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LOOKING FOR THE NEEDLE IN THE HAYSTACK: BIOLUMINESCENCE AND CULTURE MEDIA AS A COMBINED APPROACHED TO EVALUATE MICROBIAL LOAD IN ICUS OF A BRAZILIAN MEGALOPOLIS

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Abstract: Hospital-acquired infections are one of the leading causes of mortality, and their source can be the environment itself. Proper monitoring of cleaning quality in ICUs has been used in specific situations. In Brazil, hospitals are committed to reporting cases of infection and microbial resistance, yet validations of environments have been recommended. In order to evaluate the luminometer, five surfaces in the ICU of a public hospital in São Paulo were monitored using RODAC® plates as a standard. Spearman correlation and ROC curve analyses were used for statistical analysis; however, these analyses did not show significance. Nonetheless, using the A NOVA methodology, they were validated with significance ≤ 0.001. From this study, we conclude that some surfaces are more susceptible to dirt accumulation, requiring greater control over the frequency and methodology of cleaning, and that the methodology used for this monitoring can be one of the methods utilized or the use of both when possible, resulting in continuous improvement.
Keywords: Microbiology; Hospital Infection; Bioluminescence; RODAC; Biodesinfection.

INTRODUCTION

Hospital-acquired infections are a leading cause of mortality worldwide and in many times have the environment as a source, since it can be considered a reservoir of microorganisms, especially in healthcare settings, contributing to potential infection risks (1–4). The hygiene maintenance services within healthcare institutions, have, as a consequence, structured cleaning protocols based on the scientific literature. Nevertheless, practical execution often falls short, primarily due to labor issues and various factors contributing to process failure (5). Adequate monitoring of cleaning quality is imperative, and has been utterly important under very specific situations, for instance, hospital outbreaks and in intensive care units (hereafter, ICUs).

The commitment of multidisciplinary teams to record and validate cleaning methods and techniques, along with environmental control, is necessary to prevent cross-infections. These actions have been evidenced and adopted by several hospitals during and after the COVID-19 pandemic to strengthen patient safety. They are even more important under ICUs' conditions, given the poor condition of many patients and the risk to their lives. However, many of the methods lack standardized values to guide teams during score validation (6–9), something that not only impairs the development of safe measures and scientific knowledge but also might be evasive allowing infections to spread out.

In Brazil, hospitals are obliged to report cases of infection and microbial resistance to the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA) (10) On the other hand, the validation of hospital environments is only recommended but not enforced (3,11,12). Moreover, some regulations such as those provided by ANVISA (2003) and CONAMA (2018) address environmental control. Still, they do not adequately normalize the process of monitoring hospital environmental cleanliness, leaving gaps, especially in interpreting results obtained from existing tests.

Methodologies for controlling environmental hygiene, have already been established and used in the food and pharmaceutical industries for a longer period, were implemented in the Brazilian healthcare sector in recent years with the same objective Two of the most common tests used are luminescence detectors and contact plates, which are cultured in clinical laboratories after collection (13–18). Under this context, here, we provide the first-of-its-kind assessment of fluorescence detector and the application of surface collection plates in Brazil, in order to evaluate possible relationships between the two methods, aiming for the luminometer to prove to be a suitable instrument for routine control in ICUs of a public hospital in São Paulo megalopolis.

METHODOLOGY

SAMPLING PROTOCOL

A total of 180 surface samples from the adult and pediatric ICUs of a public hospital were collected during the period of three months (JUL-JAN/2024). The evaluated surfaces included countertops, windows, doorknobs, equipment towers, and bed arms in randomly selected areas, with 36 samples collected for each surface type. No sample was collected more than once in the same place.

MICROBIAL EVALUATION METHODS

The first method used was the RODAC® plate, considered the gold standard in this evaluation, as its capacity allows for the growth of viable microorganisms, which are quantified in Colony Forming Units (hereafter, CFU/m^3). Collection was carried out by pressing the agar surface in the collection area, and then the plates were incubated at 37 °C for 24 hours in a bacteriological incubator (3). For this method, a total of 180 plates were analyzed.

The second method used was bioluminescence evaluation by a luminometer, which aims to detect organic residues, including adenosine triphosphate (ATP), used as an indicator of biological residues. A swab was used to collect the surface sample, then placed in the equipment to measure the relative light unit (RLU). The sample was exposed to a chemical agent, and the light-producing substrate was activated by ATP, where the amount of emitted light is directly proportional to the amount of ATP present in the collected material. This amount of light is recorded as RLU. This methodology does not differentiate microorganisms from other organic cells, and the result is given in real-time (11,19).

STATISTICAL ANALYSIS

Since the assumptions of normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test) were not satisfied, data on CFUs/m^3 were log-transformed. To compare differences in the CFUs/m^3 by local of sampling (e.g., countertops, windows, doorknobs, equipment towers, and bed arms), we used Analysis of Variance (i.e., ANOVA), followed by Tukey's HSD *post-hoc* test. These analyses were performed with a significance level of $\alpha = 0.05$, using Statistica v. 14.0.0.15 (20).

RESULTS

Both methodologies indicated that the highest indices of infection (i.e., microbial load/burden) were found in the equipment tower area, highlighting the need to monitor this location when focusing on surface hygiene (Fig. 1 and 2). Either by RODAC © ($F_{(4,175)} = 8.44$; $P < 0.001$) or ATP © $F_{(4,175)} = 6.26$; $P = 0.0001$) the tower showed the highest CFUs number, respectively. The countertop, on the other hand, showed different data in the studied methodologies, making it difficult to choose the best method for monitoring its cleanliness (Fig. 1 and 2).

DISCUSSION

This study focused on evaluating only two types of methodologies, bioluminescence (ATP) and RODAC®, as they are recognized and commonly used for validation in healthcare facilities (11,21) B, C e D.

The discrepancy in results between samples collected from the countertop and the tower can be attributed to various external factors, for example, equipment composition, given that both the countertop and the tower are predominantly composed of metal. This fact can facilitate the circulation of microbial particles, thus allowing their removal during routine cleaning and the fixation of particles where surface sanitation is not carried out because they are not touched, such as the object's indentations. (22)

Thus, some surfaces are more prone to dirt adhesion, requiring greater control over cleaning frequency and methodology. Our study suggests that at least one of these surfaces is the tower of equipment, which is similar to the research performed by (23,24).

The proper selection of cleaning methods and sanitizers aid in the maintenance of high-hygiene levels in hospital facilities. Standardizing these systems impact product consumption and the risks of physical, chemical, ergonomic, and biological hazards, consequently affecting absenteeism rates, as well as their monitoring (3,17).

The methodologies employed in this study were suitable and can be used for ICU cleaning control. However, it is important to note that RODAC® results are not immediate and cannot be used for real-time cleanliness assessment, only for report composition. After the growth of CFU/m^3, microorganisms can be identified and correlated with cross-infections (3,25). The bioluminescence method yielded similar results to RODAC® plates. With this finding, we concluded that the technique could be recommended as an indicator of hygiene condition, informing whether organic matter is present on the analyzed surface, but it cannot determine microbiological concentration. Its advantage lies in providing immediate and real-time results, allowing for immediate cleaning correction. This method can be safely used only for bio-decontamination control, assisting quality improvement programs (17).

CONCLUSION

Just as cases of healthcare-associated infections (HAIs) are monitored by the epidemiological surveillance centers of each state, microbiological monitoring of critical areas in healthcare facilities is necessary as a preventive action for hospital infection control. Methodologies used for environmental control, such as those used in this study, can be successful provided that cutoff values are standardized by regulatory agencies, considering the criticality of the environment being monitored. Additionally, updating current legislation is crucial to standardize and improve the effectiveness of ICU hygiene assessment.

AUTHOR'S CONTRIBUTION

DMCS and VBDF conceived and designed the research; DMCS and VBDF collected data; DMCS, VBDF, and MCM performed the statistical analyses; DMCS, VBDF, EDC, ACSRC, MCM, AO, and NJFC wrote and edited the manuscript; DMCS led the writing of the manuscript. Critical contribution to drafts and final approval for publication was given by all authors.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest.

DATA AVAILABILITY

Data will be made available upon request.

REFERENCES

1. De Moraes BA, Cravo CAN, Loureiro MM, Solari CA, Asensi MD. Epidemiological analysis of bacterial strains involved in hospital infection in a University Hospital from Brazil. Rev Inst Med Trop Sao Paulo. 2000;42 (4) :201–7.

2. Cabo Verde S, Almeida SM, Matos J, Guerreiro D, Meneses M, Faria T, et al. Microbiological assessment of indoor air quality at different hospital sites. Res Microbiol. 2015;166 (7) :557–63.

3. Cristiane Schmitt, Maria Clara Padoveze, Denise Brandão de Assis, Ariana Maria da Silva Felix, Amanda Luiz Pires Maciel, Ana Rubia Guedes Vinhole, Claudia Vallone Silva, Ligia Maria Abraão MMB. Melhores práticas para higiene e limpeza em ambiente hospitalar [Internet]. São Paulo; 2022 [cited 2022 Aug 23]. Available from: http://saude.sp.gov.br/resources/cve-centro-de-vigilancia-epidemiologica/areas-de-vigilancia/doencas-de-transmissao-respiratoria/coronavirus/2022/abril/coronavirus080422_situacao_epidemiologica.pdf

4. Monegro AF, Muppidi V, Regunath H. Hospital-Acquired Infections. Cambridge Handb Psychol Heal Med Second Ed [Internet]. 2023 Feb 12 [cited 2024 Jan 29];736–8. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441857/

5. Boyce JM. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrob Resist Infect Control [Internet]. 2016;5 (1) :1–10. Available from: http://dx.doi.org/10.1186/s13756-016-0111-x

6. Macher JM. Positive-Hole Correction of Multiple-Jet Impactors for Collecting Viable Microorganisms. Am Ind Hyg Assoc J. 1989;50 (11) :561–8.

7. Flores MEB, Medina PG, Camacho SPD, De Jesus Uribe Beltran M, De La Cruz Otero MDC, Ramirez IO, et al. Fungal spore concentrations in indoor and outdoor air in university libraries, and their variations in response to changes in meteorological variables. Int J Environ Health Res [Internet]. 2014;24 (4) :320–40. Available from: http://dx.doi.org/10.1080/09603123.2013.835029

8. Amodio E, Kuster SP, Garzoni C, Zinkernagel AS, Sax H, Wolfensberger A. Disinfecting noncritical medical equipment—Effectiveness of hydrogen peroxide dry mist as an adjunctive method. Am J Infect Control. 2020 Aug 1;48 (8) :897–902.

9. Dhama K, Patel SK, Kumar R, Masand R, Rana J, Yatoo MI, et al. The role of disinfectants and sanitizers during COVID-19 pandemic: advantages and deleterious effects on humans and the environment. Environ Sci Pollut Res. 2021;28 (26) :34211–28.

10. Manual de Limpeza e Desinfecção de Superfícies.pdf — Português (Brasil) [Internet]. [cited 2021 Jul 1]. Available from: https://www.gov.br/anvisa/pt-br/centraisdeconteudo/publicacoes/servicosdesaude/publicacoes/manual-de-limpeza-e-desinfeccao-de-superficies.pdf/view

11. Oliveira T de, Canettieri ACV. Eficiência dos métodos microbiológicos e de ATP-bioluminescência na detecção da contaminação de diferentes superfícies. Rev Inst Adolfo Lutz. 2010;69 (4) :467–74.

12. Batista Duo Filho V, Alvarez Ferro Research V, Gade Hospitalar Marcos Antônio Cyrillo Hospital do Servidor Público Municipal D, Paulo S. Monitoring the Action of Aerosolized H 2 O 2 and the Impact on Fungi and Bacteria in the Indoor Air of São Paulo Environments. Int J Heal Sci. 2022;1–12.

13. Ribeiro H. Saúde Pública e Meio Ambiente : evolução do conhecimento e da prática , alguns aspectos Public Health and Environment : Evolution of the knowledge and the practice , some ethical aspects. 2004;70–80.

14. Boyce JM, Havill NL. Evaluation of a New Hydrogen Peroxide Wipe Disinfectant. Infect Control Hosp Epidemiol. 2013 May;34 (5) :521–3.

15. Ferreira PDG. Avaliação da atividade micobactericida de desinfetantes de alto nível registrados e comercializados no Brasil [Internet]. Fundação Oswaldo Cruz. Instituto Nacional de Controle de Qualidade em Saúde; 2017 [cited 2022 Apr 7]. Available from: https://www.arca.fiocruz.br/handle/icict/39416

16. Guerrero FGR. AVALIA ÇÃ O DA EFICI Ê NCIA DE LIMPEZA EM PONTOS CHAVES DE AMOSTRAGEM DE UMA F Á BRICA. 2018.

17. Furlan MCR, Ferreira AM, Rigotti MA, Guerra OG, Frota OP, Sousa AFL de, et al. Correlação entre métodos de monitoramento de limpeza e desinfecção de superfícies ambulatoriais. Acta Paul Enferm. 2019;32 (3) :282–9.

18. Cordero PRF, Bayly K, Man Leung P, Huang C, Islam ZF, Schittenhelm RB, et al. Atmospheric carbon monoxide oxidation is a widespread mechanism supporting microbial survival. ISME J [Internet]. 2019;13 (11) :2868–81. Available from: http://dx.doi.org/10.1038/s41396-019-0479-8

19. Cumhur YE, Esra ZEN, Doktora K, Ve UM, Hastaliklari K, Dani D, et al. Epidemiology of bacterial contamination of inert hospital surfaces and equipment in critical and non-critical care units: a Brazilian multicenter study. J Wind Eng Ind Aerodyn [Internet]. 2019;26 (1) :1–4. Available from: https://doi.org/10.1007/s11273-020-09706-3%0Ahttp://dx.doi.org/10.1016/j.jweia.2017.09.008%0Ahttps://doi.org/10.1016/j.energy.2020.117919%0Ahttps://doi.org/10.1016/j.coldregions.2020.103116%0Ahttp://dx.doi.org/10.1016/j.jweia.2010.12.004%0Ahttp://dx.doi.o

20. Gao Y, Liu W, Wang H, Wang X, Yan Y, Zhang R. A review of distributed statistical inference. Stat Theory Relat Fields [Internet]. 2022;6 (2) :89–99. Available from: https://doi.org/10.1080/24754269.2021.1974158

21. De Abreu CS, De Jesus Andreoli Pinto T, De Oliveira DC. Áreas limpas: estudo de correlação entre partículas viáveis e não-viáveis. Rev Bras Ciências Farm [Internet]. 2003 [cited 2024 Jan 23];39 (2) :177–84. Available from: https://www.scielo.br/j/rbcf/a/nJ5PTz446MwVZyjzCgNkDdy/

22. Sozzi JSR, Miguel YC de S, Morais L de O, Gomes-Júnior MV, Ferreira AP, Garcia PG. Pesquisa de Bactérias Patogênicas em Superfícies e Equipamentos de Ambulâncias. Rev Médica Minas Gerais [Internet]. 2019 [cited 2022 Aug 23];29 (1) :1–7. Available from: http://www.rmmg.org/artigo/detalhes/2592

23. Montagna MT, Rutigliano S, Trerotoli P, Napoli C, Apollonio F, D'Amico A, et al. Evaluation of air contamination in orthopaedic operating theatres in hospitals in Southern Italy: The IMPACT project. Int J Environ Res Public Health. 2019;16 (19) :1–11.

24. Siciliano B, Dantas G, da Silva CM, Arbilla G. The updated Brazilian national air quality standards: A critical review. J Braz Chem Soc. 2020;31 (3) :523–35.

25. Huslage K, Rutala WA, Gergen MF, Sickbert-Bennett EE, Weber DJ. Microbial Assessment of High-, Medium-, and Low-Touch Hospital Room Surfaces. Infect Control Hosp Epidemiol [Internet]. 2013 Feb [cited 2022 Oct 4];34 (2) :211–2. Available from: https://www.cambridge.org/core/journals/infection-control-and-hospital-epidemiology/article/abs/microbial-assessment-of-high-medium-and-lowtouch-hospital-room-surfaces/80B5517EDD71849CC4F041A7C66C4767

Sample number	Bed arm		Countertop		Tower		Doorknob		Window	
	RODAC®	ATP	RODAC®	ATP	RODAC®	ATP	RODAC®	ATP	RODAC®	ATP
1	0	27	0	2	0	14	0	16	0	4
2	0	64	0	0	0	10	0	7	0	56
3	0	4	0	7	2	12	0	0	0	79
4	0	10	0	10	3	55	0	5	0	12
5	0	13	0	7	3	43	0	7	1	58
6	1	12	1	0	3	111	0	4	1	8
7	1	14	1	0	3	24	0	18	2	18
8	1	21	2	2	4	7	0	16	3	39
9	1	27	3	14	5	45	0	68	5	119
10	2	6	3	0	5	60	1	63	5	7
11	2	9	5	37	6	22	1	23	5	22
12	2	52	6	2	6	0	1	0	5	107
13	3	21	8	51	7	182	2	2	5	4
14	3	105	9	11	9	14	2	11	6	4
15	3	22	10	3	10	10	2	12	6	37
16	3	54	10	17	12	38	2	16	7	46
17	3	11	15	98	15	20	2	12	7	199
18	4	22	16	10	23	37	2	74	8	89
19	5	46	18	22	25	365	3	2	9	18
20	5	34	18	18	30	10	3	23	10	73
21	6	5	18	6	30	30	3	50	11	24
22	6	41	18	10	38	1311	4	79	12	78
23	6	41	20	3	40	339	4	46	13	102
24	7	8	23	5	42	22	6	7	15	4
25	8	195	25	2	45	37	6	94	15	13
26	9	5	27	6	48	46	6	127	16	96
27	10	87	28	102	53	120	7	6	16	182
28	10	171	28	182	53	51	7	16	28	73
29	10	37	36	120	60	128	7	12	30	17
30	12	0	40	40	68	200	12	118	30	10
31	15	39	42	11	75	93	15	209	30	98
32	17	142	56	42	94	600	15	43	31	180
33	20	2375	56	108	120	6	15	17	35	10
34	30	9	65	48	230	329	25	45	37	95
35	41	32	150	9	280	91	30	29	83	178
36	180	52	230	18	300	244	38	17	*220*	52

Table 1 – Colony Forming Units (CFU/m3) of the 180 surface samples using RODAC® plates and bioluminescence (ATP) in the ICU of a Public Hospital in São Paulo, Brazil.

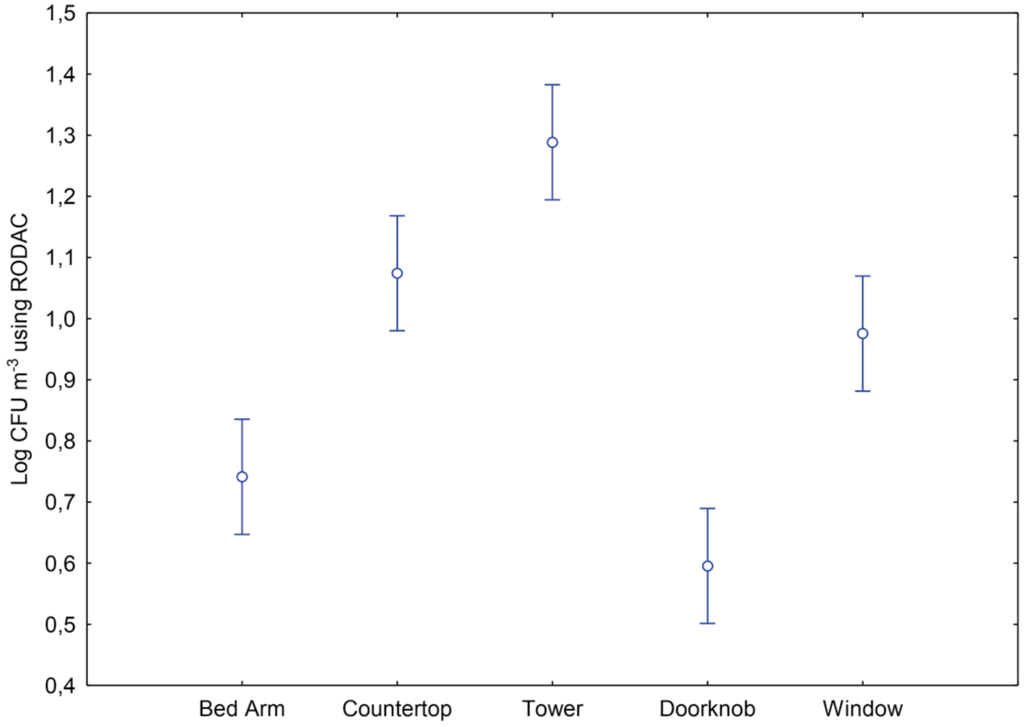

Figure 1 – Number of colony forming units (Log-transformed CFUs/m³) collected in different surface samples using RODAC in the ICUs of a public hospital in São Paulo, Brazil.

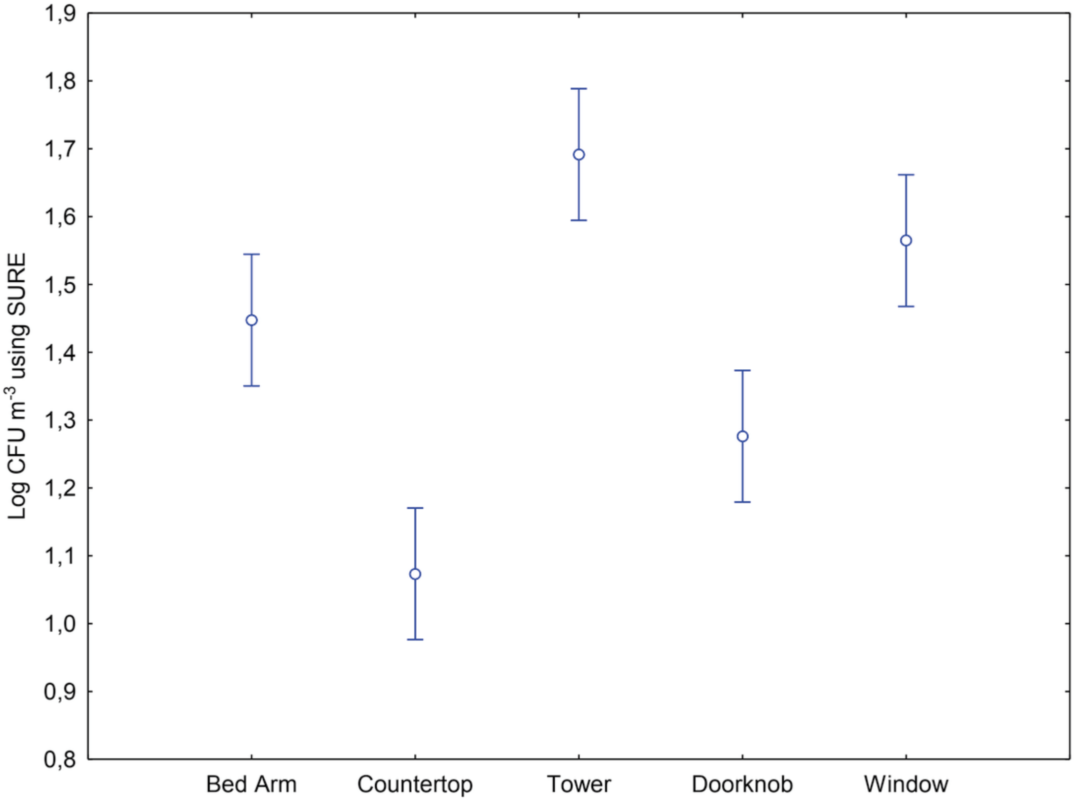

Figure 2 – Number of colony forming units (Log-transformed CFUs/m³) collected in different surface samples using SURE bioluminescence (ATP) in the ICUs of a public hospital in São Paulo, Brazil.