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Brief report

Decrease of microbial contamination by application of routinely glycerol and phenol solution on human cadavers

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Abstract

Human cadaver is imperative learning material for medical students. The cadavers expose to microbial contamination every time anatomists work with them. The microorganisms found in preparations' cadavers could be pathogenic. Although the cadaver has supported a preservation method but does not inhibit microbial germination. The purposes of this study were evaluation the use of the routine solution to decrease potential contamination. Samples were collected air dissection hall and cadavers' sections after drying the cadavers, and after use of routinely glycerin and phenol solution (1.5 g phenol in 100 cc glycerol), inoculated on eosin methylene blue agar (EMB), blood agar (BA), Sabouraud dextrose agar (SDA), and SDA with the addition of chloramphenicol (50 mg/l in ethanol solution) (SC), in a Petri dish and incubated at 25 °C and 37 °C. After 3-5 days, the morphology was studied macroscopically and microscopically. We found *Sporothrix schenckii*; *Streptomyces*, *Bacillus subtilis* and *Aspergillus flavus*, *Geotrichum candidum*, on the samples of dryness cadavers and air dissection hall, respectively. But fungus and bacteria contamination was decreased after the use of glycerin and phenol solution. Based on the results of this study, it is suggested that glycerol and phenol solution can be used at least once a week to reduce microbial contamination and prevent the spread of the disease from the cadavers to students and professors.

Keywords: Cadaver, Microbial contamination, Glycerol, Phenol

1. Introduction

Cadavers are the main studying materials of anatomists. Cadavers are a teaching tool for teachers and students but may infect persons who work with them. All of those are potentially in danger of exposure to pathogenic microorganisms like fungi, bacteria, and viruses. The infectious agent in the cadavers includes *Mycobacterium tuberculosis*, hepatitis B and C viruses, human immunodeficiency virus, *Aspergillus*, *Candida*, *Penicillium*, etc. Specific care protections are necessary to avoid accidental disease transmission from cadavers before and during dissection [1]. Therefore, the human cadavers in medical universities usually were protected for a very long time in a fixative solution, which contains formalin, alcohol, phenol, water, and glycerin. Many of the laboratories in the departments of human anatomy ignore the damaging effect of air dryness on the cadaveric sections [2]. Airborne fungi are the most important microorganism in the air, which causes contamination problems in the environment. Generally, saprophytic' fungi in many ecosystems can cause respiratory system infections, especially in immunosuppressed people [3].

Drying and hardening cadaveric tissue and fungi contamination is one of the problems predictable that to occur due to a long time of exposure cadaver to air drying, but this problem probably resolves with processed cadaveric with routinely use glycerin and phenol solution. Due to the activity of bacteria and fungi on cadavers that are drying, probably many cases of destruction will be seen in cadavers. In addition to economic damage and trouble in teaching, this subject can be cases of contamination in the dissection hall and tools, and subsequently infection the staff, students, and professors [4].

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This study aimed to evaluate the use of routinely phenol and glycerin solution to decrease the fungal contamination in the university dissection hall.

2. Materials and Methods

2.1 Study design

The cadavers used for this study were obtained from the Department Of Human Anatomy, in the dissection Hall of Guilan University of Medical Sciences, Anzali International Campus. The dissection hall was 2.7 meters in height, 10.8 meters in length, and 10 meters in width. The room has a single door and five windows with 1.4 by 1.5 meters. The floor and the walls had been tile up to the ceiling. The room has two autopsy tables made of stainless steel. The air conditioning dissection hall is administered by a simple fan system on the south wall. The dissection hall has three radiator heating systems and they were on at the time of the experiment (Figure 1).

2.2 Sample collection

All samples were taken from different surfaces of cadavers by the sterile cotton swap, after drying the cadavers, and one week after the use of routinely phenol and glycerin solution (1.5 g phenol in 100 cc glycerol). In addition, Petri dishes with a diameter of 8 cm were put at dissection hall for taken airborne microorganisms for 24 h [5, 6].

2.3 Culture

After collection, each sample was used to inoculate four different media: Eosin methylene blue agar (EMB) to suppress Gram-positive bacterial growth, Blood Agar (BA), Sabouraud dextrose agar (SDA), SDA with the addition of chloramphenicol (50mg/l in ethanol solution) (SC) to suppress bacterial growth. Agar samples were incubated at 25 °C and 37 °C and colony growth was checked daily.

2.4 Samples Staining

All fungi isolate were stained by lactophenol cotton blue and bacterial isolate were stained by Gram stain and acid-fast stain. All samples were identified by comparing the microscopic characteristics of fungi and bacterial standard produedures.

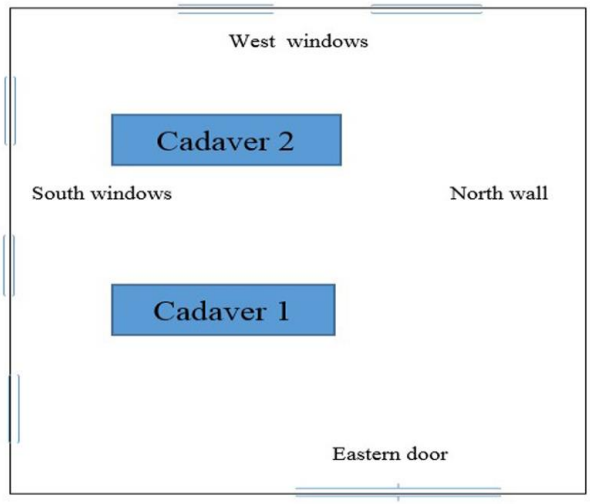

Figure 1. The plan of the autopsy room in the dissection hall of the university

Table 1. The culture results of samples from the surface of the cadavers and the air-dissection hall

Sample source	Fungi growth in SC					Bacterial growth in BA				
	Sample of head	Sample of neck	Sample of hand	Sample of foot	Sample of visceral	Sample of head	Sample of neck	Sample of hand	Sample of foot	Sample of visceral
Cadaver 1	No growth	No growth	No growth	*Sporothrix schenckii*	No growth	No growth	No growth	No growth	No growth	No growth
Cadaver 2	No growth	No growth	*Sporothrix schenckii*	*Sporothrix schenckii*	No growth	No growth	No growth	No growth	*Bacillus subtilis*	*Streptomyces* spp.
	Fungi growth in SC and SDA									
	South windows		West windows		Eastern door		North wall		Floor	
Air-dissection hall	*Aspergillus flavus*		*Sporothrix schenckii*		*Geotrichum candidum*		*Geotrichum candidum*		*Aspergillus flavus*	

EMB: Eosin methylene blue; BA: Blood agar; SDA: Sabouraud dextrose agar; SC: Sabouraud dextrose agar with the addition of chloramphenicol (50 mg/l in ethanol solution)

3. Results

All the samples were culture in the department of microbiology in Anzali International Campus. All of the sample results from the air-dissected hall were positive for fungal growth, also five samples from different surfaces of the preserved cadaver were positive for fungal and bacterial growth (Table 1). Two different bacterial colonies were observed after 72 h on BA and three different fungi colonies were observed after 3-5 days on SDA and SC media (Table 2).

All samples culture from the cadaver surface took after using glycerin and phenol solution were negative. Also, the samples culture from the dissection hall was taken after cleaning were still positive, but they were reduced.

Table 2. Colonial and microscopic morphology and biochemical identification of organisms

Organism	Colonial morphology and characteristics			Biochemical identification	Microscopic characteristics
	BA	EMB	SC and SDA		
Bacillus subtilis	Brown color, mucoid surface, wrinkled - shaped, hemolytic effect	Inhibition	-	Catalase (+), indole reaction (-), lactose (-)	Gram-positive, rod shape
***Streptomyces* spp.**	Red-pink color, mucoid surface, soil smell	Inhibition	-	Indole reaction (-), SH2 (-), motility (+), Gas (-) , citrate (+), catalase (+) Acid from lactose(-), Acid from glucose (+), Urea hydrolysis(+)	Gram-variable, motile, filamentous, non - acid-fast
Aspergillus flavus	-	-	Granular, flat, with radial grooves, white at first but quickly becoming yellow to dark yellow-green with age	-	Typically radiate, loose columns, biseriate or uniseriate, directly on the vesicle (uniseriate). Conidiophores are hyaline and roughened, often more visible near the vesicle. Conidia are spherical, pale green, and echinulate
Geotrichum candidum	-	-	Fast-growing, flat, white to cream, dry, suede-like with no reverse pigment	-	Hyaline hyphae with septate branched and break up into chains of hyaline, smooth, one-celled, and cylindrical arthroconidia
Sporothrix schenckii	-	-	Slow-growing, compact and suede-like or floccose, dry, flat, brownish-black, grey color, dark-brown diffusible pigment	-	Hyaline and produce mostly simple awl-shaped erect phialides with inconspicuous collarettes. Conidia are hyaline or pigmented, spherical to cylindrical, one-celled, and typically aggregated in heads at the apex of each phialide. chlamydospores may be present.

EMB: Eosin methylene blue; BA: Blood Agar; SDA: Sabouraud dextrose agar, SC: Sabouraud dextrose agar with the addition of chloramphenicol (50 mg/l in ethanol solution)

4. Discussion

The human cadavers in medical colleges usually were preserved in a fixative solution, which contains formalin, alcohol, phenol, water, and glycerin but after staying in the dissection hall for a long time, they will dry out. In this research study, not only the surfaces of the cadavers were examined for the presence of viable microorganisms but also the air-dissection hall was examined for dangerous infectious agents. Three different fungal colonies identified as *Sporothrix* spp. (from the surface of the cadavers), *Aspergillus* spp. and *Geotrichum* (from the air-dissection hall) and two bacteria colonies identified as *Streptomyces* and *Bacillus* (from the surface of the cadavers), the source of contamination. Those are microorganisms that transmitted by air and cause opportunistic disease [7-11]. This is a matter of concern because students and anatomists may be exposed to the infectious disease every time they work with a cadaver [1].

Normally, 10% formalin or 4% formaldehyde in a water solution is used to prevent the contamination of microorganisms. Furthermore, formalin-fixed human cadavers are routinely used for study and research in hospitals and universities. Airborne fungi have become a serious problem in dissection hall. Therefore, the presence of airborne fungi on embalmed human cadavers could be perilous for teachers and students and necessary measures must be taken to reduce fungi contamination. One report has suggested that formalin concentration for inhibiting fungal growth should not be less than 4% (5–7.5 percentage) [12]. Previously, it was showed that 15% formalin was very effective in the prevention of growth of bacteria, fungi, and also decay and discoloration [3]. The main factors leading to fungal contamination in the dissection hall are room humidity, the concentration of airborne fungal spores, and the duration of time to fungal inoculation. All instructors use routinely glycerin and phenol solution in the dissection hall to prevent drying and decrease microbial contamination of human cadavers after work with cadavers.

Glycerol (glycerin) is a simple polyol complex that uses in the dissection hall. It is colorless, odorless, sweet-tasting, and non-toxic. Multiple journals describe the antimicrobial and antiviral effects of glycerol [13], as well as, Glycerol catalyzes spore germination at about 64–70% humidity for microbial life. The germination of fungal xerophiles can occur at 60% moisture but for the most dangerous fungal xerophiles, metabolic activity and cell division typically stop between 64–70% humidity. These findings describe an inconsistency. On the one hand, glycerol can be particularly stressful and prevent cellular development. On the other hand, may be essential for cells (microbiota) to function at the water-activity limit for life, and used routinely in cadaver dissection hall [14].

Phenol derivatives isolated from the plants showed good antifungal activity that uses in the dissection room for the elimination of microbiota contaminations [15]. Phenol, or carbolic acid, is a colorless or white crystalline solid with a partly low melting point. It has been shown that phenol becomes bactericidal/fungicidal at concentrations of 1.0–1.5% and destroys cell walls. Phenol is an excellent fungicide and bactericide but it denatures proteins, with resultant drying and discoloration of tissues, and has a nasty odor. Phenol is harmful to the esophagus, causing vomiting, and headache, and faintness, loss of blood pressure, pulmonary edema, and cyanosis [16].

Despite the use of phenol for decreased microbiota contamination, and the use of glycerin to decrease resulting phenol's dryness, we cannot prevent the spread of bacteria and fungi in the indoor air and cadavers' contaminations, even by closing the windows and routinely use of glycerin and phenol solution and installing a proper ventilation system, but only we can decrease contaminations. On the other hand, the phenol has side effects, nevertheless is still used in universities to disinfect cadavers.

The use thymol for moistening the cadavers at the end of every dissection course, at least once a week. Thymol (2-isopropyl-5-methyl phenol) is naturally occurring and found in thyme oil. A thymol-ethanol solution as a humidifying solution is keeping at room temperature. Thymol has no carcinogenic or other serious effects on health but it has bactericidal and fungicidal effects and it is better than phenol [16, 17]. Suitable preservation is critical to the effective use of cadavers in educational settings. Everybody, those works in the anatomy department must ensure the safety of the cadaver from harm, decay, or disintegration [18].

In the present study, three different types of fungus (*Sporothrix*, *Aspergillus*, and *Geotrichum*) and two bacteria (*Bacillus* and *Streptomyces*) were identified. The potential infectious hazard from human cadavers is one of the main hazards associated with the anatomy department. Safe working conditions for handling cadavers can be provided through proper education, use of protective clothing, and practice of hygienic measures. Embalming decreases the risk of transmission of

potentially infectious microbial agents, but it is not possible to prevent the cadavers from drying out in the cold season due to the heating system are work. The use of glycerin for moistening and prevent drying of the cadavers and use of thymol (instead of phenol), because it is not toxic and side effects, externally at the end of every dissection course or at least once a week, is necessary and recommended.

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Author Contributions

All authors contributed equally to this manuscript and approved the final version of the manuscript.

Conflict of Interests

The authors declare that they have no conflicts of interest.

Ethical declarations

This study was in accordance with the declaration of Helsinki and an ethical permission was sought from the Head of the Institute (Anzali International Campus).

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