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ORIGINAL ARTICLE

Formalin Resistant Fungi Isolated from Cadavers at a Medical School's Dissection Hall in Malaysia.

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Abstract

Introduction: Utilization of the cadavers as part of teaching methodology in anatomy are equally important either for undergraduate or postgraduate students. Microorganisms such as bacteria (*Mycobacterium tuberculosis*) were previously reported to have a survival rate up to 36 days after the death of the host. Recent studies have suggested the difference in the degree of efficacy in formalin to halt the growth of different microorganisms in the formalin fixed tissues. Hence this study aims to determine the presence of specific bacteria and / or fungi on the surface of a formalin-fixed cadavers.

Methodology: Swab samples were taken from the nose, oropharynx, ear, axilla, and anal canal of five cadavers in the dissection hall at ainternational Medical School (IMS) of Management & Science University (MSU). Samples were incubated at 37^{o} for 24-48 hours in various culture media which includes blood agar, nutrient agar, chocolate agar, and MacConkey agar for bacterial culture and Sabouraud dextrose agar (SDA) for fungal culture. Blood agar plates were incubated in anaerobic jars to create low oxygen conditions to allow the growth of anaerobic bacteria. The culture media were taken outside the incubators and put under room temperature and observed for another 7 days to observe for any fungal growth.

Results: Fungal growth was found in two cadavers in the regions of the ear and oropharynx. Fungal growths were stained with lactophenol cotton blue solution and examined under the light microscope. No bacterial growth was detected in this study. **Conclusion:** This study showed that the growth of fungus corresponds with *Aspergillus* species isolated from two cadavers.

Keywords: Bacteria, Fungi, Formalin resistant, Cadavers.

Introduction

Human cadavers are excellent educational tools used by anatomists in teaching medical undergraduate students.[1] The anatomy cadavers are used in anatomy teaching either by using prosected specimen demonstration or by allowing the medical student to practice the art of dissection by themselves.

In medical schools of Malaysia, undergraduate and postgraduate medical students study human anatomy by dissecting anatomy cadaver at the dissection halls under the supervision of their lecturers. The experience of dissecting anatomy cadavers gives medical students a better appreciation of three-dimensionality of human body. The students can examine the external as well as internal structures of human body by observing, touching, and palpating the cadavers. These processes will impart and cultivate the students to attain better comprehension on the structure of human body as human cadavers exhibit divergence of anatomical variations.[1,2]
Starting in the 1960s and 1970s, people started to donate their bodies as the deceased would give informed consent during their lifetime. The source of cadavers used for anatomy teaching comes from body donation, unclaimed bodies and some countries will import cadavers from abroad.[3]

Depending on the cause of death, for example, death due to diseases, accidents, suicide, or homicide, the corpses are considered infectious material. Some bacteria, such as *Mycobacterium tuberculosis*, can survive in the bodies of their host for up to 36 days after the death.[4] Therefore, to warrant the practise of dissection among medical students using cadavers without the risk of decay, tissue loss, and transmission of potential pathogens, these cadavers need to be embalmed and preserved.

Modern embalming practices of human cadaver involve the use of buffered formalin with or without addition of glycerol, salts, disinfectant agents, and water as fixative agents. The embalming procedure must have qualities such as good preservation of organs and tissue with minimal structural alterations in order to acquire a decent view of body structure from a cadaver. Formalin is a 37% solution of formaldehyde gas in water (University of Memphis). It was found that formalin is a potent disinfectant.[5]

The formalin targets the amine functional groups in proteins, and then denaturing them.[6] They found that formaldehyde has a strong bactericidal, sporicidal and virucidal properties. Even at low concentrations, formaldehyde kills fungi and inhibits the growth of bacteria.

Formaldehyde is a highly toxic substance to certain microorganisms. According to the Centers of Disease Control and Prevention (CDC) in the United States of America, microorganisms can be inactivated by formaldehyde due to its action of alkylating the amino and sulfhydryl groups of protein and the ring nitrogen atoms of purine bases. For example, application of 25% formaldehyde for 10 minutes can cause *Salmonella typhi* to become inactive in the presence of organic matte.[7] Some of the advantages of using formalin include that it helps to harden proteins and prevent them from being discomposed; it is also inexpensive.
The issue arises when recent studies have suggested that formalin might not be very effective in inactivating all kinds of microorganisms in the formalin-fixed tissues and that the microbes may develop growth on the surface of formalin in long-preserved tissue in formalin.[8,9]

This study is to determine the presence of any formalin-resistance bacterium and/or fungus on the surface of cadavers at MSU's IMS dissection hall. This study is a concern because students, lecturers and staff who handle the cadavers may be exposed to potentially pathogenic organisms.

Malaysia has not reported any research on the identification of formalin-resistant organisms in cadavers, and only few related studies have been conducted in another countries. A study conducted by Claudio et al., in Chile found that there were live bacteria on the surface of formalin-fixed cadavers.[9] Due to a lack of studies on formalin-resistant microorganisms in cadavers, previous studies suggested additional research to explore whether the bacteria obtained from cadavers are formalin-resistant or due to environmental pollution.

Materials and methods

This a descriptive study of the cadavers that are used for anatomy teaching. The anatomy cadavers are embalmed according to the guidelines from the Department of Anatomy at the National University of Malaysia. The embalming solution for the anatomy is composed of 5% formaldehyde (53ml), distilled water (48 ml/l), phenol (67g/l) and glycerol (207 ml/l). A total of five cadavers were used in this study. This research was approved by the Management & Science University (MSU) Research Committee Under the Code Ethics Number (MSU-RMC-02/F R01/04/L1/039).

Before sample collection, the cadavers were taken out from a tank containing embalming solution and placed on a dissection table for two days. Sterile swabs were used by rubbing and rolling them firmly several times across the sampling area of the cadaver. Twenty-five swab samples were taken from the nose, oropharynx, ear, axilla, and anal canal.

Culture Procedure

After the samples inoculated on different culture media such as blood agar, nutrient agar, chocolate agar, and MacConkey agar for bacterial culture and Sabouraud dextrose agar (SDA) for fungal culture then incubated at 37 ºC under oxygen conditions. Blood agar plates were incubated in anaerobic jars to create low oxygen conditions. This is to allow the growth of anaerobic bacteria. Sterile culture media were used and incubated as negative controls. These culture media was purchased from Next Gene Scientific.

Colony Morphology Identification

The following terms were used to describe the microbial growth patterns: circular, irregular, filamentous, and rhizoid to describe the colony form; raised, convex, flat, umbonate, or crateriform to describe the colony elevation; entire, undulate, filiform, curled, or lobate to describe the colony margin; smooth, glistening, wrinkled, dull, or rough to describe the surface; white, buff, red, purple, or other pigmentations; translucent, transparent, or opaque to describe the various opacities of the colony.

Staining of Fungi

Lactophenol cotton blue stain was used in this study to examine fungal elements following either a tape preparation or a scraping. It contains phenol that kills other organisms, while lactic acid preserves fungal structures, and cotton blue stains chitin in the fungal cell walls. The ingredients of this lactophenol cotton blue solution were phenol crystal, cotton blue, lactic acid, glycerol, and distilled water. The solution has a limitation that does not allow to observe the early differentiation stage of the fungus and cannot be stored in long period. Also, during the sampling disruption of fragile fungal architecture may occur.

Lactophenol cotton blue solution was applied onto a clean glass slide, followed by a scraping from the fungal growth on the agar medium, and mixed well. The smear was covered using a cover slip and the slide was observed under a light microscope.
The hyaline fungi were stained as blue by the lactophenol, and the dematiaceous fungi were stained as brown due to the pigment.

Results

Among 25 samples collected from five different parts of five formalin-fixed anatomy cadavers, two culture samples were positive with fungal growth. Two different colonies of fungi were identified from cadaver A (Table 1 and Figure 1) and cadaver B (Table 2 and Figure 2).

Fungal growth was isolated from the ear of cadaver A. The fungal growth was isolated from the oropharynx of cadaver B. After two days of incubation, the colony emerged and was observed for up to one week. The colony isolated from cadaver A is round in shape, opaque, umbonate, and rough. It is white in colour with a black centre.No growth was detected in cadavers C, D, and E (Table 3). No growth was detected from the controls.Microscopically, hyphae were septate and branched and stained blue with lactophenol cotton blue dye solution. The colony isolated from cadaver B appear whitish, irregular shape, umbonate, curled, opaque, glistening and show presence of hemolysis on blood agar. Microscopically, hyphae were septate and branched (Figure 3). The colony of the fungi seen in this study was very similar to the *Aspergillus species*. There was no growth of bacteria detected from all the samples isolated.

Discussion

The goal of this study was to determine if formalin-resistant bacteria or fungi that might grow on formalin-fixed anatomy cadavers used by medical students at MSU. Previous studies have shown that fixed anatomy cadavers could be the source of different bacteria and fungi.[10]

From the five anatomy cadavers used in this study, two colonies of fungi were found growing on blood agar culture media. This result confirmed the study conducted by Yaragalla, S. and Rajput, A., when they identified fungal growth from the internal organ of preserved human cadavers.[12] They had described three species of fungal growth in their study includes *Penicillium*, *Trichophyton* and *Aspergillus* species. In a study conducted by Ishii, K. et. al., they identified fungi flora detected on the skin and bones of two human cadavers.[6] Another study done by Tabaac, B. et., al., they confirmed the presence of both bacteria and fungi on the different areas of the cadavers used in teaching of human anatomy.[11]

In this study the cadavers were previously fixed with 5% formalin. From the observation of the colony and microscopic morphology, the appearance and characteristics of the fungus correspond with *Aspergillus* species. It is commonly found in soil, dust, and decaying vegetation. According to Centre for Disease Control and Prevention (CDC), *Aspergillus* is a type of fungi that is very common both indoors and outdoors.[1] Due to this, most people may breathe in fungal spores every day. It is not harmful to people with a healthy immune system, but it is found to cause disease in immunocompromised people and people with lung disease.

The possibility of the source of the fungus in the cadaver being a contamination from the air or surrounding environment might be there, as this was mentioned in previous study conducted by Langroudi, M. and Farzanegan, A. .[7] They found that both bacterial and fungal infections decreased after the usage of glycerine and phenol solution. They also suggested that glycerol and phenol solutions can be added to the formalin solution once a week to minimize the contamination of the cadavers and to protect students and staff from the spread of these microbes among them.

It is also possible that in cadaver B, the source of fungi may be due to airborne contamination. It may be either come from the anatomy cadavers, clothing, or dissection table during the process of culturing the sample on culture media.

Further studies are needed to confirm the ability of the fungus to grow on the formalin-fixed

human cadavers at the dissection hall at IMS of MSU. It is also much better to take these samples by swabbing these cadavers immediately after taking them out of the formalin tanks, and not waiting for two days to take the samples. This may give more accurate results and minimize the possibility of contamination of cadavers by these air-born fungi. In cadavers C, D, and E there was no growth of neither bacteria nor fungi (Table 3).

The result of this study shows that the cadavers which were used to teach the medical and paramedical students were not safe. Students and staff are recommended to wear masks and gloves to avoid the transfer of fungal and bacterial infection to healthy individuals.

Conclusion

This study showed that there was fungal growth in the ear and oropharynx in two cadavers. Precautionary measures should be taken when the staffs and students work with these cadavers by wearing masks and gloves during dissection sessions.

Acknowledgement

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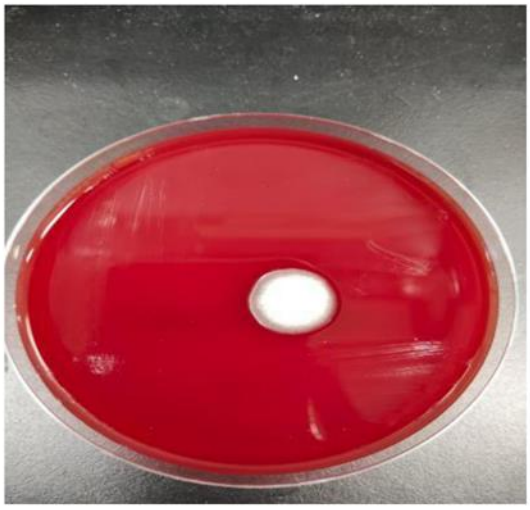

Figure 1: Cadaver A (Ear). After five days of incubation. The appearance of the fungus is opaque, circular, and white in colour.

Table 1. The microbial growth pattern on Cadaver A.

Body region	***MacConkey Agar***	***Blood Agar***	***Chocolate Agar***	***Nutrient Agar***	***Sabouraud dextrose agar (SDA)***
Nose	No growth	No growth	No growth	No growth	No growth
Oropharynx	No growth	No growth	No growth	No growth	No growth
Ear	No growth	**Fungal growth**	No growth	No growth	No growth
Axilla	No growth	No growth	No growth	No growth	No growth
Anal canal	No growth	No growth	No growth	No growth	No growth
Control media	No growth	No growth	No growth	No growth	No growth

The result demonstrates fungal growth on blood agar media where the sample is taken from the ear area.

Figure 2: Cadaver B (Oropharynx). After 1 week incubation, the colony of the fungal appear whitish, irregular, umbonate, curled, opaque and presence of hemolysis on blood agar.

Table 2. The microbial growth pattern on Cadaver B.

Body region	***MacConkey Agar***	***Blood Agar***	***Chocolate Agar***	***Nutrient Agar***	***Sabouraud dextrose agar (SDA)***
Nose	No growth	No growth	No growth	No growth	No growth
Oropharynx	No growth	**Fungal growth**	No growth	No growth	No growth
Ear	No growth	No growth	No growth	No growth	No growth
Axilla	No growth	No growth	No growth	No growth	No growth
Anal Canal	No growth	No growth	No growth	No growth	No growth
Control media	No growth	No growth	No growth	No growth	No growth

This result demonstrates fungal growth on blood agar media where the sample is taken from the oropharynx area.

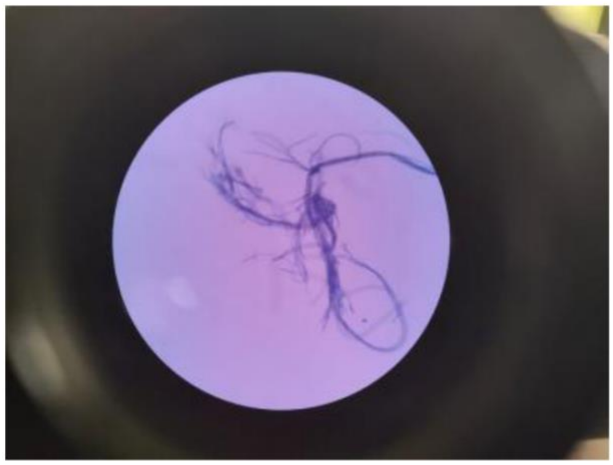

Figure 3: Microscopic appearance as seen in cadavers A (Ear) and B (Oropharynx). Septate hyphae and fungal spores-stained blue were seen.

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