Chapter 285

Identification of microorganisms present in an antineoplastic manipulation environment in a tertiary hospital in Brasília

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ABSTRACT
There is a growing concern about good practices for compounding sterile drugs in hospitals, due to the compounding room itself and the specific public that will use these products. The identification of the cleanroom microbiota in the Antineoplastics Compounding Unit (ACU) of a tertiary hospital in Brasília, aimed to assess the quality of the service executed and suggest corrections if necessary. The methodology used was based on the National Health Surveillance Agency resolutions: n° 67/2007 and n° 220/2004, as well as the United States Pharmacopoeia (USP), considering two types of monitoring: environmental and operational. Environmental monitoring was performed with the identification of microorganisms in the air and surfaces, under operating conditions and at rest, using Petri dishes with nutrient agar; and operational monitoring was carried out based on the microbiological assessment of the pharmacist's glove, which was authorized by the Human Ethics Committee of the University of Brasília. In both situations analyzed - in process and at rest there was a microbial count of 6.09 and 1.58 colony-forming units (CFU), respectively. As a result, it was found that the greater the number of people moving within the cleanroom and the number of drugs compounded during the process, the more difficult it is to maintain the conditions required by law. The identified microorganisms were common in the sterile compounding area, due to the presence of the pharmacist and the structural conditions of the cleanroom, hence the constant need for personnel training and adaptation of the structural conditions of the environment to better meet the requirements of the legislation.

Keywords: Microorganisms, Good Manufacturing practices, Antineoplastics, Cleanroom, Contamination.

1 INTRODUCTION

The antineoplastic handling unit of the hospital under study has a daily frequency of handling various antineoplastic drugs whose purpose is to treat cancer patients. Cancer in the juvenile population predominantly has an embryonic origin and generally affects the cells of the hematological system1, the highest incidence of cases in the analyzed hospital were patients with leukemia and lymphoma. However, this hospital also treats patients with primary neoplasms in different regions of the body: central nervous system (neuroblastoma, medulloblastoma, astrocytoma), bones (osteosarcoma), kidneys (nephroblastoma),

etc. As a result, the Antineoplastic Drugs Handling Unit (UMA) is responsible for handling drugs for different oncological protocols, which makes it necessary to maintain a large stock of drugs, of various classes, such as alkylating agents, antimetabolites, platinum compounds, antibiotics, alkaloids, among others.

Microbial contamination of injectable products is one of the most serious problems currently faced in the areas of drug manipulation, whether in hospitals or the pharmaceutical industry2. The biggest challenge when handling sterile drugs in hospitals or clinical pharmacies is to maintain aseptic conditions within the appropriate biosafety levels for administration to immunosuppressed patients3. Injectable drugs bypass several immune defenses associated with the gastrointestinal system. Therefore, to ensure the sterility of these products before patient administration, hospitals and clinics must follow government regulations regarding good handling and quality control practices. Maintaining and following a robust quality control program is an integral part of meeting quality standards and meeting regulatory requirements2.

There are many sources of contamination and the types of microorganisms (OM) can indicate the source of contamination. The microbiota in the environment and the finished pharmaceutical product can also originate from raw materials, including water, equipment, air installations, personnel, ambient air and production processes, and/or from the product's primary packaging4.

The manipulation of non-sterile products does not involve as many rigorous asepsis criteria as the manipulation of parenteral products, whose risks of contamination are higher and their consequences more harmful. This type of manipulation poses significant risks to patients if the requirements of relevant legislation, guidelines, and standards are not strictly followed throughout the manipulation process.

When handling sterile oncological drugs, the professional must comply with certain relevant legislation and guidelines: such as, for example, the PIC/S Guide to Good Manipulation Practices (PE 009)5 and Chapter 797 – Pharmaceutical Manipulation of the American Pharmacopoeia6, 7. regulations and guidelines guide the pharmaceutical professional regarding definitions related to the handling of sterile products and establish requirements and regulations for the acquisition and quality control of raw materials, storage, handling, fractioning, conservation, transport and dispensing of compounded drugs5-7.

Given the risk related to handling sterile products, this process must be carried out in a classified and appropriate environment to avoid microbial contamination as much as possible, that is, in areas called "clean rooms". The American Pharmacopoeia defines a clean room as: "a room in which the concentration of airborne particles is controlled to meet a certain class of particulate cleanliness, in which microorganisms in the environment are monitored so that the microbial level of the air, surface and personnel does not exceed a certain cleanliness class"7. Industries, hospitals and clinics that manufacture both sterile and non-sterile pharmaceuticals and related products are asked to demonstrate the effectiveness of the practices they employ to minimize the risk of cross-contamination. Particularly important is the monitoring of processes that are intended to obtain sterile products. The purpose of a monitoring program is to control

microorganism and particulate levels within specified limits, air monitoring (active and passive air sampling), surface monitoring (swabs or contact pads) and personnel monitoring (microbiological assessment of the glove)7.

The monitoring program must be prepared by current regulatory guidelines and standards, risk assessment and knowledge of the critical points of the controlled process. Critical points are the places that represent the greatest microbiological risk for the aseptic process. Therefore, samples from the surface and the air must be collected in the places and in the stages of the process where the product is more exposed and the risk is, therefore, greater8.

This work was an observational, qualitative and quantitative study, which aimed to carry out the identification of the microbiota of the clean room in the Antineoplastic Manipulation Unit of a hospital in Brasília.

2 MATERIALS AND METHODS

2.1 STUDY DESIGN

The study was characterized by evaluating the process of handling antineoplastic drugs in the clean area of a tertiary hospital in Brasília, considering the conditions of good practices for handling sterile drugs, with a daily routine of handling in both shifts (morning and afternoon). The clean sterile handling area of the hospital under study has a total of five spaces demarcated according to the purpose of the activity to be performed. Study planning, considering its characteristics, required submission to the Research Ethics Committee of the Faculty of Health Sciences of the University of Brasília (CEP-FS, UnB) (CAAE 12028919.9.0000.0030) (opinion 3.394.511) that gave a favorable opinion for carrying out the tests involving human beings. All procedures adopted follow Resolution 196/96 of the National Health Council, regarding the Term of Free Consent (TLC) applied to the personnel responsible for handling antineoplastic agents in the evaluated unit.

2.2 PLACE OF STUDY AND PERIOD OF ANALYSIS

The environment of the Antineoplastic Therapy Service of the evaluated hospital is composed of 5 places: an administrative room, a room for large-volume solutions and medications, a hygiene room, an antechamber and a manipulation room. UMA has seven employees (two pharmacists, four assistants and an intern) and a monthly average of eight hundred and fifty manipulations. The study was carried out in the antineoplastic drug manipulation room, which is composed of three carts containing the material to be used in the manipulation (syringes, gloves, needles, etc.), a computer, two chairs and the Biological Safety Cabinet type BII (Veco, Biosafe B2). Samples were collected daily on six days of the week, during the manipulation process – in process; And for two days at rest – at rest, that is, without anyone entering the clean room, thus totaling eight collections.

2.3 MONITORING OF THE AREA AND COLLECTION OF SPECIMENS

The methods chosen for monitoring were those cited by the United States Pharmacopoeia and the Brazilian Pharmacopoeia and are based on two types of monitoring: the environment and the handler. The analysis of the environment was carried out based on the identification of microorganisms in the air and on surfaces; and the analysis of the handler was performed based on the microbiological evaluation of the glove. For environmental monitoring, two methods were used: air sedimentation (90x15 mm petri dishes) and surface sampling (swabs); and for operational monitoring, the microbiological evaluation of the glove was used (imprinting of the glove in a petri dish)6, 7, 9.

The swab method was used to monitor the surfaces of the trolleys, the biological chapel, the pass-through and the clean room floor, whose sterile swab was dragged over the analyzed surface, in a range of 24-30cm6, then this swab was dipped in a tube falcon containing 5 mL of sterile Soy Casein Broth (CCS – Kasvi®), then it was evaluated whether or not there was microbial growth. The air sedimentation method consisted of exposing an open petri dish with sterile Soybean Casein Agar medium (ACS – Acumedia®) for four hours4-6 in the environments analyzed during the manipulation process (IN PROCESS) and without any process being carried out in the room (AT REST). Microbiological evaluation of the glove was performed by gently pressing the handler's gloved fingers on the petri dish containing the ACS medium. Table 1 describes the sampling sites and type of monitoring.

It should be clarified that the in-process experiments were carried out on five days of the week. The only day of the week on which the collection was repeated was on Wednesday. This was done to improve data sampling, since on the first Wednesday not all sampling points were reached. During the manipulation process, the medicines are manipulated according to the arrival of the prescriptions. Therefore, the pharmacist can sometimes enter the clean room to handle the products. These variables (entry into the room and amount of manipulated medication) were monitored for all in-process experiments. These data were not collected for the at-rest experiments because in this method no people enter the clean room.

Table 1 - Sampling locations and type of monitoring.

Local	Monitoring	No. of Plates	Swabbing
CAR1	Environmental	3	CTM
CAR2	Environmental	3	CTM
CAR3	Environmental	3	CTM
CSB_DENTRO	Environmental	4	CTM
CSB_TETO	Environmental	2	SCTM
SALA_LIMPA	Environmental	4	SCTM
PT.HIG_SL	Environmental	1	SCTM
PT.SL_EXT	Environmental	1	SCTM

LUVA_ME	Operational	1	-
LUVA_MD	Operational	1	-

CAR1: cart 1; CAR2: cart 2; CAR3: cart 3; CSB_INTERIOR: Interior of the Biological Safety Cabinet; CSB_CEOF: Ceiling of the Biosafety Cabinet; SALA_LIMPA: Floor of the Clean Room; PT.HIG_SL: Pass-Through Hygiene Room – Clean Room; PT.SL_EXT: Pass Through Clean Room – External Area; LUVA_ME: Left Hand Glove; GLOVE_MD: Right Hand Glove; CTM: Contamination; SCTM: No contamination.

2.4 IDENTIFICATION OF MICROORGANISMS

The petri dishes with nutrient medium distributed in the places according to Table 1 were exposed for 4 hours and then incubated at 32.5±2.5°C in an oven (DeLeo laboratory equipment) for 3 to 5 days. From the microbial growth that developed in the culture medium, the initial identification was performed using the Gram staining technique of the colony-forming units that were presented in the sedimentation plates10.

For a more precise identification regarding the genus/species of the microorganism stained by the Gram technique, the Vitek MS system was used. The procedure was performed at the Central Public Health Laboratory of the Federal District (LACEN-DF). Vitek MS is a microbial identification system that uses Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF)11 technology.

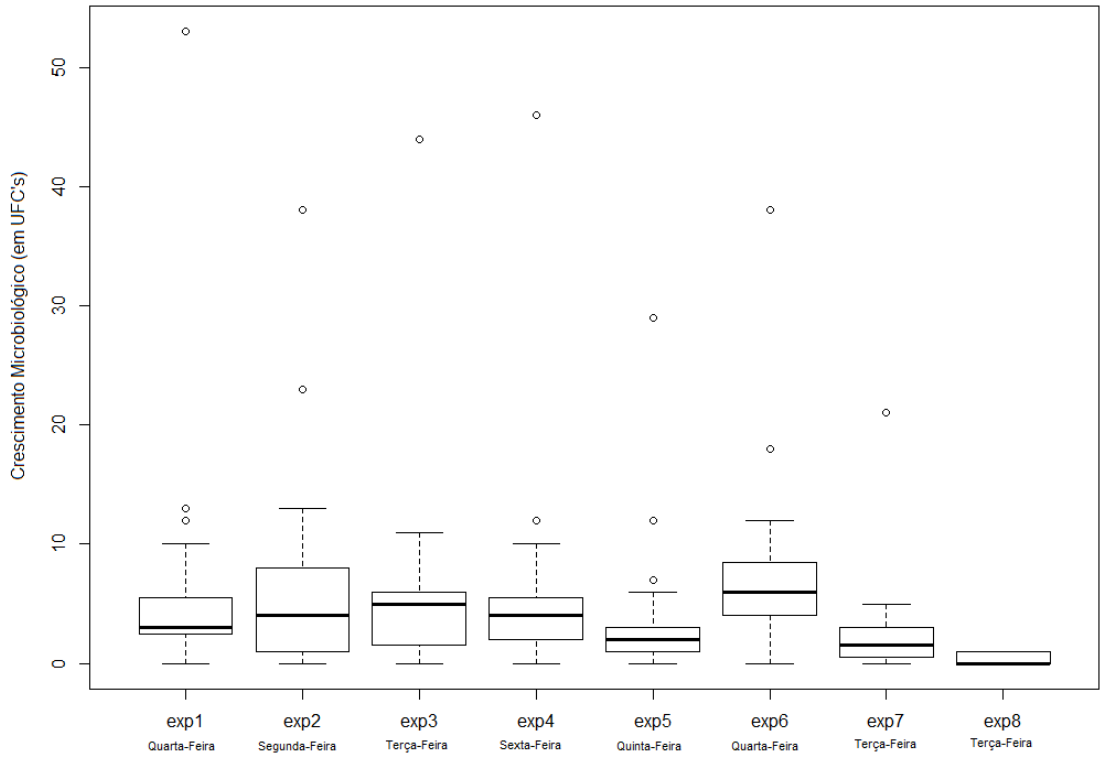
2.5 STATISTICAL ANALYSIS

The collected data were analyzed using the statistical analysis program R version 3.4.3. Numerical variables were presented as measures of central tendency and dispersion, and nominal variables as absolute numbers and proportions.

3 RESULTS AND DISCUSSION

It is possible to observe that of all the experiments, those that took place on Wednesday (experiments 1 and 6) and on Monday (experiment 2) were the experiments in which there was greater microbial contamination, with means of 6.95; 7.31 and 6.73 CFU, respectively. The lowest microbial count was found in the processes at rest: on Tuesday (experiments 7 and 8), with means of 2.75 and 0.47 CFU, respectively, as shown in Figure 1.

Figure 1: Microbiological growth per experiment performed.



The places where the highest rates of microbial growth occurred were in the pass throughs (PT), that is, in the connection between the hygiene room and the clean room (PT.HIG-SL) and between the clean room and the external part for dispensing medicines (PT.SL_EXT); with an average of 11.37 and 33.62 CFU, respectively. It is worth mentioning that the flow of both PTs is unidirectional, that is, materials only enter the clean room at PT.HIG-SL and materials/finished products only leave through PT.SL_EXT. Microbial growth in PTs is because they connect the clean room with places where there is no particle control, whether viable or not. Inside the Biological Safety Cabinet (CSB_INTERIOR), it was the place where there was the lowest CFU growth.

With this result, it is important to clarify that the sterility of a product produced by aseptic techniques is not completely guaranteed due to the numerous sources of contamination that an environment can provide during the production/handling process3. The connection between the environments is an area of greatest vulnerability, so attention must be paid to monitoring pass throughs. Table 2 shows the average microbial growth, grouping the data by location.

Table 2 - Mean microbial growth per location sampled with petri dishes.

Local	**Monitoring**	**Average Microbial Growth (in CFU/g)**
CAR1	Environmental	5,25
CAR2	Environmental	3,21
CAR3	Environmental	3,21
CSB_INTERIOR	Environmental	1,37

CSB_TETO	Environmental	2,94
SALA_LIMPA	Environmental	4,68
PT.HIG_SL	Environmental	11,37
PT.SL_EXT	Environmental	33,62
LUVA_ME	Operational	1,2
LUVA_MD	Operational	0

CAR1: cart 1; CAR2: cart 2; CAR3: cart 3; CSB_INTERIOR: Interior of the Biological Safety Cabinet; CSB_CEOF: Ceiling of the Biosafety Cabinet; SALA_LIMPA: Floor of the Clean Room; PT.HIG_SL: Pass Through Hygiene Room – Clean Room; PT.SL_EXT: Pass Through Clean Room – External Area; LUVA_ME: Left Hand Glove; LUVA_MD: Right Hand Glove.

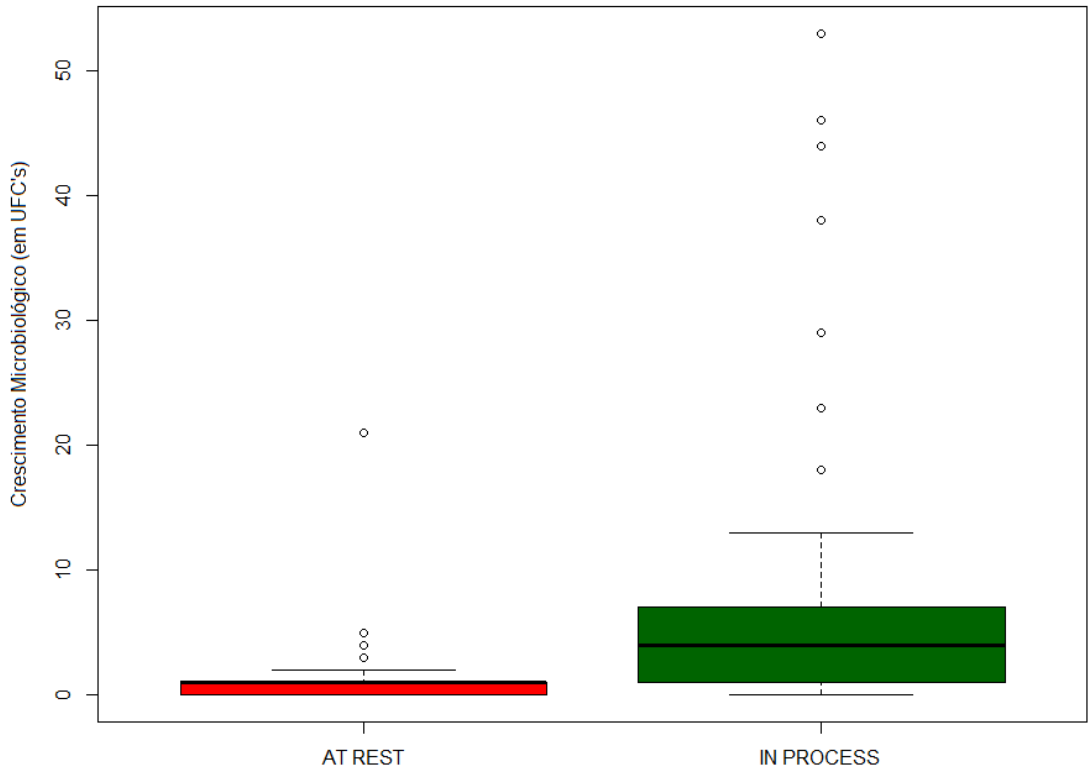
The condition of an aseptic environment in the work area of CSB class II, type B2, for handling sterile antineoplastic drugs must be certified, that is, the supply air must guarantee, in operation, an air cleanliness classification as ISO class 5, according to ISO 14644-112 for particles of size equal to or greater than 0.5 µm and less than or equal to 5 µm. According to the last validation carried out, before this analysis, the Biological Safety Cabinet present in the UMA was classified as ISO 5. The place where the CSB II, B2, indicated for handling antineoplastic drugs, must have an ISO class air classification 7 and negative differential pressure about adjacent environments, as recommended by RDC 67/0713 and the Food and Drug Administration14. The UMA cleanroom was not classified for the presence of particles.

In a study published by Costa in 2014, the sanitary framework of health services in Brazil, especially the hospital sector, shows deficiencies mainly in the context of the inadequacy of the physical structure, processes and results, presenting a percentage of 55% of adequacy in the evaluated period from 2004 to 2006. For the period from August 2008 to July 2009, 31.7% of the 186 evaluated health services presented several sanitary irregularities that compromised the quality of the services, highlighting again the hospital service, being these related to the physical structure, product quality, documentation, human resources, preventive maintenance and calibration of equipment, and storage of health waste15.

As shown in Table 2, we see that the study revealed a high rate of contamination in all the analyzed locations, mainly in the critical points, that is, those points where there is a greater risk of contamination8: places where there is a greater movement of people or places where there is a direct flow of air conditioning, for example, the carts that have the materials used for handling. The Biological Safety Cabinet was classified as ISO 5 or Grade A, according to the recommended contamination limits present in the guide for good handling practices of the Pharmaceutical Inspection Convention5, and therefore it must not have any type of growth by the sedimentation methods of the air in petri dishes. Also according to PIC (2018), in operational monitoring, the gloves should also not have shown the levels of contamination shown in this study (>1 CFU). RDC 220/0416 stipulates that during the handling process, two pairs of sterile gloves must be used, changed every hour or whenever their integrity is compromised. This routine is occasionally not followed by the handlers, which may have contributed to the occurrence of contamination in the gloves.

Microbiological growth was also compared between processes – at rest and in process. The mean microbiological growth of the in process experiments was 6.09 CFU and that of the at rest experiments was 1.58 CFU. In Figure 2, these results are compared in a box plot.

Figure 2: Box diagram comparing the processes analyzed within the clean area.

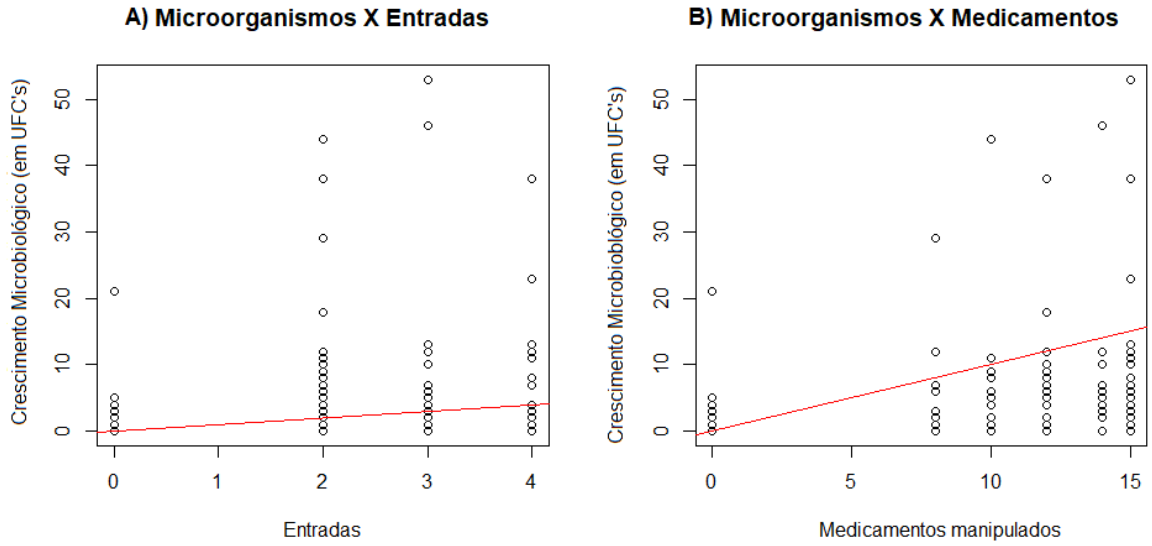


Contamination in a cleanroom can originate from many different sources, but the biggest source of contamination in a pharmaceutical facility can be traced back to people working in the cleanroom17. This is evidenced from the association of microorganisms present on the skin, being the main isolates from environmental monitoring in controlled environments18. The human body loses a large number of skin cells and the cleanroom suits worn by staff cannot contain all human waste.

Surface sampling (swabbing) was performed on Monday (experiment 2 – in process) and on Tuesday (experiment 7 – at rest). In the in process experiment, only two sites showed microbial growth: CAR1 and CAR3. In the at-rest experiment, there was no microbial growth using this method.

Figure 3 shows the relationship between microbiological growth in CFU/g with the entry of people into the clean room (a) and the amount of manipulated drugs (b). Pearson's correlation coefficient between microbiological growth with inputs is 0.948 and microbiological growth and compounded drugs is 0.921 ($p < 0.05$). According to Figure 3 and Pearson's correlation statistics, for both variables, there is a very strong positive correlation with microbiological growth, that is, the greater the entry of people, or the number of manipulations, the greater the growth. microbiological.

Figure 3: Relationship between microbiological growth in CFU with the entrance of people (a) and with the amount of manipulated drugs (b).



Therefore, according to a published study correlated with the qualification and classification of clean areas, the initial qualification should include an assessment of the air quality as-built, static conditions, and dynamic conditions. Thus, the qualification of the area needs to place greater emphasis on data generated under dynamic conditions, that is, with people present, equipment in place and operations in progress. The facility needs to be properly maintained, monitored and used for its intended purpose, as environmental monitoring may be able to identify potential routes of contamination, and it is then possible to implement corrections19.

Table 3 - Classification of microorganisms identified by Gram staining technique.

Morphological classification of microorganisms	**Gram stain**	**Amount**
Coccus	Positive	50%
Coccus	Negative	1%
Bacillus	Positive	27%
Bacillus	Negative	19%

According to Table 3, the findings data are similar to those found in the literature: the most common microorganisms in clean rooms are Gram positive bacteria20. And these usually have a close phylogenetic affiliation, as indicated by the comparative analysis of partial 16S rDNA studies, such as between Micrococci and Staphylococci21, both microorganisms identified in this study.

Table 4 - Identification of microorganisms by the Vitek MS system in the in process situation.

Identified microorganisms	**Collection location**

Staphylococcus hominis	Clean room
Paenibacillus lautus	Clean room
Bacillus pumilus	CSB
Micrococcus luteus	CSB
Brevibacillus	CAR1, 2, 3

The microorganisms identified in the analyzed sites were those shown in Table 4, using the mass spectrometry technique using the Vitek MS system.

The Staphylococcus hominis identified in the clean room are Gram positive cocci, they usually occur as harmless, non-pathological commensals on human skin in immunocompetent persons. However, it is an opportunistic pathogen capable of causing a wide variety of diseases, including bacteremia, septicemia and endocarditis, especially in immunocompromised patients22. This microorganism has a subspecies that is resistant to antibiotics: Staphylococcus hominis subsp. Novobiosepticus, is especially dangerous for cancer patients, causing them septicemia23. Bacillus pumilus is a Gram positive, aerobic, non-pathogenic bacillus commonly found in soil. It is a spore-forming bacterium, exhibiting an abnormally high persistence in bactericidal environments. In its dormant state, it is capable of withstanding doses of ultraviolet radiation (UV) or hydrogen peroxide, which are lethal for the vast majority of microorganisms24. And for this reason, it is used as a biological indicator in the sterilization process by ionizing radiation9.

The species Paenibacillus lautus is characterized as aerobic or facultatively anaerobic, formed by Gram positive rods or Gram variable endospores. Paenibacillus species have been isolated from a variety of sources including soil, fresh and salt water25. The species Paenibacillus lautus has cellulase activity26, which may be useful in the textile and food industry27. Micrococcus luteus is a Gram positive (and Gram variable) coccus found in soil, dust, water and transiently on human skin. It is commonly used for the detection of antimicrobial compounds and may be associated with the occurrence of infections such as abscesses, pneumonia, septic arthritis, meningitis, bacteremia and septic shock in immunocompromised patients28. Some studies show that the types of microorganisms recovered from clean rooms are very similar and are usually the following: Staphylococcus hominis, Staphylococcus epidermidis, Micrococcus spp., Bacillus spp. and yeast18,29. This information is similar to that found in this work. Most of the microorganisms identified in this work are environmental contaminants, however, one should consider the type of patient treated by the studied institution: patients with cancer, that is, patients with compromised immune systems. And so, even though these microorganisms are classified as environmental contaminants, they can behave as opportunistic pathogens, which can cause serious infections in immunocompromised patients30.

A study carried out to verify air contaminants in the operating room in a hospital environment showed the presence of several microorganisms, including: Staphylococcus hominis and Micrococcus

luteus, which were also found in our study31. This reinforces the argument regarding the need to carry out daily and frequent monitoring of hospital clean areas, reinforcing the training of the team that performs activities within these environments. Despite being a clean area for handling sterile medications or performing surgeries, these microorganisms are common in hospital environments and can cause harm to immunocompromised patients.

As seen in the results of this study, one of the biggest contamination problems in clean rooms is the movement of people. To minimize the risks of contamination related to people, some precautions must be taken. Staff must be in appropriate clothing, which must be low shedding of particles, allow the body to breathe while trapping particles within the clothing, be flexible enough for comfortable wearing, and withstand repeated cleaning and sterilization cycles. In addition to adequate clothing, constant training should take place to guide employees in topics such as: microorganisms and microbiological contamination control, entry and exit from handling facilities, personal hygiene training and microbiological risks associated with specific production tasks5.

There was a significant amount of contamination in all monitored areas. However, there are two very important factors to be mentioned in this study: first, the manipulated drugs are never exposed to air, even inside the cabin. Injectable drugs presented in vials have a rubber cap that allows proper sealing with the aid of an aluminum seal, so the prescribed dose is drawn through the syringe and released directly into the bag, where the equipment will be connected to administering the final product to the patient; which reduces the risk of contamination considerably. And second, the Infection Control Service (SCI) investigates all infections that affect hospital patients, and no case of infection has ever had any UMA process as a cause, until the completion of this study.

4 CONCLUSIONS

Based on the results obtained both in the environmental and operational spheres, it was possible to verify the swift need for adjustments both in the area of handling antineoplastic products and about the training of employees who actively participate in the process at the hospital unit under study. Considering the requirements of the legislation in force, RDC n° 67/2007 and RDC n° 220/200413,16, as well as the other guidelines available in the American and Brazilian Pharmacopoeia6,7,9, it is necessary to ensure strict control regarding the count of viable and non-viable particles to maintain clean area classification within the biological safety cabinet and surrounding areas.

It is important to pay attention to the situation of the unit under study, where oncological drugs are handled for immunocompromised patients. The microbial identification results bring an alert to the situation of most hospital units, with a large volume of manipulations and an excessive daily frequency, which often overload the responsible personnel and leave room for relevant contaminations that may compromise the state of health. patients' health.

There must be an update of the legislation in force so that situations that may pose risks to hospitalized patients who are already in a vulnerable situation are minimized, with stricter and more frequent control by the supervisory bodies so that these areas of manipulation can adapt to environmental and occupational conditions.

Our study justifies the need for changes in the structure of the analyzed location. In this way, the sector will need to undergo reforms in the clean room and surrounding areas to adapt to the requirements present in current legislation and guidelines, both national and international. It will certainly be necessary to continue further evaluation studies to verify possible improvements in asepsis conditions and training of handlers.

INTEREST CONFLICTS

There are no conflicts of interest involved in this research.

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