# Identification of Fungal Growth in Formalin Fix Human Cadaver among Faculties of Medicine at Khartoum Stat

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**Abstract:** Cadavers are main teaching tools in Anatomy and are handled by the staff and students routinely. One of the problems that face anatomists is the growth of fungi on cadavers. The aim of this study is to detect fungi that growth on formalin fix human cadaver in different medical colleges in Sudan. Fifty one swabs from issecting room was collected from 13 faculties of medicine, swabs inoculated into Sabouraud dextrose agar with the addition of chloramphenicol to suppress bacterial growth and incubated at $25^0$C. Five different *species*, *Microsporum spp*, *Aspergillus spp*, *Cryptococcus spp, Trichophyton spp* and *Candida spp*, the source of our isolated fungi, could be air or water, or they could have been brought with the dead bodies in the first place. Instructors and student should use face masks and rubber gloves for protection.

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## 1. Introduction

Sudan has more than 25medical colleges in different states which show variations in their climatic conditions. Gross anatomy is one of the fundamental topics in medical education; dissection courses are one of the most important roles in learning it. Cadavers remain teaching tool for instructor and students, but may pose infection hazards to people who handle them, include pathologists, nurses, mortuary attendants, embalmers, funeral directors and members of the emergency services. All of these are potentially at risk of exposure to pathogenic microorganisms Such as fungi, bacteria and viruses. To date no published studies had been carried out to overcome these problems in Sudan.

The embalming fluid used in anatomy departments contains fixatives, disinfectants, surfactants, buffers, glycerol, salts, and water. The most frequently used fixatives and disinfectants are formalin, ethanol, and phenol. Formalin, a 37% aqueous solution of formaldehyde gas, inactivates infectious agents by forming covalent cross-links with several organic functional groups on proteins. (1)

Ethanol is one of the most commonly used alcohols to control microbial growth. Its mechanism of action involves protein denaturation and lipid dissolution. Ethanol can be used alone in concentrations of 60 to 95% or in combination with other antimicrobial agents in lower concentrations. It is known to be effective against bacteria and fungi but not endospores, nonenveloped viruses, or prions (1).

Phenol and its derivative phenolics exert antimicrobial activity by inactivating essential cell enzymes and injuring lipid-containing plasma membranes, which results in leakage of cellular contents. At concentrations above 1%, phenol and phenolics have an antibacterial effect. They have a broad spectrum of activity against bacteria, viruses, and fungi, but they are ineffective against prions. These fixatives and disinfectants were shown to be effective against most of the bacteria and viruses (2). However, it is not clear whether they are also effective in cadavers, for several reasons include, concentration of the embalming fluid components decreases as they diffuse throughout the human body, protein inactivation to some classes of product (formalin, alcohols, and phenolic agents) sensitivity to organic load suggests that the efficiency of the disinfectants will be much lower in cadavers than in vitro tests (3).

Formalin is most chemical uses in Sudan for cadavers embalm ding to preserve human cadavers, for teaching and research purposes. Concentration of formalin always is problem to the instructor and students in medical colleges, when they raising the concentration of formalin they suffering from irritation on the other hand lowering the concentration, make them have problem of fungal and other microorganism growth.

Fungi are saprophytic, parasitic or commensal organisms. Fungi are widely spread in nature and thrive on every climate on earth, most live in the soil on decaying matter helping to recycle organic matter. Most fungi grow well in temperature range between (20-30 $^0$C) [4].

The incidence of fungal infections (mycoses) is increasing throughout the world as a result of modern medical advances that use immunosuppressive therapies, broad spectrum antibiotics, and central venous access devices, as well as a rise in the population of individuals at risk. Many fungi can produce illness through inhalation of the mold or throw mycotoxins, some fungi are toxic to people who handling them during embalming procedure or dissection of cadavers [5, 6, 7].

Risk can be reduced by protocols include the layout, construction, ventilation and operation of necropsy rooms, Hands should be washed routinely after each procedure and the environment cleaned with a phenolic disinfectant daily. The instruments should be washed in a washer-disinfector, autoclaved or immersed in a phenolic disinfectant for 20 minutes. A phenolic disinfectant is preferred to hypochlorite because hypochlorite is corrosive and may damage surfaces and instruments. [8].

The aim of this study is to detect fungal that growth in fixed human cadaver, call for attention to this fungal problems in medical colleges in Sudan and to suggest safety guidelines for the protection of all who handle cadavers.

## 2. Materials and Methods

**Samples collection:**

Total numbers of 51 swabs from dissecting room were collected from 13 faculties of medicine in Khartoum state during the period (jeanery 2013 to may 2013). Fungal colonies were collected by swabbing exposed parts of cadavers and formalin pools.

**Culture:**

All samples were inoculated into Sabouraud dextrose agar with the addition of chloramphenicol to suppress bacterial growth. [10] All samples were incubated at 25$^0$C, colony growth was checked daily.

**Colonial morphology:**

Different type of fungi was produce in different-looking colonies describe depended on**:**

- The basic shape of the colony circular, filamentous, etc.
- Size the diameter of the colony.
- Elevation - This describes the side view of a colony. Turn the Petri dish on end.
- Margin/border – The edge of a colony
- Surface - the surface of the colony appearance smooth, glistening, rough, wrinkled, or dull.
- Opacity - transparent (clear), opaque, translucent (like looking through frosted glass)
- Color - (pigmentation) - white, buff, red, purple, etc.

**Microscopical identification of the isolated fungi:**

All isolate were stained by lacto phenol cotton blue and identify by the presence of:

- Fungal hyphae (branched filaments) making up a mycelium
- Arthrospores
- Arthroconidia
- fungi were identified by comparing micrographic characteristic of fungi to stander mycology text book

## 3. Result

Thirty four fungi were isolated from 13 faculties of medicine, isolated include five different *species*, *Microsporum spp* 10, *Aspergillus spp* 8, *Cryptococcus spp* 6, *Trichophyton spp* 5 and *Candida spp* 5. (Table 1)

Concentration of formalin used in the fixation of cadaver was 10%, 37%, 40%, 70% respectively.

Low formalin concentration 10%, showed highly isolated fungi which include Aspergillus, Microsporum, Trichophyton, Candida and Cryptococcus. On the other hand at high concentration70% there was no isolated fungi. (Table 2).

Preservation time of cadaver range between 3, 4 and 5 years, 5 years showed highest isolated include *Microsporum spp*, *Aspergillus spp*, *Cryptococcus spp, Trichophyton spp* and *Candida spp*. only two species isolated at 3 years of preservation (Table 3).

Table 1. Showing frequency of isolated fungi

| Fungi Isolated | Frequency |
|---|---|
| Aspergillus | 8 |
| Trichophyton | 11 |
| Microsporum | 12 |
| Candida | 6 |
| Cryptococcus | 7 |

## 4. Discussion

Five different fungal species were isolated; *Microsporum spp* was the highest isolated fungi 29.4%, *Candida* and *Trichophyton spp* were the lowest isolate 14.7%, while , *Aspergillus spp* and *Cryptococcus spp represented* 23.5%, 17.6% respectively. Our founding almost agree with other study done by Elsebai and his colleague which they

isolated Aspergilli, Penicillium, *Trichophyton, Epidermophyton* and *Cryptococcus* [9]. Koyoshi *et al* reported that the main isolated fungi were *Eurotium repens*, *Eurotium rubrum*, *Eurotium chevalieri* and *glicocladium spp* [10]. the genus *Eurotium* usually inhabits soil and exhibit osmophilic properties. The source of our isolated fungi could be air or water, or they could have been brought with the dead bodies in the first place.

Our study showed that fungal growth effected by concentration of formalin, highest concentration 70% showed no fungal growth, while lowest concentration of formalin 10% showed isolated of five different species. This result indicates that the isolated fungi have some sort of adaptation and resistance to low formalin concentrations. Our result in agree with Sarsilmaz *et al* they suggested formalin concentration effective enough to prevent fungal growth should not be less than 5% [11].

Table.2 showing relationship between concentration of formalin and isolated fungi

| University | Isolated Fungi | Concentration of formalin |
|---|---|---|
| 1 | Aspergillus, Microsporum, Trichophyton, Candida, Cryptococcus | 10% |
| 2 | Aspergillus, Microsporum | 40% |
| 3 | Aspergillus, Microsporum, Candida | 40% |
| 4 | Cryptococcus, Microsporum, Trichophyton | 10% |
| 5 | Cryptococcus, Microsporum | 37% |
| 6 | Aspergillus, Microsporum | 40% |
| 7 | Aspergillus, Microsporum, Candida | 40% |
| 8 | Microsporum, Trichophyton, Candida, Cryptococcus | 40% |
| 9 | Aspergillus, Microsporum, Cryptococcus | 40% |
| 10 | Aspergillus, Microsporum, Cryptococcus | 37% |
| 11 | Trichophyton, Microsporum | 37% |
| 12 | Non | 70% |
| 13 | Trichophyton, Candida, Trichophyton | 40% |

Table.3 showing relationship between Time of preservation cadaver and isolated fungi

| University | Isolated Fungi | Time of preservation cadaver |
|---|---|---|
| 1 | Aspergillus, Microsporum, Trichophyton, Candida, Cryptococcus | 5 years |
| 2 | Aspergillus, Microsporum | 3 years |
| 3 | Aspergillus, Microsporum, Candida | 3 years |
| 4 | Cryptococcus, Mibsporum, Trichophyton | 3 years |
| 5 | Cryptococcus, Microsporum | 3 years |
| 6 | Aspergillus, Microsporum | 5 years |
| 7 | Aspergillus, Microsporum, Candida | 5 years |
| 8 | Microsporum, Trichophyton, Candida, Cryptococcus | 4 years |
| 9 | Aspergillus, Microsporum, Cryptococcus | 4 years |
| 10 | Aspergillus, Microsporum, Cryptococcus | 4 years |
| 11 | Trichophyton, Microsporum | 4 years |
| 12 | Non | 4 years |
| 13 | Trichophyton, Candida, Trichophyton | 5 years |

## 5. Conclusion

Five different fungal colonies identify as *Microsporum spp*, *Candida*, *Trichophyton spp, Aspergillus spp* and *Cryptococcus spp*, the source of this strain may be environmental contamination, instructor or cadaver itself. Special care for handling cadavers must be taken to reduce hazard from human cadavers, updating literature in the field to helping reduce risks of infectious hazard of cadaver to a minimum level.

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