-cost techniques that can be adopted for routine use in low-income countries is still needed. Among these, PCR combined with matrix-assisted laser desorption ionization time-of-flight mass spectrometry analysis can represent a promising approach for clinical sample identification and epidemiological surveillance. Other possibilities have been demonstrated for determining characteristics linked to resistance and intervention genes, using techniques that are both robust and yield sufficiently precise data, as has previously been demonstrated by high performance and specificity obtained via the integration of multiplex PCR with the CRISPR-Cas matrix (Wang et al. 2021). However, given the costs associated with implementation and maintenance, adopting such approaches is still far from practical realization in developing countries.

A notable limitation of the present study is that we did not characterize the alpha and beta diversity of the samples, as we lacked sufficient information regarding the identity and quantity of other agents present in assessed samples, which can potentially influence the performance of the test we used. Although we were able to estimate the origin of samples with high biodiversity, gaining a better understanding of microbial biodiversity will undoubtedly contribute to clarifying the potential of the PCR technique in detecting *A. baumannii* against a background of variability in frequencies of other microbial agents.

5. Conclusions

Based on our findings, *Acinetobacter baumannii* could be found both within hospitals and more widely in the areas surrounding these facilities, although populations tended to be more prevalent in the hospital environment. Moreover, we detected a notably greater quantitative variability in *A. baumannii* populations among those samples collected in a hospital setting, which was found to be dependent on the surface assessed. In addition, the conventional PCR method applied in this study enabled us to detect positive and negative samples with both high sensitivity and specificity.

The prevalence characterized in this study emphasizes the need to intensify the control of *A. baumannii* infection in both intra- and extra-hospital environments. Specifically, efficient hospital surface cleaning protocols should be established as a measure to minimize the spread of infectious agents. Moreover, given its high accuracy and relatively low cost, conventional PCR could be widely implemented in routine epidemiological surveillance.

Authors' Contributions: SILVA, K.F.B.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; MIGUEL, C.B.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; DE OLIVEIRA JÚNIOR, R.R.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; ARAÚJO, M.C.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; AGOSTINHO, F.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; UEIRA-VIEIRA, C.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; ; LAZO-CHICA, J.E.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; RODRIGUES, W.F.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: This study was approved by the Research Ethics Committee of the Clinical Hospital of the Federal University of Triângulo Mineiro (approval number: 3,050,072).

Acknowledgments: The authors would like to thank or the technical support from Prof. Dr. Paul Roberto da Silva (CEFORES/UFTM). WFR receives a postdoctoral fellowship from the National Council for Scientific and Technological Development (CNPq; process: 152889/2022-1).

References

AFUNWA, R., et al. Multiple antibiotic resistant index of Gram-Negative bacteria from bird droppings in two commercial poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology*. 2020, **10**(4), 171–181. https://doi.org/10.4236/ojmm.2020.104015

ANANE, A.Y., et al. Prevalence and molecular analysis of multidrug-resistant *Acinetobacter baumannii* in the extra-hospital environment in Mthatha, South Africa. *Brazilian Journal of Infectious Diseases*. 2020, **23**(6), 371–380. https://doi.org/10.1016/j.bjid.2019.09.004

ANDINI, N., et al. "Culture" shift: broad bacterial detection, identification, and antimicrobial susceptibility testing directly from whole blood. *Clinical Chemistry*. 2018, **64** (10), 1453–1462. https://doi.org/10.1373/clinchem.2018.290189

ARANGO, H.G., 2001. Bioestatística teórica e computacional. In: Bioestatística teórica e computacional. pp. 235–235.
ARGUDÍN, M.A., et al. Bacteria from Animals as a Pool of Antimicrobial Resistance Genes. *Antibiotics.* 2017, **6**(2), https://doi.org/12.10.3390/antibiotics6020012

BONNIN, R.A., et al. Phenotypic, biochemical, and molecular techniques for detection of metallo-*β*-lactamase NDM in *Acinetobacter baumannii*. *Journal of Clinical Microbiology*. 2012, **50**(4), 1419–1421. https://doi.org/10.1128/JCM.06276-11

CHIDAMBA, L. and KORSTEN, L. Antibiotic resistance in *Escherichia coli* isolates from roof-harvested rainwater tanks and urban pigeon faeces as the likely source of contamination. *Environmental Monitoring Assessment.* 2015, **187**(7), 405. https://doi.org/10.1007/s10661-015-4636-x

CHIN, C.Y., et al. A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. *Nature Microbiology*. 2018, **3**(5), 563–569. https://doi.org/10.1038/s41564-018-0151-5

DADGOSTAR, P. Antimicrobial Resistance: Implications and Costs. *Infection and Drug Resistance*. 2019, **12**, 3903–3910. https://doi.org/10.2147/IDR.S234610

DE OLIVEIRA XAVIER, A.R.E., et al. Detection and identification of medically important microorganisms isolated from pigeon excreta collected in a university in a newly industrialized country. *Biotemas*. 2019, **32**(1), 11–20. http://dx.doi.org/10.5007/2175-7925.2019v32n1p11

DONSKEY, C.J. Beyond high-touch surfaces: Portable equipment and floors as potential sources of transmission of health care-associated pathogens. *American Journal of Infection Control*. 2019, **47S**, A90–A95. https://doi.org/10.1016/j.ajic.2019.03.017

ESPINAL, P. et al. Dissemination of an NDM-2-producing *Acinetobacter baumannii* clone in an Israeli rehabilitation center. *Antimicrobial Agents and Chemotherapy*. 2011, **55**(11), 5396–5398. https://doi.org/10.1128/AAC.00679-11

GHADERI, Z., EIDI, S. and RAZMYAR, J. High Prevalence of *Cryptococcus neoformans* and isolation of other opportunistic fungi from pigeon (*Columba livia*) droppings in Northeast Iran. *Journal of Avian Medicine and Surgery*. 2019, **33**(4), 335–339. https://doi.org/10.1647/2018-370

GUIMARÃES, M. and CAROLINA, S. Laboratory tests: sensitivity, specificity and positive predictive value. *Revista da Sociedade Brasileira de Medicina Tropical*. 1985, **18**(2), 117–120. https://doi.org/10.1590/S0037-86821985000200009

HOU, C. and YANG, F. Drug-resistant gene of *blaOXA-23*, *blaOXA-24*, *blaOXA-51* and *blaOXA-58* in *Acinetobacter baumannii*. *International Journal of Clinical and Experimental Medicine*. 2015, **8**(8), 13859.

LEUNG, L.M., et al. A prospective study of *Acinetobacter baumannii* complex isolates and colistin susceptibility monitoring by mass spectrometry of microbial membrane glycolipids. *Journal of Clinical Microbiology.* 2019, **57**(3), e01100–18. https://doi.org/10.1128/JCM.01100-18

LIU, S., et al. Rapid and accurate detection of carbapenem-resistance gene by isothermal amplification in *Acinetobacter baumannii*. *Burns & Trauma*. 2020, **8**, tkaa026. https://doi.org/10.1093/burnst/tkaa026.

ŁOPIŃSKA, A., et al. Occurrence of Acinetobacter baumannii in Gulls and Songbirds. Polish Journal of Microbiology. 2020, **69**(1), 1–6. https://doi.org/10.33073/pjm-2020-011

MORAKCHI, H., et al. Molecular characterisation of carbapenemases in urban pigeon droppings in France and Algeria. *Journal of Global Antimicrobial Resistance*. 2017, **9**, 103–110. https://doi.org/10.1016/j.jgar.2017.02.010

MOREIRA, D.D., et al. Ants as carriers of antibiotic-resistant bacteria in hospitals. *Neotropical Entomology*. 2005, **34**, 999–1006, 2005. https://doi.org/10.1590/S1519-566X2005000600017

PELEG, A.Y., SEIFERT, H. and PATERSON, D.L. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews*. 2008, **21**(3), 538–582. https://doi.org/10.1128/CMR.00058-07

RASHID, T., et al. Mechanisms for floor surfaces or environmental ground contamination to cause human infection: a systematic review. *Epidemiology & Infection.* 2017, **145**(2), 347–357. https://doi.org/10.1017/S0950268816002193

RIBEIRO, E.A., et al. Molecular epidemiology and drug resistance of *Acinetobacter baumannii* isolated from a regional hospital in the Brazilian Amazon region. *Revista da Sociedade Brasileira de Medicina Tropical*. 2020, **54**, e20200087. https://doi.org/10.1590/0037-8682-0087-2020

RODRIGUES, W.F., et al. Low dose of propranolol down-modulates bone resorption by inhibiting inflammation and osteoclast differentiation. *British Journal of Pharmacology*. 2012, **165**(7), 2140–2151. https://doi.org/10.1111/j.1476-5381.2011.01686.x

RODRIGUES, W.F., et al. Establishing standards for studying renal function in mice through measurements of body size-adjusted creatinine and urea levels. *BioMed Research International*. **2014**, 872827. https://doi.org/10.1155/2014/872827

SRIVASTAVA, J., et al. Understanding the development of environmental resistance among microbes: a review. *CLEAN–Soil, Air, Water*. 2016, **44**(7), 901–908. https://doi.org/10.1002/clen.201300975

TADA, T., et al. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* isolates from hospitals in Myanmar. *Journal of Global Antimicrobial Resistance*. 2020, **22**, 122–125. https://doi.org/10.1016/j.jgar.2020.02.011

VIJAYAKUMAR, S., BISWAS, I. and VEERARAGHAVAN, B. Accurate identification of clinically important *Acinetobacter* spp.: an update. *Future Science OA*. 2019, **5**(6), FSO395. https://doi.org/10.2144/fsoa-2018-0127

WANG, Y., et al. Integration of multiplex PCR and CRISPR-Cas allows highly specific detection of multidrug-resistant *Acinetobacter Baumannii*. *Sensors and Actuators B: Chemical.* 2021, **334**, 129600. https://doi.org/10.1016/j.snb.2021.129600

Received: 2 September 2021 | **Accepted:** 8 February 2022 | **Published:** 27 January 2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.