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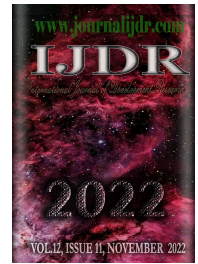
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RESEARCH ARTICLE

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USE OF ADENOSINE TRIPHOSPHATE (ATP) TO MONITOR CLEANING AND DISINFECTION OF SURFACES IN AIR MEDICAL TRANSPORT

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ABSTRACT

With the objective of evaluating the quality of disinfection of aircraft and equipment used during inter-hospital aeromedical transport using ATP bioluminescence technology, we studied the environmental contamination that represents the risk of microbial transmission between patients and professionals. This remains one of the challenges of clinical practice. Visual inspection and microbial culture are considered common methods for evaluating the cleaning and disinfection of surfaces close to patients in hospital settings, such as cardiac monitors, infusion pumps, stretchers, etc. Visual inspection is easy to perform and inexpensive, but it does not provide objective information about the level of cleanliness achieved and therefore the risk of infection. Microbial cultures are highly sensitive and specific, but they are time-consuming, expensive and require different equipment and supplies, microbiology laboratories and professionals. This is a descriptive and comparative study based on the results of cultures collected by a private aeromedical transport company in Belo Horizonte, Minas Gerais, Brazil. In this research, the samples were cultivated in two steps. For convenience, samples are collected and surfaces are established by hand and patient contact. Finally, although data on clinically relevant cutoffs for reducing microbial transmission are limited, cutoffs based on hospital studies were used, which may not be appropriate for an aeronautical medicine setting.

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INTRODUCTION

Environmental contamination represents a risk of microbial transmission between patients and professionals and remains one of the challenges for clinical practice¹. In this sense, it is considered that cleaning and disinfecting the environment reduces contamination of environmental surfaces and can contribute to reducing the occurrence of healthcare-associated infections associated with cross-infection. In this case, it is necessary to evaluate and understand the method of monitoring adenosine triphosphate (ATP)^{1,2} as a possible marker of the efficiency of aircraft disinfection and qualitative cultures performed by microbiological analysis. Visual inspection and microbiological cultures are considered common methodologies to evaluate the cleaning and disinfection of surfaces close to patients in hospital environments, such as cardiac monitors, infusion pumps, stretchers, and others. Visual inspection is easy to perform and inexpensive, but it does not provide objective information on the levels of cleanliness obtained and, consequently, on the risk of infection. Microbiological cultures have high sensitivity and specificity, but they are time-consuming, and have a higher cost, in addition to requiring different equipment and supplies, a microbiology laboratory, and specialized personnel³. Although the role of the environment in the acquisition of potential pathogens is unclear, several microorganisms of epidemiological relevance have been isolated from different locations in the healthcare environment. It is believed that once contaminated, these surfaces can favor the spread of microorganisms⁵. In this sense, hygiene measures in environments intended for health care are a primordial part of the multiple strategies necessary for the prevention and control of infections. Among these care environments, we can list the land and airmobile units for transporting the sick. Aeromedical transport is a modality used mainly when talking about seriously ill patients and, on several occasions, it represents the best option, sometimes the only one, for the individual to receive assistance in a center specialized in their affections. Its origin comes from war experiences related to the need to quickly remove the wounded from battles. In the early 1990s, the use of large-scale air medical transport for critically ill patients emerged. This took place in the search for better assistance, based on the rationality of the resource as well as on the specialization of large centers that had better treatments and the use of more advanced equipment, which facilitated the access of seriously ill patients to these services in the area of health⁸. In the last decade, the measurement of organic adenosine triphosphate (ATP) on surfaces with ATP-bioluminescence testing has gained popularity, given its speed, objectivity, provision of quantitative data, its possibility of immediate results, enabling the implementation of improvements to practices. Cleaning with minimal technical training requirements⁴. This technology consists of the reaction between the luciferase enzyme and ATP molecules, derived from organic matter from microorganisms or not, recovered from the surface from swabs. ATP concentrations are quantified using a luminometer and the results are expressed in relative light units (URL)^{5,6}. The incessant search for quality is reflected in several aspects of health care providers, and, in this way, air medical transport services increasingly aim at the use of good practices⁸. Works are scarce in the literature, especially in the aeromedical field, on best practices in aircraft cleaning and disinfection. Hence, the interest in the adoption of effective methods to evaluate the cleaning of surfaces, especially microbiological ones, and in the possible introduction of ATP bioluminescence in aeromedical services, to expand discussions on cleaning processes. The objective was to evaluate the quality of disinfection of aircraft and equipment used during inter-hospital transport. aeromedical, through the use of the technique of ATP bioluminescence.

METHODS

This is descriptive and comparative research, carried out from the results of cultures collected by a private aeromedical transport company based in Belo Horizonte, Minas Gerais, Brazil. The cultures were carried out by the company in two stages in the periods from

09/02/2017 to 11/05/2017, and 04/08/2018 to 04/23/2018. The samples were taken for convenience and the surfaces were established from the contact of the hands and the patient. In the first step, quantitative monitoring was used with the ATP bioluminescence method, later, the results were analyzed. Based on the results, it was possible to proceed with the second stage: the selection of the most relevant surfaces for the addition of microbiological analyses. The quantitative monitoring of ATP was performed using equipment that performs the rapid test of the microbiological population found on surfaces using swabs. This technology quickly measures the degree of contamination of surfaces, utensils, medical items, and hands, through the detection of cell ATP, one of the main energy molecules for all animal, plant, bacteria, yeast, fungi, and biofilm cells present in the sample. A swab moistened with a cationic substance (luciferin and luciferase) is used to assist in collecting samples and releasing ATP from intact cells. The test reading is done quantitatively, through the reaction of the reagent present in the ampoule with the ATP collected in the swab, producing light and the results are emitted in Relative Light Units (URL). Light intensity is associated with the amount of ATP collected in the sample and, therefore, with the degree of contamination⁹. For disinfection of surfaces in the immediate post-flight, a disinfectant detergent was used for cleaning and disinfection of surfaces and hospital equipment, composed of did ecyldimethylammonium chloride (0.30%), sequestering nonionic surfactants, excipient, pH regulator and water. During the disinfection procedures, the product was applied to the surface and the time of 15 minutes was respected for bactericidal activity.

The first stage was characterized by the selection of ten surfaces: (1) From the aircraft: ceiling, floor, side wall where the stretcher base is fixed, a stretcher seat belt for use on the patient, the center of the stretcher, stretcher handle, joystick and inside rear door handle. (2) Also, the non-invasive pressure cuff (NIBP) and the multiparameter monitor screen. The ceiling and floor surfaces were considered control, with the ceiling being the cleanest surface and the floor the dirtiest. The chest seat belt, NIBP cuff, center of the stretcher, and side wall of the aircraft were selected due to the high probability of direct contact with the patient. The stretcher handle, the multiparameter monitor screen, the joystick, and the internal door handle were part of the sample because they had frequent contact with the hands of the care team. The missions carried out in fixed-wing aircraft were composed of a team of 01 doctors, 01 nurses 01 pilot, and 01 co-pilot. The collections were carried out at three different and consecutive times: pre-flight, a moment that allowed the evaluation of the cleanliness of the surfaces of the aircraft and equipment that were available for the missions; post-flight, when it was possible to assess the dirt generated during the missions; and 45 minutes after disinfection of the surfaces, when it was possible to evaluate the quality of the disinfections carried out on the surfaces. The surfaces that presented the most expressive results in the first stage were selected for quantitative and qualitative investigation in the second stage of the research. The ceiling and floor were maintained as controls, the stretcher handle and joystick as reference surfaces for contact with hands, and the NBP cuff and chest seat belt related to direct contact with the patient. At this stage, in addition to the quantitative analysis using the equipment that performs the rapid test of the microbiological population found on the surfaces from the use of swabs, a qualitative analysis was performed, with the performance of laboratory microbiological analysis. For the microbiological analysis, samples were collected from the surfaces using swabs with culture medium and sent to a clinical analysis laboratory, which used the VITEK® system (bioMérieux, France) for analysis. In them, identifications and antibiograms of gram-negative and positive microorganisms and yeast cells were performed. Identifications were performed through cultures in specific media and automated identification. The antibiogram was determined with the automated minimum inhibitory concentration. The characterization of the studied sample was carried out through a descriptive analysis of the information contained in an Excel® spreadsheet, using an auto filter to identify and correct discrepancies, with mean, median, standard deviation, maximum and minimum values. The software used in the analysis was Statistical Packages for the Social Sciences (IBM SPSS

version 23) and statistical significance was considered with a p-value < 0.05 . To verify the normality of the data, the Kolmogorov-Smirnov test was used. The data set of the first stage of the research was characterized by non-normality, using non-parametric techniques with the Wilcoxon and Mann-Whitney tests. In the second stage, in addition to the data characterized by non-normality, normally distributed data were observed, implying the application of parametric techniques, such as the paired T student test, for data analysis. Ethical aspects were respected, following the guidelines expressed in Resolution 466/12 of the National Health Council, which presents the ethical standards for conducting research with human beings and was approved by the Research Ethics Committee (CEP) of the Faculdade Ciências Médicas of Minas Gerais, under CAAE: 48361321.8.0000.5134 and opinion 4,831,047 (BRASIL, 2012).

RESULTS

The first stage sample consisted of one hundred and three (103) cases. It had the same amount in the pre-flight and post-flight, 44 cases. The remaining fifteen (15) cases were recorded 45 minutes after the disinfection of the aircraft and equipment. The URL count of the analyzed surfaces showed that in the collected points: 'Teto' and 'Chão' are discrepant points of the analysis, where the first presents the lowest values for the analyzed variables and the second, almost always, presents the highest values. Possibly indicating the 'Teto' as one of the places with the lowest amounts of ATP and the 'Ground' as one of the places with the highest amount of ATP on the aircraft. The general variation between the maximum, minimum, and median values of the surfaces was represented in Table 1.

Table 1. Quantitative description of Relative Light Units (URL) by surface from September to November 2017

	Roof	Floor	stick	monitor display	stretcher handle	middle of the stretcher	pressure cuff	Chest seat belt	Aircraft side wall	Doorknob
Minimum	5	53	106	59	65	10	12	4	8	33
Maximum	432	26,606	27,330	2,339	8,104	4,289	7,440	6,954	1,053	12,060
Average	96.4	4,143.3	2,766.2	542.2	1,819.8	455.5	896.1	528.7	172.6	1,523.2
median	67	2,306	1,400	462	1,344	298	499	259	97	843
Standard deviation	91.7	4,782.2	3,907.2	431.1	1,706.6	645.9	1,261.2	900.6	200.2	1,990.9
no	103	103	103	103	103	103	103	103	103	103

Table 1. Normality Test

Surface	Statistic	p-value
Roof	0.179	0.000
Floor	0.178	0.000
stick	0.244	0.000
monitor display	0.191	0.000
stretcher handle	0.138	0.000
middle of the stretcher	0.245	0.000
pressure cuff	0.249	0.000
Chest seat belt	0.270	0.000
Aircraft side wall	0.204	0.000
Doorknob	0.217	0.000

Table 3. Comparison of the amount of Relative Light Units (URL) per surface between the pre-and post-flight phases

Surface	Statistic	p-value
Roof	-0.694	0.488
Floor	-4,715	0.000
stick	-4,295	0.000
monitor display	-3,198	0.001
stretcher handle	-4,248	0.000
middle of the stretcher	-1,179	0.239
pressure cuff	-2,883	0.004
Chest seat belt	-3,250	0.001
Aircraft side wall	-0.251	0.802
Doorknob	-5,298	0.000

The comparison between the pre-and post-flight moments showed that 'Ceiling', 'Middle of the stretcher', and 'Aircraft side wall' showed no difference in the amount of ATP units after transporting the sick (p-value > 0.05). The other surfaces analyzed were statistically

significant (p-value < 0.05), indicating that the amount of ATP units after transporting the patient was greater than before transport. The results of this comparison are shown in Table 3 below. **Willcoxon test:** The interest was to evaluate how much the medium can contribute to the generation of ATP units in the aircraft until the next mission. To this end, the differences between the amounts of RLU found on the surfaces of the samples and the post-disinfection and pre-flight records of the subsequent mission were analyzed. In these analyses, it was observed that the 'Ground' and the 'Thorax Seat Belt' were significantly affected by the environment, with differences between their results (p-value < 0.05). Table 4 presents the results by surface.

Mann-Whitney Test: The second stage was extended to transport 08 patients, in which samples were collected from six selected surfaces in the pre-flight, post-flight, and 45 minutes after disinfection. The quantitative results in RLU were similar to those presented in the first stage, with emphasis on the averages of 'Chão' which had the highest value (2,944.67), and 'Teto' the lowest value (106.96). The general variation between the maximum and minimum values was 239 in the ceiling and 16,564 in the stretcher handle. The largest standard deviations were for the surfaces 'Ground' and 'Manche', 2,440.84 and 1,405.00 respectively, indicating the large variation of ATP units on these two surfaces. Most data sets were characterized by non-normality, implying non-parametric techniques for data analysis, except for the "Ceiling" and "Manche" surfaces for which the data were normal (p-value > 0.05) using the technique parametric for analysis. The ground surface presented a p-value < 0.05 at the pre-flight moment and a p-value > 0.05 after the flight, prevailing the use of the non-parametric test since one of the variables at a given moment did not obtain normal behavior (Table 5).

For the comparison of pre-flight and post-disinfection times, two sets of data were adjusted to normality, p-value > 0.05 , which are the surfaces: ceiling and stretcher handle. The data set for the other surfaces were characterized by being non-normal < 0.05 (Table 6).

The comparison of the pre-and post-flight phases showed that, among the non-parametric variables, only the 'Land of the stretcher' surface presented a statistically significant median amount of ATP units at the end of the transport of the patients (Table 7). The parametric surfaces ceiling and joystick did not present results with amounts of URL with statistical significance (Table 8)

Table 4. Comparison of the amount of RLU between the post-disinfection and pre-flight phases of the subsequent mission

Surface	Statistic	p-value
Roof	238.5	0.111
Floor	178.0	0.008*
stick	297.0	0.566
monitor display	279.0	0.375
stretcher handle	262.0	0.237
middle of the stretcher	313.0	0.767
pressure cuff	308.0	0.702
Chest seat belt	205.0	0.030*
Aircraft side wall	290.0	0.486
Doorknob	300.0	0.602

Table 5. Normality test for the pre-flight and post-flight phases

Surface	time compared	Statistic	p-value
Roof	pre- flight	0.192	0.200*
	Post-flight	0.143	0.200*
Floor	pre- flight	0.295	0.039
	Post-flight	0.170	0.200
stick	pre- flight	0.283	0.059*
	Post-flight	0.184	0.200*
Stretcher handle	pre- flight	0.267	0.096
	Post-flight	0.381	0.001
NIBP cuff	pre- flight	0.151	0.200
	Post-flight	0.295	0.039
Seat belt	pre- flight	0.328	0.012
	Post-flight	0.314	0.019

Table 6. Normality test in the pre-flight and post-disinfection moments

Surface	time compared	Statistic	p-value
Roof	pre- flight	0.192	0.200*
	post disinfection	0.238	0.200*
Floor	pre- flight	0.295	0.039
	post disinfection	0.324	0.014
stick	pre- flight	0.283	0.059
	post disinfection	0.301	0.031
Stretcher handle	pre- flight	0.267	0.096*
	post disinfection	0.240	0.197*
NIBP cuff	pre- flight	0.151	0.200
	post disinfection	0.346	0.005
Seat belt	pre- flight	0.328	0.012
	post disinfection	0.332	0.010

Table 7. Comparison of pre-phases of the after flight

Surface	Statistic	p-value
Floor	-0.980	0.164
stretcher handle	-1,820	0.034*
NIBP cuff	0.140	0.556
stretcher chest seat belt	-0.560	0.288

Paired t-test: Again, it was of interest to assess how much the medium can generate from ATP units on the aircraft until the next mission. For this, the difference in the amount of RLU recorded on the 6 surfaces between the post-disinfection and pre-flight moments was analyzed. Tables 9 and 10 show that no statistical significance was found on any of the surfaces studied.

Culture results from surface samples: The sample consisted of 144 cultures from swabs collected in 8 aeromedical missions, in the pre-flight, post-flight and post-disinfection moments, on the 6 surfaces of the aircraft and previously selected equipment: ceiling, floor, joystick,

stretcher handle, NBP clamp, and chest seat belt, showing the growth of microorganisms in 12 (8.3%) samples, as specified in tables 11, 12 and 13. The microorganisms were identified in 4 aeromedical missions and only the stick was not affected.

Table 8. Comparison of pre-and post-flight moments of parametric surfaces

Surface	Statistic	p-value
Roof	1,339	0.888
stick	-1,317	0.115

Table 9. Wilcoxon test moments after disinfection and pre-flight

Surface	Statistic	p-value
Floor	2,240	0.987
stick	1,960	0.975
NIBP cuff	-0.104	0.444
stretcher chest seat belt	-0.560	0.712

Table 10. Paired t-test at post-disinfection and pre-flight moments

Surface	Statistic	p-value
Roof	3,760	0.996
stretcher handle	1,311	0.884

Table 11. Microbial growth in the pre-flight, post-flight, and post-disinfection moments

Moments	No	%
pre- flight	3	23
Post-flight	8	62
post disinfection	two	15
Total	13	100

The sample was relative to the missions that comprised two pediatric patients and six adults. Of this sample, seven were transported on the first day of admission and one of them 2 months after admission, and the requests came from the hospitals of origin. The variation in the time spent on patient care by the aeromedical transport team ranged from 140 to 307 minutes, with a mean of 162 minutes. Microbial growth was observed, with an average time of aeromedical transport of 192 minutes.

Table 12. Frequency of microbial growth on aircraft and equipment surfaces

Surface	Moments	GROWTH YES		GROWTH NO			
		N	%	N	%	N	%
Roof	Pre-flight	1	0,69	7	4,86	8	5,55
	Post Flight	0	0	8	5,55	8	5,55
	Post disinfection	0	0	8	5,55	8	5,55
Floor	Pre-flight	1	0,69	7	4,86	8	5,55
	Post Flight	2	1,38	6	4,16	8	5,55
	Post disinfection	2	1,38	6	4,16	8	5,55
Stick	Pre-flight	0	0	8	5,55	8	5,55
	Post Flight	0	0	8	5,55	8	5,55
	Post disinfection	0	0	8	5,55	8	5,55
Stretcher handle	Pre-flight	0	0	8	5,55	8	5,55
	Post Flight	2	1,38	6	4,16	8	5,55
	Post disinfection	0	0	8	5,55	8	5,55
PNI	Pre-flight	0	0	8	5,55	8	5,55
	Post Flight	2	1,38	6	4,16	8	5,55
	Post disinfection	0	0	8	5,55	8	5,55
Seat belt	Pre-flight	1	0,69	7	4,86	8	5,55
	Post Flight	1	0,69	7	4,86	8	5,55
	Post disinfection	1	0,69	7	4,86	8	5,55
TOTAL		13	9	131	91	100	100

DISCUSSION

In the first stage of this investigation, the highest average amounts of RLU were identified on the floor, stretcher handle, joystick and door handle. Being that, the last three were constantly touched by the hands of the crew and the medical team. The English author corroborates the findings and describes that in healthcare, hands are the main forms of propagation and contamination. Therefore, he considers proper hand washing as one of the main ways to prevent the spread of diseases.

Table 13. Characterization of bacterial growth on aircraft and equipment surfaces

	Surfaces	Moments	Microorganisms	Profile
MISSION 1	Roof	Pre-flight	Staphylococcus Haemolyticus	Intermediate to fusidic acid. Resistant to: Benzylpenicillin, Erythromycin, Rifampicin
	Floor	After flight	Bastonete Gram +	Not identified
		Post disinfection	Bastonete Gram +	Not identified
	Stretcher handle	After flight	Staphylococcus Epidermidis	Resistant to: Benzylpenicillin, Erythromycin, Gentamicin
	Belt	Pre-flight	Staphylococcus Epidermidis	Multi sensitive
	Stretcher handle	After flight	Staphylococcus Hominis	Resistant to: Benzylpenicillin, Erythromycin, Oxacillin
MISSION 2	NIBP cuff	After flight	Staphylococcus Epidermidis	Resistant to: Benzylpenicillin
	Seat belt	After flight	Staphylococcus Hominis	Resistant to: Fusidic Acid, Benzylpenicillin, Ciprofloxacin, Erythromycin, Linezolid, Moxifloxacin, Norfloxacin, Oxacillin, Rifampicin, Sulfamethoxazole/Trimethoprim, Teicoplanin, Vancomycin
	Floor	Pre-flight	Staphylococcus Epidermidis	Resistant to: Benzylpenicillin, Erythromycin
After flight		Bastonete Gram +	Not identified	
MISSION 3	NIBP cuff	After flight	Staphylococcus Epidermidis	Resistant to: Benzylpenicillin, Erythromycin
	Seat belt	Post disinfection	Staphylococcus Capitis	Intermediate to Erythromycin Resistant to: Benzylpenicillin
MISSION 5	NIBP cuff	After flight	Staphylococcus Haemolyticus	Fusidic acid intermediate Resistant to: Benzylpenicillin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Moxifloxacin, Norfloxacin, Oxacillin, sulfamethoxazole+trimethoprim, cefoxitin screening test

The variation in the amount of RLU on the floor, joystick, and handle of the stretcher, confirmed by the high values of their standard deviations, made it possible to hypothesize that at some point in the analyzed times, differences in the amount of RLU identified would be presented. After the statistical analysis, it can be seen that only the stretcher handle showed a significant difference between pre and post-flight, indicating that the amount of dirt at the end of the missions was greater. The evaluation of samples directly related to the body surface of the patients identified the highest amounts of RLU in the NIBP cuff and in the portion of the seat belt that is at the level of the patient's chest. NBP cuffs are usually in direct contact with the skin, exposed to scaling and moisture from patients' body fluids. The seat belt, on the other hand, has its contact with the skin limited by clothing, but the fabric consists of a weft of interlaced threads that can retain dirt. Although both are handled by the hands of the medical team, they did not show statistical significance when comparing the amount of RLU in the pre-and post-flight moments. The roof, center of the stretcher, and the side wall of the aircraft where the base of the stretcher is attached practically did not show any increase in the dirt at the end of the aeromedical missions, which can be considered non-critical points of the aircraft. In the evaluation of the samples at the post-disinfection and pre-flight moments, only the ground and *seat belts showed an increase in the amount of RLU with statistical significance.*

This finding allows the hypothesis that the manipulation of surfaces by professionals involved in the preparation of aeromedical missions has contributed to this result. Furthermore, the proliferation of microorganisms while the new mission was awaited, as the presence of dirt, especially organic matter, can serve as a substrate for the proliferation of microorganisms¹⁰. It is verified that the cutoff of RLU is not established to determine the cleanliness of the surfaces and the correlation between ATP levels, as well as, it is uncertain about microbial contamination^{4,7}. On the other hand, some studies considered clean when the ATP index was <5 RLU/cm², collected from an area of 100 cm², that is, <500 RLU/surface^{13,14}. The analyzes of this study, after cleaning and disinfecting the surfaces, showed a median ranging between 49 and 1018 RLUs. It is considered that differences in fabrics, surface coatings, chemicals used, and cleaning and disinfection techniques can influence the results of ATP counting using the bioluminescence method^{4,5,11}. Therefore, the results may have been influenced by the high amounts of RLU evidenced before cleaning and the use of a product that has did ecyldimethylammonium chloride (0.30%) in its composition.

Another point of discussion is the stability of ATP. The study demonstrated that, in the absence of cleaning and disinfection, ATP residues from both organic matter and living or dead microorganisms do not deteriorate rapidly. After 29 days, surfaces contaminated with the suspension of *P. aeruginosa*, *E. faecalis*, and *C. Albicans* maintained, respectively, 65%, 69%, and 96% of the ATP level originally present in the solution. Surfaces soiled with blood showed 100% and 8% of their original ATP after 4 and 29 days, respectively¹⁵. So, if the dirt removal method fails, ATP can remain stable for more than 24 h on environmental surfaces; the microorganisms, however, are killed by the action of the disinfectant⁴. This study showed that 62% of the microbial growth was concentrated in the post-flight phase, coinciding with the time when the surfaces were most dirty. On the other hand, microbial growth occurred in only 8.3% of the samples, and in several of them, high amounts of RLU were observed. Consequently, this result was contrary to the use of the luminometer to identify microorganisms, but it allows us to infer that there is quality in the aircraft disinfection process, given the low frequency of identification of microorganisms. After analyzing the investigated surfaces, only the stick showed no microbial growth, a surface that is highly touched by the pilots' hands and presented high amounts of URL, a finding that corroborates the result that some studies showed low sensitivity of Clean trace for microbial identification^{5,11,12}.

The floor was the surface that showed the highest frequency of microbial growth, being identified as gram + rods and *Staphylococcus epidermidis* in two aeromedical missions, distributed in pre-flight, post-flight, and post-disinfection moments. The stretcher handle, which is a surface that is highly manipulated by the hands, presented high amounts of RLU, especially in the post-flight moment. As well, two missions were affected by bacterial growth found on the skin, *Staphylococcus epidermidis* and *Staphylococcus hominis*. Interestingly, the few samples that showed microbial growth were collected in the first missions (1, 2, 3, and 5), a situation that reinforces the quality of surface disinfection, however, it allows us to question whether there was no measurement bias. Therefore, the cleaning and disinfection process may have been optimized during the research, since those responsible for the procedure were aware of the development of the study. Furthermore, the hypothesis of interference related to environmental temperature in the low bacterial proliferation cannot be ruled out, since the samples for culture were carried out in autumn, the season with milder temperatures. It is known that, in general, gram-positive microorganisms present optimal growth around 30 to 37 degrees centigrade. Another point to be considered is

the characteristic of transport, as most patients (87%) stayed for a short time in the hospital of origin, being transported on the first day of hospitalization, and had a short time of contact (average of 162 minutes) with the aircraft and equipment, a condition that can minimize the spread of multidrug-resistant microorganisms. The transport of the patient with a longer hospital stay was the only one with isolation of microorganisms with a broader antimicrobial resistance profile, where a *Staphylococcus* was identified as *haemolyticus*, *intermediate* to fusidic acid and resistant to the respective antibiotics: Benzipenicillin, ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Moxifloxacin, Norfloxacin, Oxacillin, sulfamethoxazole+trimethoprim, and cefoxitin. It is known that hospital stay increases the risk of cross-infection and the relation to colonization by resistant microorganisms, its implication in the development of nosocomial infections is high. The presence of dirt, mainly organic matter, can contribute to the proliferation of microorganisms¹⁰, however, the findings of this study showed that the measurement by ATP units may not properly correlate with the presence of dirt or the presence of dirt does not necessarily contribute to the proliferation of microorganisms.

This study showed high amounts of RLU in several samples of surfaces with no microbial growth, corroborating studies that described that the relationship between RLU and colony-forming units is not linear^{11,12}. It is considered that the modest sample and study design did not allow for establishing a relationship between the results obtained by quantitative monitoring of ATP expressed by RLU and microbiological tests. Although the surfaces sampled by ATP-bioluminescence swab and swab for culture before the flight, after the flight, and after disinfection was adjacent, it is possible that different soiling levels could have occurred on different areas of the same surface. The quantitative monitoring of ATP captures microbial organic matter or not, as well as whether the solution routinely used in the disinfection process of aircraft and equipment has didecyltrimethylammonium chloride in its formulation and this compound that can interfere with the results of measurements expressed in RLU. Finally, although there are limited data on clinically relevant cut-off values for reducing the transmission of microorganisms, cut-off values based on hospital studies were used, which may not be suitable for the aeromedical environment.

CONCLUSION

It is concluded that this study showed that the disinfection process of aircraft and equipment intended for the inter-hospital transport of critical patients was effective, given the small percentage of identification of microorganisms on the surfaces analyzed. To this end, the monitoring of ATP bioluminescence showed no association with microbial growth on aircraft and equipment used at times that permeated the transport. Therefore, the importance of preventing cross-transmission of healthcare-associated infections in the aeromedical environment is undeniable. However, additional investigations involving environmental aspects, inputs, and surfaces are necessary to better deepen the topic of cleaning and disinfection, in line with the use of adenosine triphosphate as a marker of the efficiency of aircraft disinfection and microbiological analysis.

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