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QUALITY OF INDOOR ENVIRONMENT OF HOSPITAL DIAGNOSTIC IMAGING SERVICES: LOW DOSES RADIATION EXPOSURE

QUALIDADE DO AMBIENTE "INDOOR" DE SERVIÇOS DE DIAGNÓSTICO POR IMAGENS EM HOSPITAIS: EXPOSIÇÃO À BAIXAS DOSES DE RADIAÇÃO

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ABSTRACT

Estimates of radiation risks at low doses and low dose rates are important, for example for populations living in contaminated areas after nuclear accidents or in high natural background radiation areas, or for radiation protection of individuals occupationally exposed to ionizing radiation This work aimed to study the indoor environment regarding on Radiological Safety and on microbiological contamination due airborne fungi, by monitoring the potential mutagenic effects caused by possible low doses of radiation exposure. As a bioindicator of mutagenicity it was chosen the micronuclei production by *Tradescantia pallida*, one of the most sensitive organisms for the detection of mutagens in the air; the fungi contamination research was performed by spontaneous deposition in plate. We monitored five Hospitals image services in the Sorocaba city, state of São Paulo, Brazil, two of them are public services and the other three, are private ones. The results pointed mutagenic activity in the images rooms in the two public hospitals, but not in the three private image services. It was found spores, mainly of the genres *Aspergillus, Penicillium, Cladosporium* and *Fusarium*, in all rooms, indicating risk of opportunistic infections, especially related to the presence of *Penicilium sp* and *Fusarium sp* genres. The *T. pallida* bioassay represents a adjuvant tool in monitoring these indoor environments and showed that there is a mutagenic activity, despite being controlled environments. Therefore, even in these controlled environments the risks related to exposure to radiation are still imponderable.

Keyword: Ionizing radiation. *Tradescantia pallida.* Mutagenesis. Anemophilus fungi.

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RESUMO

Estimativas de riscos de radiação em baixas doses e baixas taxas de dose são importantes, por exemplo, para populações que vivem em áreas contaminadas após acidentes nucleares ou em áreas de alta radiação natural de fundo, ou para proteção de radiação de indivíduos ocupacionalmente expostos à radiação ionizante. Este trabalho teve por objetivo estudar o ambiente interno no que diz respeito à Segurança Radiológica e à contaminação microbiológica por fungos aerotransportados, monitorando os potenciais efeitos mutagênicos causados por possíveis baixas doses de exposição à radiação. Como bioindicador de mutagenicidade, escolheu-se a produção de micronúcleos por *Tradescantia pallida*, um dos organismos mais sensíveis para a detecção de mutagênicos no ar; a pesquisa de contaminação por fungos foi realizada por deposição espontânea em placa. Foram monitorados cinco serviços de imagem de Hospitais da cidade de Sorocaba, estado de São Paulo, Brasil, dois deles públicos e os outros três privados. Os resultados apontaram atividade mutagênica nas salas de imagens dos dois hospitais públicos, mas não nos três serviços privados de imagens. Foram encontrados esporos, principalmente dos gêneros *Aspergillus*, *Penicillium*, *Cladosporium* e *Fusarium*, em todas as salas, indicando risco de infecções oportunistas, principalmente relacionadas à presença dos gêneros *Penicilium sp* e *Fusarium sp*. O bioensaio de *T. pallida* representa uma ferramenta coadjuvante no monitoramento desses ambientes internos e mostrou que existe atividade mutagênica, apesar de serem ambientes controlados. Portanto, mesmo nesses ambientes controlados os riscos relacionados à exposição à radiação ainda são imponderáveis.

Palavras-chave: Radiação ionizante. *Tradescantia pallida*. Mutagênese. Fungos Anemófilos.

INTRODUCTION

The main objective of Occupational Toxicology is the prevention of health changes in workers exposed to various risk factors. The presence of potentially toxic substances in the workplace requires that exposure to be systematically evaluated[1]. The emission of pollutants in the air above pre-established concentrations has effects on human health, leading from simple irritation to lung cancer[2,3].

Air pollution is not restricted to visible pollutants in urban air, and the concentration of air pollutants in indoor environments can be a thousand times worse than open areas, due to the location of potential sources of internal issue and the lack of suitable ventilation systems that dilute or disperse pollutants[2]. The indoors pollution is not easily noticed by the population, since this kind of pollution is hardly visible, usually characterized the smoke and particulates or even other sources such as ionizing radiation.

The atomic energy use has grown, leading to additional environmental radiation emissions, generating an increased risk of changes in future generations, induced by radiation. Estimates of radiation risks at low doses and low dose rates are

important, for example for populations living in contaminated areas after nuclear accidents or in high natural background radiation areas, or for radiation protection of individuals occupationally exposed to ionizing radiation[4]. Thus, systems correlation between low dose radiation and mutations often has been developed in order to estimate this risk[5]. Radiation is emission and transfer of energy in the form of waves or particles [6]. The use of radiation in medicine or research is constantly increasing, contributing immensely to occupational environment pollution[7]. Radiation interaction with living tissues results in excessive generation of free radicals termed as reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, singlet oxygen and hydrogen peroxide[8]. ROS can cause detrimental effects on cellular macromolecules leading to cellular dysfunction[9]. Ionizing radiations like X- and gamma rays have akin physical characteristics showing similar biological effects. X-rays have longer wavelength than gamma rays and thus are more penetrating with outspoken cellular effects[10].

The earliest occupational groups exposed to radiation exposure were radiologists and radiologic technologists. It was the observation of earliest radiologists that led to the recognition of radiation-induced skin cancer, the first solid cancer linked to radiation in 1902. In 1940s and 1950s, excess mortality from leukemia in radiologists was recognized. This led to a rising concern about the effect of chronic radiation exposure and led to some landmark studies of radiologists[11]. In A cohort study it was shown that contrary to the belief that high dose rate exposures are substantially more dangerous than low dose rate exposures, the risk per unit of radiation dose for cancer among radiation workers was similar to estimates derived from studies of Japanese atomic bomb survivors. Cancer risks that are associated with protracted radiation exposures can help strengthen the foundation for radiation protection standards[12].

To assess the risk and prevent the presence of potential residual toxic effects of environmental radiation, sensitive biomarkers are used to detect the action of these compounds. Micronucleus test, using *Tradescantia pallida* (TRAD-MN assay) is a widely employee bioassay, which is considered one of the most sensitive and efficient organisms for mutagens detection in the air[13]. Micronucleus (MN) are formed during cell division and the chromosome fragmentation is the responsible for that. The fragmentations are not included in the nuclei of daughter cells, remaining in the cytoplasm of interphase cells after division[14, 15]. Micronuclei formation is related to various environmental deleterious factors[14] and the numerous similarities between the genetic constitution of higher plants and mammals may lead to believe similar effects on a mutagenic DNA of plant and mammal[16,17,18,19,20].

Therefore, the aim of this work was to evaluate the potential risks of the indoor environment of imaging services, related to radiation exposure and microbiological contamination of the staff in five Hospitals in Sorocaba city, State of São Paulo, Brazil.

MATERIALS AND METHODS

STUDY SCENARIO

There was studied Medical Image services rooms of five Hospital of Sorocaba City, in southeast of Brazil. Two hospitals were public and the other three were private hospitals. In the public hospital 1 (H1), the study was carried out in the ***X-ray, Mammography, Computed Tomography (CT) and Densitometry rooms. In the public hospital 2 (H2) the sites studied were Xray, Tomography and Mamography rooms. In the private hospital (H3) the analysis was carried out only um the X-ray and Mamography rooms, in private hospital (H4), the X-ray and Tomography rooms were evaluated and in private hospital (H5) only in the X-ray room was possible realize the test. T***he Research Ethics Committee (COEP-02/2014) of the hospital approved the project.

ASSESSMENT OF MUTAGENICITY

For monitoring the impacts of potential radiation exposure, the bioassay with *Tradescantia pallida* was carried out, by expose three tradescantia vessels in each room. The vessels remained in these sites for two period of 30 days. The mutagenicity test was performed according to Ma (1981) with some adjustments[16, 13].

There were required 15 intact inflorescences for each experimental group. The stems were cut with 10-15cm in length and subsequently placed in beakers with 150 mL of water for 24 hours, for repair time. The negative control was carried out with inflorescences taken from unexposed vessels, the positive control was carried out by exposing the inflorescences to the Trifluralin herbicide (1,68 ppm), which is known as a mutagenic compound[21]. After the plants exposure, the inflorescences were removed and fixed in acetone-ethanol solution (1:3 V:V) for 24 hours and then, they were stored in 70% ethanol until the analyses. The selected button received two drops of 2% acetic carmine, and it was dissected and macerated after that. "All debris" were removed before the cover slip was placed. After observing the presence of cells in tetrad stage, using the optical microscope, the cover slip is set, and a rapid flow is performed in the Bunsen burner in order to that fix the cells to the slide and allow the dye excess evaporation.

The counting of micronucleus was performed at 400X magnification. For each experimental group, 5 slides were prepared and 300 tetrads of each blade were examined. The frequency of the micronucleus was calculated by dividing the total number of micronucleus by the total number of counted tetrads. The value is given in number of micronuclei / 100 tetrads. The mean and standard deviation were calculated for each group and the statistical analysis used was ANOVA one way, followed by non-parametric Tukey posttest.

MICROBIOLOGIC ANALYSIS

For the analysis of air microbiological quality, it was used fungi identification technique in air samples in simple sedimentation. In the analyzes, six Petri dishes containing potato dextrose agar were sampled in each room. The plates were opened and exposed for fifteen minutes. Although simple sedimentation technique does not recover some types of microorganisms present in the air, it is a technique recommended in environments evaluation[22]. The potato dextrose agar plates were incubated for 7 days at room temperature. After the incubation period, macroscopic characteristics of fungi that grew in the sampled plate were observed.

For recovery of fungi, one agar potato tube, cut with a hot platinum loop was placed on a sterilized slide, contained in a sterile Petri dish. The fungi in the four sides of the agar cube were seeded. It was covered with a sterile cover slip. A wet chamber was made with the addition of 1 to 2 mL of sterile distilled water at the bottom of the plate lined with filter paper to prevent desiccation of the culture medium during the growth of fungi[23]. The plate was closed and left at room temperature for 7 days, until to be seen a development of hyphae with or without pigmentation. After this period, the microscopic analysis was performed in the common optical microscope.

For microscopic analysis, it was placed on the slide two drops of lactophenol (blue cotton). It was removed with histological clamp the cover slip that was over the agar microcultivation and put it on the slide containing the dye. The microscopic fungi structures were observed with a magnification of 100x and 400x.

RESULTS AND DISCUSSION

The first solid epidemiological evidence of the stochastic effects of irradiation came from a study of occupational exposure to medical x-rays that was reported in 1944, which demonstrated an increased risk of leukemia among US radiologists, but the general lack of dose records for early medical staff who tended to experience the highest exposures hampers the derivation of risks per unit dose received by medical workers[24]. In recent years there has been a growing awareness of the possible effects of chronic exposure to low dose of ionizing radiation[5, 25]. This is due to the fact that the treatment of cases of trauma is increasingly dependent on imaging diagnosis. Radiographs of the chest and pelvis, as well as computed tomography scans are frequently requested in an emergency for life-threatening assessment, and are routine radiological examinations in trauma services[11].

Although deterministic effects are generally well known, the potential long-term risk associated with ionizing radiation (such as cancer and genetic effects) is usually more difficult to assess[4, 26, 27].

In our study it was demonstrated that the *Tradescantia* micronucleus assay is a good tool adjuvant to the dosimeter for monitoring the effects of chronic exposure to radiation. It was found micronucleus formations as illustrated in the images of Figure 1 F and G. The results of the TRD-MN assay showed that there was

mutagenic activity in exposed plants in the X-ray, Computerized Tomography and Mammography rooms, but not in the Densitometry room in H1 (Figure 1 A). In the H2, mutagenic activity was detected only in the X-ray room (Figure 1 B). In all three private services (H3, H4 e H5) it was not found any micronuclei production (Figure 1C, D and F).

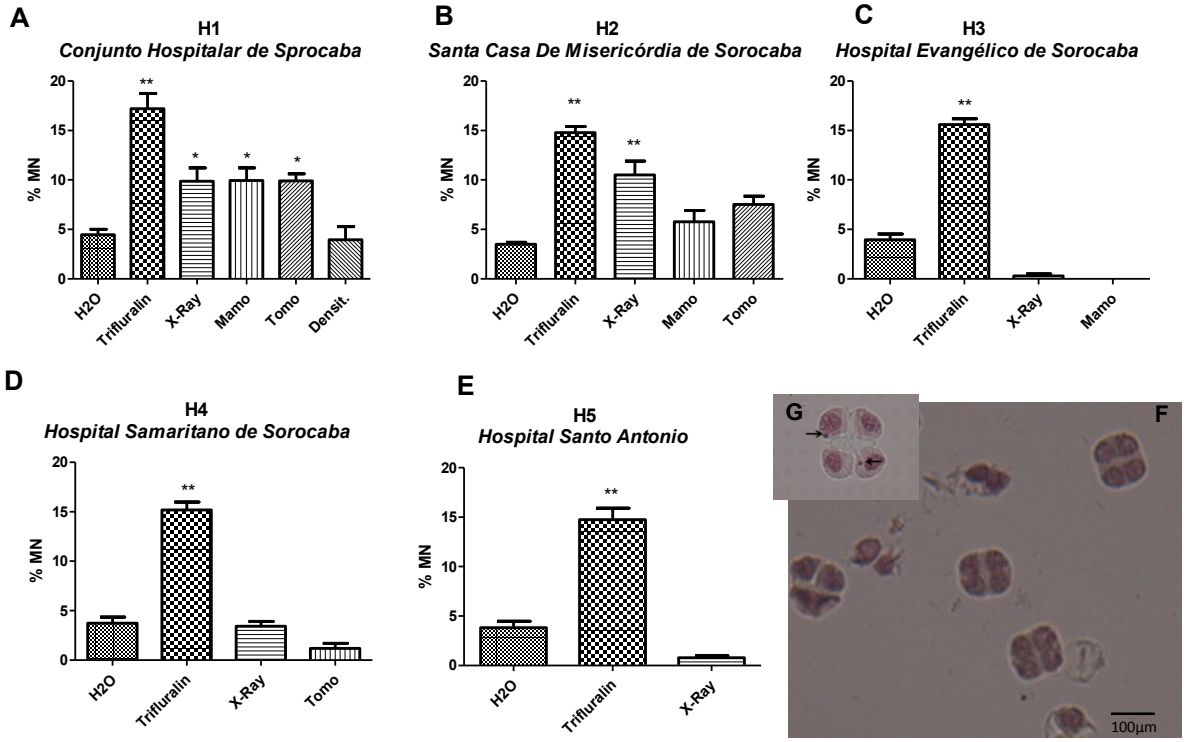

Figure 1 - Mutagenic Activity in the imaging diagnostic rooms by TRAD_MN assy. In A the data is relative to H1, and B, C, D and E are relative to H2, H3, H4 and H5, respectively. In F it is shown a microscopic image of *Tradescantia´*s tetrads (400Xx) and in G, a detail with a tetrad with two micronuclei. **, p<0,01; *, p<0,05.

Although studies with *T. pallida* admit their development under various environmental conditions[28], in this study we found that the *T. pallida* plants exposed in the image's rooms presented major changes in the macroscopic appearance of its foliage. Its leaves became thinner, clearer and the direction of stem growth was contrary to natural phototropism. We assign these observations to the exposure conditions, in which plants were exposed in a poor lighting room due to its hermetic constitution, only with artificial lighting. However, these characteristics of grow remained in our control conditions. The stem growth continued to occur downward and the plant sprouting was even more exuberant than the controls. Interesting to point that besides these growth alterations of behavior it was not seem additional MN formation. It seems that de radiation exposition can also influence these macroscopic characteristics of the *Tradescantia.*

Mutagenic activity was found only in the imaging services of public hospitals, which can be attributed to factors such as the greater demand for exams (Table 1),

as well as the conditions and types of equipment existing in these services. It was observed that in public services the technologies were older and with worse maintenance conditions. On the contrary, in private services, modern and well-calibrated equipment ensure greater safety to operators and patients.

Table 1 – Monthly average of imaging exams in each hospital

	Média mensal			
Serviço	**RX**	**TOMO**	**MAMO**	**DENS**
Conjunto Hosptalar de Sorocaba H1	5500	1900	270	240
Sta Casa de Misericordia H2	5000	900	350	DH*
Hospital Samaritano H3	350	160	DH	DH
Hospital Evangélico H4	50		60	DH
Hospital Santo Antonio H5	1400	35	DH	DH

*DH= It doesn't have

Recent work[29] evaluated the radiation exposure to patients and workers in a vascular hybrid operating room during X-ray-guided procedures. The working conditions in the hybrid operating room ware safe in terms of patient and staff radiation protection. Nevertheless, these authors affirm that the doses are highly dependent on the workload, thus, further research would be necessary to evaluate any possible radiological deviation of the daily working conditions in this environment.

For the operator and the other personnel, it is assumed that scattered radiation from the patient is the main source of exposure and directly relates to the dose area product (DAP) value of the patient. Therefore, the patient's constitution has a major influence on the dose rate for all persons in the room, for instance[30] and reducing the patient will directly translate into reduced exposure of the cath lab personnel[31]. Thus, the evaluation and monitoring of staff radiation doses in these places are an important subject to consider for the safety of professionals[32].

Our data demonstrate that, despite the adherence to the standards and guidelines regarding radiological protection, it is essential to continue monitoring the exposure levels of employees especially by means of adjuvant tools. These results reinforce the importance of sensitive biological indicators, as complementary tools for indoor environment monitoring in these services and thus to reduce the uncertainties of possible effects caused by residual doses of ionizing radiation.

Regarding to the samples collected by simple sedimentation of the hospital's image sectors, the fungi research in all five imaging diagnostic services revealed the presence of spores of different genres, and the most prevalent genres were *Aspergillus, Penicillium, Cladosporium, Fusarium* and *Nigrospora* as shown in Figure 2.

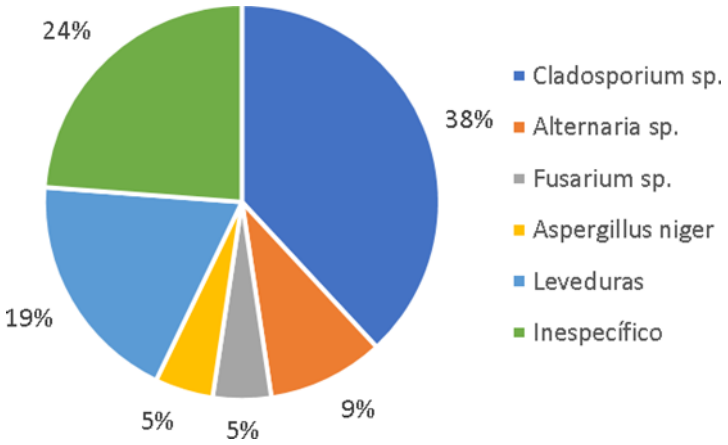

Figure 2 – Prevalence percentage of the different fungi genre in image rooms analyzed in the five image diagnostic services.

The prevalence of fungal genres found were determined by the classification of the colonies found in each plate of all rooms analyzed, as it is shown in Figure 3A-I. In this figure it can be seen the opportunistic genres of *Aspergillius sp.,* characterized by aspergillary “heads” (Figure 3A) and *Fusarium sp*., as identified by the “banana shape” of macroconidia (Figure 3B). The genre *Alternaria sp*, which is responsible for some types of hypersensitivity reactions, was also identified by your morphologic aspect, like your “bladder-shaped” macroconidia, with constrictions along the fruiting body (Figure 3C). The common genre of air microbiota *Cladosporum sp*., showing its recent reproduction hypha of oval shapes (Figure 3 D and E). In G, H and I, fungi at 200x could not be recognized, due to their distorted morphology and the lack of typical structures for identification and it was considered as unspecific. They also represent common types present in the anemophily microbiota.

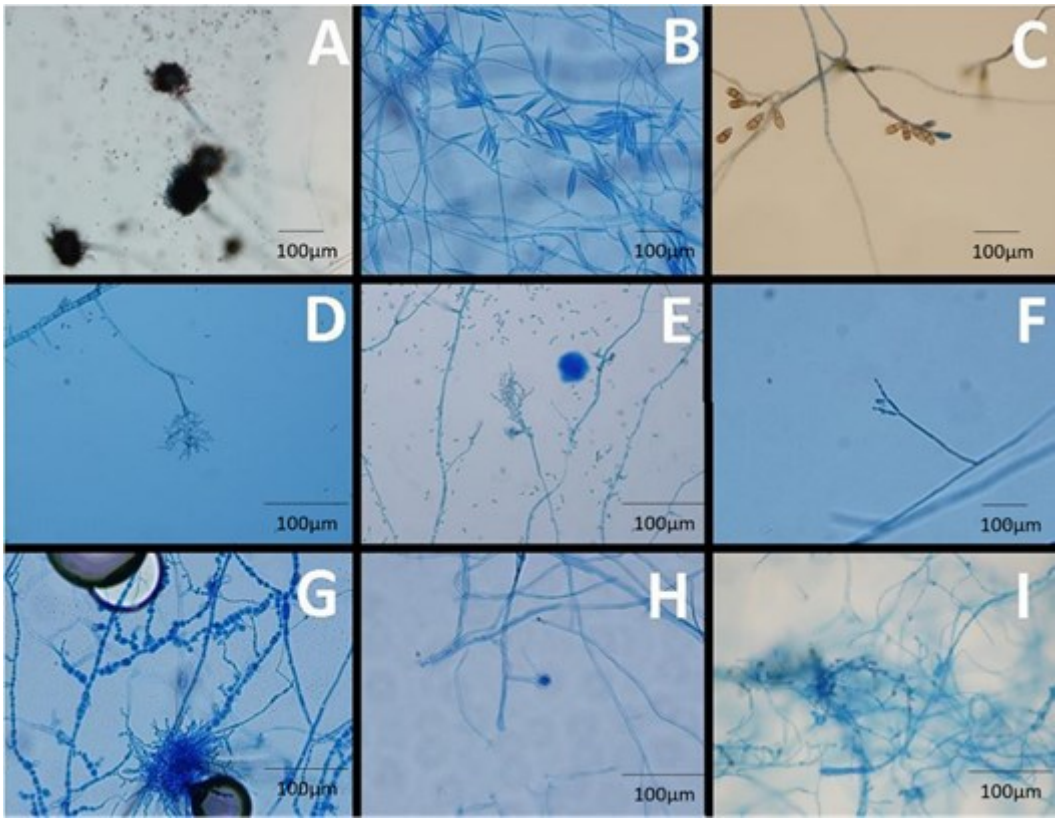

Figure 3 – Microscopic images of the fungi genres identified in the samples collected with lactophenol staining (400X). In A it is shown the *Aspergillius sp*; in B. the "banana shape" of macroconidia of *Fusarium sp*.; in C the "bladder shape" of *Alternaria sp*.; in D, E (200X) and F (400X), it is shown the *Cladosporum sp*.; in G, H and I images of unspecific fungi(400X).

As described in the arrangement of Figure 3, it was ignored the strains that contained yeasts, which are not interesting since they were not of *Candida* genre. The remaining filamentous fungi converged to an unspecific normal microbiota of an environment, such as the *Cladosporium sp.,* fungi that do not represent hazard. Although this fact, the occurrence of fungi *Cladosporium sp*. with a deformed aspect of the structure of its hyphae is not common and rise a question about if it would be due effects of radiation on fungal structures. Therefore, among the most important findings the opportunistic genera Aspergillus sp. and *Fusarium sp*., whose presence represents a major concern for immunocompromised individuals, due to the risk of difficult-to-treat infections, as well as to the occupational health due the possibility of aggravate allergies resulting from the long period of exposure of technicians. In the microscopic reading of the fungi, fruiting structures were sought, typical characteristics of reproduction of certain genera of fungi. These macroconidia function as a fingerprint of fungi. Thus, the following images demonstrate the representativeness of the fungi according to their clinical relevance. In the Figure 11 shows the fungi in order of relevance of clinical interest. as imagens

Considering the pathogenic potential of *Penicillium* and *Aspergillus* genres, the presence of these organisms in the tested environment is cause for concern for patients and staff attending the image room studied. Inhalation of *Penicillium spp*. and *Aspergillus spp*. conidia can generate pathologies such as penicilliosis and

aspergillosis, respectively. Both of which are characterized by a lung disease, which can spread to neighboring blood vessel, the cerebrospinal fluid, kidney and endocardial, featuring a usually fatal form, especially in immunocompromised individuals, such as is the case for many patients undergoing testing in this sector[33].

These results coincide with those of Bosquez-Molina *et al*., (2010)[34], in which they studied hospital environments and found *Cladosporium* and *Penicillium* genres followed by non-sporulating fungi were more frequent. Our results agree with a previous study[35] in hospitals of the same nature where the prevalent genres were *Penicillium, Cladosporium, Rhodotorula* and *Aspergillus*, the exception was the genre *Rhodotorula,* which was found in this paper, however, it is not reported in the literature as a fungi commonly found in hospital environments. The *Rhodotorula* fungi, is characterizes as an opportunistic pathogen capable of infecting patients with the immune system suppressed in several factors[36], thus running the risk of potential infections, especially in a hospital setting.

The CNEN Resolution (27:2004, of 06\01\2005) sets out the "Basic Guidelines on radioprotection"[37].

CONCLUSIONS

The results with *T. pallida* showed that there is a physical risk associated with the residual radiation on the evaluated local, since the plant exposure was able to induce mutagenicity as demonstrated by the micronucleus production by the plant, mainly in public administration services, where technologies are generally older and poorly maintained.

As the long-term effects are still unknown and multifactorial, prevention measures and further studies on the risk of occupational exposure to radiation are needed.

The microbiologic contaminations of the air conditioning equipment, in all rooms studied were predominantly from spores of the genres *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium* and *Nigrospora*. Considering the pathogenic potential of *Penicillium* and *Aspergillus*, it is of utmost urgency for greater attention to hygiene, mainly in the air conditioning system in this sector for staff and patient safety.

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