Formaldehyde did not prevent the growth of microorganisms in the brains of a human anatomy laboratory

O formaldeído não impediu o crescimento de microrganismos nos cérebros de um laboratório de anatomia humana

El formaldehído no impidió el crecimiento de microorganismos en los cerebros de un laboratorio de anatomía humana

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ABSTRACT
The conservation of cadaverous parts of human beings in formaldehyde is classically used through an aqueous solution of formaldehyde, reported in the literature as having the best action against microorganisms. However, studies have shown that some microorganisms have potential for resistance to formaldehyde, such as some fungi and bacteria, which are able to colonize man and animals, causing various infectious conditions and pathologies. In order to verify the possible growth of these microorganisms in human anatomy pieces preserved in formaldehyde, from the anatomy laboratory of a private Faculty in Bauru (São Paulo, Brazil), samples were collected through superficial scraping in the parts of the central nervous system (brain). After collection, an analysis of the growth of fungi and bacteria was carried out. It was found in the brain samples studied that 10% formaldehyde was not able to totally prevent the growth of microorganisms, fungi and bacteria, but it guarantees the non-growth of microorganisms in large quantities.

Keywords: anatomy, bacteria, formaldehyde, fungi, microorganism.

RESUMO
A conservação de partes cadavéricas de seres humanos em formaldeído é classicamente utilizada por meio de uma solução aquosa de formaldeído, relatada na literatura como tendo a melhor ação contra microrganismos.

Entretanto, estudos têm demonstrado que alguns microrganismos têm potencial de resistência ao formaldeído, como alguns fungos e bactérias, que são capazes de colonizar o homem e os animais, causando diversos quadros infecciosos e patologias. Com o objetivo de verificar o possível crescimento desses microrganismos em peças anatômicas humanas preservadas em formol, do laboratório de anatomia de uma Faculdade Particular de Bauru (São Paulo, Brasil), foram coletadas amostras por meio de raspagem superficial nas partes do sistema nervoso central (cérebro). Após a coleta, foi realizada uma análise do crescimento de fungos e bactérias. Verificou-se nas amostras de cérebro estudadas que o formaldeído a 10% não foi capaz de impedir totalmente o crescimento de microrganismos, fungos e bactérias, mas garante o não crescimento de microrganismos em grandes quantidades.

Palavras-chave: anatomia, bactérias, formaldeído, fungos, microrganismo.

RESUMEN

La conservación de partes cadavéricas de seres humanos en formaldehído es clásicamente utilizada a través de una solución acuosa de formaldehído, reportada en la literatura como la de mejor acción contra microorganismos. Sin embargo, estudios han demostrado que algunos microorganismos tienen potencial de resistencia al formaldehído, como algunos hongos y bacterias, que son capaces de colonizar al hombre y a los animales, causando diversas condiciones infecciosas y patologías. Con el objetivo de verificar el posible crecimiento de estos microorganismos en piezas de anatomía humana conservadas en formaldehído, del laboratorio de anatomía de una Facultad privada de Bauru (São Paulo, Brasil), se recogieron muestras a través de raspado superficial en las partes del sistema nervioso central (cerebro). Tras la recogida, se realizó un análisis del crecimiento de hongos y bacterias. Se constató en las muestras de cerebro estudiadas que el formaldehído al 10% no era capaz de impedir totalmente el crecimiento de microorganismos, hongos y bacterias, pero garantiza el no crecimiento de microorganismos en grandes cantidades.

Palabras clave: anatomía, bacterias, formaldehído, hongos, microorganismo.

1 INTRODUCTION

Traditionally, the teaching of human anatomy occurs through the study of dissected cadavers, which primarily aim at learning the structures and functions of the human body (SUGAND *et al*., 2010). Continuous study, using pieces of human anatomy in the laboratories, is only possible due to the conservation process (BALTA *et al*., 2017). There are several types of substances used for the preservation of structures and among them, formaldehyde is the most used by

the academic community, due to the low cost, ease of use and good antimicrobial characteristics (BRENNER, 2014).

Formaldehyde is a flammable and colorless gas, sold in its 30% or 50% aqueous form and is classically used in 10% aqueous solution, where there seems to be better bactericidal, fungicidal, virucidal and sporicidal actions. There is a considerable number of studies pointing out the dangers of acute and chronic occupational exposure to formaldehyde (VOHRA, 2011), such as the association of the substance with cancer (COSTA *et al*., 2019) and liver toxicity (BEALL; ULSAMER, 1984), however there are scarce microbiological studies in pieces of human anatomy, analyzing the potential of formaldehyde in educational laboratories.

Therefore, studies have shown that some fungal genera, spores and bacteria have shown potential for resistance to formaldehyde under certain conditions of temperature and humidity. Thus, this fact can be accentuated in the human anatomy laboratories, explained by the laboratory routine that includes the washing of the pieces and their exposure to the didactic activities of human anatomy (BALTA *et al*., 2017).

The cutaneous microbiota of the human being is populated by several species of fungi and bacteria, including some opportunists or pathogens, most of which are inhibited by the organism itself, but this situation has been changing over time. Fungi and bacteria are beings dispersed in the environment, atmospheric air, soil and water, presenting thousands of species, capable of colonizing man and, when they are in host-parasite imbalance and outside their natural habitat, they can cause several infectious conditions and several pathologies, from small to great complexity and prognosis (LI *et al*., 2017). There is a study that shows the presence of fungi, mainly filamentous and bacteria, in pieces of human anatomy preserved in formaldehyde (TABAAC *et al*., 2013).

Filamentous fungi produce some secondary metabolites that can be responsible for multiple health problems, especially when exposure becomes prolonged. The main metabolites produced are mycotoxins, which act as immunosuppressants. Filamentous fungi are also responsible for releasing spores, important contaminants in closed environments, which are better known

as fungal allergens (KÖHLER *et al*., 2017). Among the bacteria, one can exemplify the staphylococci belonging to the *Micrococcaceae* family, being represented in two groups, positive coagulase, where *Staphylococcus aureus* and negative coagulase are found, these are the ones most associated with human infections. (POLLITT *et al*., 2018). Coagulase negative *Staphylococcus* is common in human flora, found in the skin and mucous membranes, and can become pathogens if they have access to other tissues in the human body. The infections are varied, depending on the colonization site and in individuals with immune deficiency, they can be more serious (KURIZKY *et al*., 2020).

Therefore, knowing the possible harmful potential of fungi and bacteria to the health of employees, teachers, visitors and students of the Human Anatomy laboratories, and due to little research on the action of formaldehyde as a fungicide and bactericide in anatomical parts conserved in this substance, usually used, this experimental study was developed with the purpose of verifying the bacterial and fungal growth in human anatomical parts of the brain of a human anatomy laboratory of a Brazilian Faculty.

2 MATERIAL AND METHOD

The anatomical pieces used in this study came from the anatomy laboratory of a Private Faculty in the city of Bauru (São Paulo, Brazil), with all 23 pieces of the central nervous system present in the laboratory being selected, more specifically the brain, in the region of the grooves and rotations, where two samples per piece were taken, one for the analysis of fungi and the other for bacteria. The samples were collected immediately after removal of the 10% formaldehyde with sterile lancets.

The collected samples were stored in a sterile test tube and later transferred to sterile Eppendorf tubes containing sodium chloride (9% sterile saline), being properly identified, packaged and transferred to the microbiology laboratory to be processed in culture media for biological analysis.

For the analysis of fungi, a direct examination with Potassium Hydroxide (KOH) or Caustic Potash at 20% was performed to verify the presence of fungi, when positive, were later sown in Saboraud Dextrose Agar (SDA) culture media

– (Himedia™, Mumbai, India) Chlorophenicol (50mg/l, Sigma-Aldrich™, USA) which is a non-selective medium that presents the microbial inhibitor and allows the growth of opportunistic and pathogenic fungi and facilitates the increase of sporulations and characteristic colonial morphology.

The culture medium was placed in sterile 90x15mL glass Petri dishes, left in an oven at 37 ºC for 24 hours for sterility control. After this period, they were sown by the depletion technique and kept for 7 to 15 days at room temperature to check the fungal growth and allow macroscopic analysis of the colonies through direct observation.

Positive samples were classified as HCF (there was growth of fungi) and NHCF (there was no growth of fungi). After this procedure, the microculture technique was used in slides for the filament test performed with potato dextrose agar – BDA (Merck™, Darmstadt, Germany), left in an incubation period of 24, 48 and 72 hours. The slides were stained in Lactophenol and examined under an optical microscope (Olympus™, BX-50, Tokyo, Japan) with 400x magnification, where the fungus species was identified through the observation and morphological comparison of hyphae, conidia, conidiophores, septa and phialide.

For the analysis of the bacteria, the samples stored in Eppendorf™ tubes with 9% Sodium Chloride, were added in Brain Heart Infusion – BHI Broth (Merck™, Darmstadt, Germany) which is a nutritious medium for the development of demanding microorganisms and if cloudy when it is indicative of bacterial growth, and after 72 hours in a controlled greenhouse from 35 ºC to 37 ºC, they were sown in specific media for bacterial growth. Blood agar (Merck™, Darmstadt, Germany) was used, which is a differential and non-selective culture medium rich in nutrients, used for the isolation of non-fastidious microorganisms, it is a test of satellites and verification of hemolysis of *Streptococcus spp* or *Staphilococcus spp*.

MacConkey Agar (Himedia™, Mumbai, India) which is a selective medium for gram-negative bacteria and an indicator of positive and negative lactose fermentation by changing its color. Mannitol Agar (Himedia™, Mumbai, India) which is a selective medium for gram-positive coconuts, being

differential for *Staphilococcus aureus* and coagulase negative *Staphylococcus*, also changing their color.

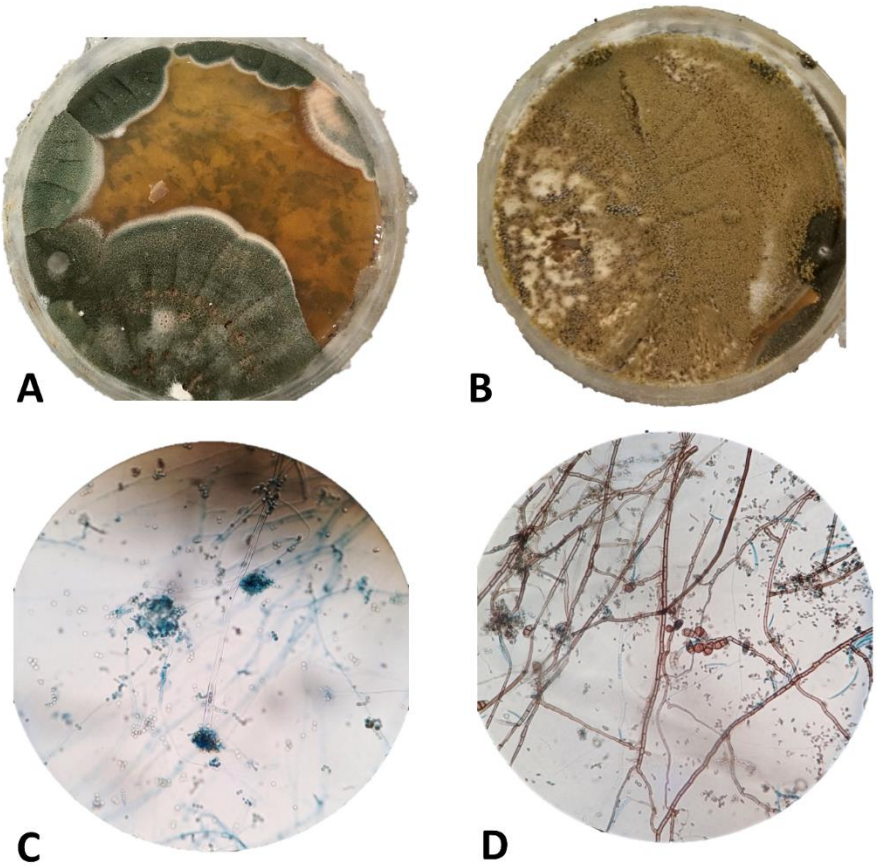
In addition to the culture media, the catalase test was used to distinguish between *Staphylococcus* and *Streptococcus*, and the coagulase test, which is used to differentiate between *Staphylococcus aureus* and *Staphylococcus* negative coagulase. The culture media were placed in sterile 90x15mL glass Petri dishes, left in an oven at 37 ºC for 24 hours for sterility control. After this period, they were sown by the depletion technique and kept in the greenhouse, to verify bacterial growth over a period of 72 hours. After this period, the samples were analyzed to identify whether there was bacterial growth or not, and when they were positive, they were subsequently identified.

3 RESULTS

In fungi, of the 23 samples collected in the study, 6 of them responded positively to the direct examination in Potassium Hydroxide. In this way, the 6 positive samples were sown on Saboraud Dextrose-Chlorophenicol Agar, and after the growth period the morphological characteristics of the colonies of the study samples were observed, the greenish-yellow color was found in 5 samples, the most usual being, and a gray colony was also found (Figure 1A-B).

In the microscopic analysis with the microculture technique, the filament proof revealed septate hyphae in the 6 samples of the study and analyzing the microscopic morphology of the samples, they were compatible in most of the samples with the species *Penicillium spp*, and one sample was compatible with the species *Aspergillus spp*, equivalent to 85% and 15%, respectively (Figure 1C-D).

Figure 1. Morphology of fungal colonies found in the study. Macroscopic morphology in (A) *Penicillium spp.* and (B) *Aspergillus spp.* Microscopic anatomy in (C) *Penicillium spp.* and (D) *Aspergillus spp.*



Source: Authors

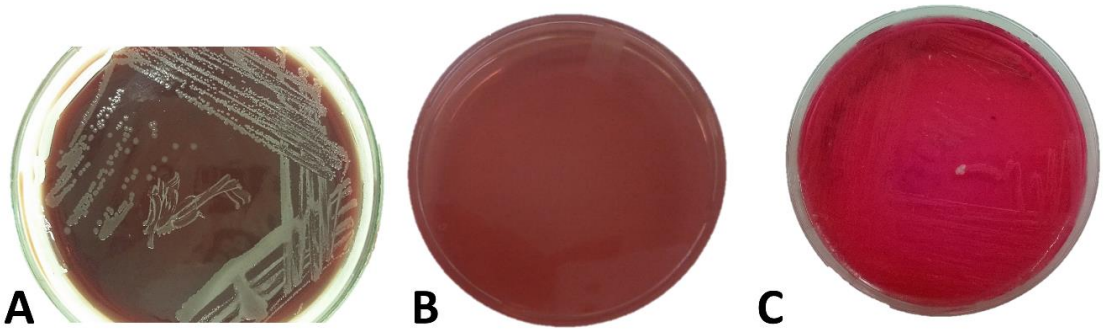
In bacteria, of the 23 samples collected, 8 samples became cloudy after being immersed in BHI broth for 72 hours, being indicative of bacterial growth. The positive samples were then sown by depletion technique in Blood Agar, MacConkey Agar, Mannitol Agar.

When the growth in Blood Agar was analyzed, growth of colonies of small formation and whitish color was observed in all samples, being indicative of Gram positive bacteria (Figure 2A). In the MacConkey medium, it was observed that there was no bacterial growth in the study samples, this medium being specific for Gram negative bacteria, it was concluded that the study samples were not Gram negative bacteria (Figure 2B).

After this process, when analyzing the Mannitol Agar medium, bacteria growth was observed, concluding that it was Gram positive bacteria, and as the medium did not change its staining characteristics in any sample, it was indicative

of negative coagulase bacteria. To prove that the coagulase negative bacteria were involved, the coagulase control was performed, being a negative analysis, that is, it did not coagulate, concluding that the coagulase bacteria were negative (Figure 2C).

Figure 2. Mediums used in this study. (A) Blood agar, where there was growth with white colonies; (B) MacConkey Agar, where there was no growth; (C) Mannitol agar, where there was growth, but without changing the color of the medium.



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Another control performed to prove if the bacteria in the sample were *Staphylococcus* or *Streptococcus*, which all samples were positive, that is, it agglutinated, showing that it was *Staphylococcus.* At the end of all tests, which allowed us to conclude that the 8 positive samples found in the study were Gram positive *Staphylococcus* coagulase negative bacteria, all the processes of the positive samples can be seen in Table 1.

Table 1 – Bacteria analysis demonstrating tests on positive samples.

Sample number	Blood agar	MacConkey agar	Mannitol agar	Final result
3	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
5	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
8	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
13	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
18	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
20	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
21	PBG	NBG	PBG	*Staphylococcus* coagulase (-) catalase (+)
23	PBG	NBG	PBG	*Staphylococcus*. coagulase (-) catalase (+)

NBG: negative bacterial growth; PBG: positive bacterial growth.
Source: Authors

4 DISCUSSION

The objective was to analyze the possible growth of microorganisms in pieces of human anatomy, more precisely, of the central nervous system, preserved in formaldehyde 10% of a didactic laboratory, as well as to differentiate them, if any, in fungi and/or bacteria. It was found that 10% formaldehyde was not able to totally prevent the growth of microorganisms, both fungi and bacteria, in the 23 brain samples studied, despite guaranteeing the non-growth of microorganisms in a good number of samples, demonstrating the possible occupational danger of infections in anatomical parts of the central nervous system fixed in formaldehyde.

Some factors may have interfered with the microbial power of 10% formaldehyde and, thus, provide conditions for fungi and bacteria to be present. Such factors are routinely found in the human anatomy laboratories of educational institutions, which are: the daily removal of 10% formalin pieces for washing in water for human anatomy classes, and thus it is possible, that the concentration of formaldehyde be diluted in water and, in addition, be exposed for long periods in half. Another inevitable factor is the handling of the parts by students, teachers and employees, who, when unprotected, can contaminate the parts and allow, along with other factors, the proliferation of fungi and bacteria.

The fungi found in the study were the species *Aspergillus spp* and *Penicillium spp*. According to Armstead *et al*., (2014) *Aspergillus spp* is a filamentous fungus, found in the soil, in vegetables and in decomposing organic matter, it is carried by air, coming from humid environments, justifying its identification in the parts of the nervous system of the human anatomy laboratory.

There are 900 species of *Aspergillus spp* identified, classified in 18 groups, 12 of which cause diseases in humans, such diseases of pulmonary and extrapulmonary origin, disorders in the central nervous system, mucous membranes, respiratory usually in an opportunistic manner, mainly in individuals with immune deficiency (KOULENTI *et al*., 2014)

The species *Penicillium spp*, on the other hand, is a fungus present in decomposing soil, air and vegetation, some of the species have potent mycotoxins and are considered opportunistic for both plants and rarely for

humans; however, it has become common in individuals with low immunity, mainly with AIDS (ROOHANI *et al*., 2018). Therefore, in Brazil, although rare, a case of multiple brain abscesses has been reported in an HIV negative patient caused by infection by *Penicillium spp*, which was responsible for the individual's death (NORITOMI *et al*., 2005).

In the case of bacteria, the presence of coagulase-negative *Staphylococcus* in the nervous tissue was identified by the analyzes made in the study. These types of bacteria initially seemed to be of no clinical importance; however, nowadays with the increase in virulence and antibiotic resistance, it has become worrying (FRANÇA *et al*., 2021). Coagulase negative *Staphylococcus* is common in human flora, and is found in the skin and mucous membranes with a symbiotic relationship with the host (PIETTE; VERSCHRAEGEN, 2009). However, they can acquire a pathogenic character if they have access to other tissues of the human body, such as, for example, in wounds and ulcers that can occur in people who visit human anatomy laboratories. The infections caused can be urinary, endocarditis of natural and prosthetic heart valves, osteomyelitis, post-surgical endophthalmitis and, in patients with immune deficiency, cause bacteremia (BECKER *et al*., 2014). Currently, coagulase staphylococci, positive or negative, have high resistance to penicillin G worldwide (POLLITT *et al*., 2018), therefore, it is necessary to alert these microorganisms by adopting care and protection when using the human anatomy laboratory.

We believe that there should be an obligation to use personal protective equipment (PPE) when using and handling parts of the human anatomy laboratory to protect against contamination by microorganisms, not only by students and by teachers, but also by employees, who have daily direct contact with anatomical pieces. Special attention to individuals with immune compromise, as they are more susceptible to infectious agents, which can cause serious infections and bacteremia, as discussed in this study.

Likewise, handling with PPE not only protects those who manipulate the parts from possible contamination, but also helps in the conservation of anatomical parts, as it avoids contamination by microorganisms found in the hands of laboratory users (BUCHAIM *et al*., 2014).

In future studies samples will be taken with a greater amount of tissues from the human body, identifying the microbiota in which they are located and if there are similarities between them, as well as short and long term tests with various concentrations of formaldehyde and different tissues, to be carried out. in order to obtain an adequate concentration for each type of human body tissue and less contamination of anatomical pieces.

In conclusion, based on the results of the study, there was microbial growth in some anatomical samples of the brain in the study, demonstrating fungi and bacteria that have pathogenic potential, demonstrating the importance of personal protective equipment (PPE) and caution with users of human anatomy laboratories with immune deficiency.

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