Student's Journal of Health Research Africa
e-ISSN: 2709-9997, p-ISSN: 3006-1059
Vol. 5 No. 12 (2024): December 2024 Issue
https://doi.org/10.51168/sjhrafrica.v5i12.1456
Original Article

COMPREHENSIVE MICROBIOTA ANALYSIS AND UV-C INTERVENTION IN EMBALMED CADAVERS: A RETROSPECTIVE STUDY ON ANATOMICAL TEACHING AND PRESERVATION

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Abstract

Background

Post-mortem specimens used for anatomy teaching are commonly embalmed using chemical compositions to maintain tissue quality and avoid putrefaction. This study was conducted during a dissection course on embalmed dissected cadavers, involving the cultivation of samples before and after exposure to UV-C radiation to elucidate microbiota growth. This study aimed to provide scientific insights into formalin embalming, guiding future research on mitigating microbiota growth through interventions like ultraviolet-C radiation and other modifications.

Materials and methods

This retrospective study was conducted at the Department of Anatomy and Microbiology, Diamond Harbour Government Medical College and Hospital, West Bengal, India. 96 samples were collected at each step of the study.

Results

Samples were collected from six sites of two embalmed cadavers along with a cadaver transferring trolley, Mackintosh gown used for dissections & cadaver tank fluid and processed for bacteriological and mycological culture. A total number of incubations were done in 96 samples, for the growth of anaerobic, aerobic & mycological species before & after UV-C radiation on the cadaver. After UV-C exposure for one and a half hours, mycological growth was reported from only three sites of cadavers but no bacteriological growth was found in any of the samples.

Conclusion

It has been concluded from the study that there were viable bacteria and fungi found on multiple sites and surfaces of embalmed cadavers & dissection hall materials which impose significant health hazards to healthcare professionals & medical students.

Recommendations

This study recommends integrating routine UV-C radiation treatment into disinfection protocols for embalmed cadavers and dissection materials to reduce microbiota growth. Additionally, it suggests further research on the efficacy of non-toxic chemical disinfectants as a potential intervention to enhance safety in anatomical education environments.

Keywords: *Cadaver, Anatomy, swab stick, mycological species, bacteriological species, UV-C radiation.*
Submitted: *2024-11-10* ***Accepted:*** *2024-12-11*

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Introduction

To preserve tissue quality and prevent putrefaction, post-mortem specimens used in anatomy classes are frequently embalmed using chemical compositions. Monitoring for bacterial or fungal contamination is becoming more and more crucial, particularly as steps are taken to reduce exposure to formaldehyde and other toxins [1]. The main goal of embalming is to lessen or eradicate the presence of microorganisms that could raise the danger of infection, given that it is done to stop the human body from decomposing. When comparing various embalming methods, anatomists would also take into account the tissue's quality, pace of decomposition, and fixative. Regarding the prevention of autolysis and putrefaction,

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formaldehyde consistently yields favorable outcomes at a reasonable price. Nonetheless, it is a recognized carcinogen that poses several health risks. In 2019, Waschke J et al. published recommendations urging anatomical institutes to use less formaldehyde. It is recommended that the formaldehyde concentration be brought down to 2% or less. Additionally, a 2% phenoxyethanol solution is advised for the preservation of cadavers in the dissection hall [2]. To reduce its harmful consequences, rigorous adherence to safety procedures is necessary. There are several substitute preservatives under investigation because of the increasing worries about environmental safety and health. Effective alternatives to embalming have been used, including plastination and cryopreservation.

The impact of ultraviolet light (UV-C irradiation) on the bacterial load on the surface of a preserved human cadaver was examined by Müller et al. (2019), who concluded that it was important for preserving cadavers without posing any risks to workers or the environment [3]. It is generally accepted that ultraviolet light, especially in the UV-C band (200–280 nm), is a commonly used radiation type for inactivating viruses and other microbes. During this process, their genetic material deteriorates and their ability to reproduce is inhibited [4]. The effectiveness of UV-C at 254 nm in lowering bacteria on surfaces has been noteworthy; the extent of the decrease depends on the particular device, cycle period, and kind of pathogen [5].

Numerous investigations have demonstrated the susceptibility of specific microbes to UV-C treatment, typically at 254 nm, as well as logarithmic decreases [6]. Nevertheless, there is a wide range in the UV-C dosage values required to achieve a particular decrease [7].

This study was conducted during a dissection course on embalmed dissected cadavers and involves cultivating samples before and after exposure to UV-C radiation to elucidate microbiota growth, including aerobic and anaerobic bacteria, and fungi. The findings were expected to provide scientific insights into formalin embalming, guiding future research on mitigating microbiota growth through interventions like ultraviolet-C radiation and other modifications.

Methodology

Study Design

It was a retrospective study.

Study Setting

This study was carried out at Diamond Harbour Government Medical College and Hospital's Department of Anatomy and Microbiology in West Bengal, India. It lasted six months during students' dissection classes.

Sample Collection

A total of 96 samples were collected in each step of the study. 48 samples each were taken before and after UV-C radiation on 2 cadavers where 6 sites were selected from each cadaver surface & 3 common sites were identified which are closely associated with cadavers for aerobic, anaerobic, and fungal growth.

Study Procedure

Samples were collected from dissected cadavers that had already been embalmed with existing embalming solutions. Cadavers were taken out from the formalin tank and a multi-site probe acquisition was performed using sterile bacterial swabs before UV-C radiation. Samples were taken from the lateral wall of the axilla, lower back at the lumbar vertebral column region, inferior surface of the liver, exposed biceps brachii muscle after dissection, gluteal cleft, the inner aspect of the lower lip, cadaver transferring trolley, mackintosh gown, tank fluid.

After sampling, the swab sticks were streaked out on MacConkey Agar and Blood Agar for aerobic bacteriological identification. The plates were then incubated in an aerobic atmosphere containing approximately 3 to 10% CO2 at a temperature of 35 ± 2°C for a duration of 18 to 72 hours. The initial reading of the plates was taken after 18 to 24 hours. They were re-incubated for further growth. Identification was made according to the Gram stain features and the bio-chemical activity shown by the isolates. Using Mueller Hinton Agar, the Kirby-Bauer disc diffusion technique was used to test for antibiotic susceptibility following CLSI'2024 guidelines.

To facilitate anaerobic growth, the swab-sticks were inoculated into Robertson's cooked meat media which were boiled beforehand in a water bath at 80°C for 30 minutes to make it O2 free. Sterile liquid paraffin was poured over the press to prevent the entry of O2 and to permit the growth of strict anaerobes. Subsequently, the media was incubated for 7 days at a temperature of 37°C to promote the growth of anaerobic bacteria. According to the Gram stain features and the bio-chemical reactions revealed by the isolates, identification was performed.

For the identification of fungal growth, one swab stick was to be placed in a test tube containing 3-4 ml of 10-20% KOH solution. A drop of the sample-containing solution was placed on a clean glass slide with a cover slip on top after an hour. The slide was then examined under a bright-field microscope using low-power and high-power objectives to look for signs of fungal elements, fungal hyphae, budding yeast cells, spores, pseudo-hyphae, etc. among the cleared specimen debris. All samples were then cultured on paired tubes of Sabouraud's dextrose agar with chloramphenicol (SDCA) slants in test tubes. All inoculated tubes were incubated for aerobic fungal culture at 25° C in a BOD incubator and at room temperature. The samples showing yeast cells in Gram stain or KOH mount were incubated for

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aerobic fungal culture at 37° C. The cultures were examined for growth daily during the first week and then twice weekly for a further four weeks before being considered culture-negative. Significant colony morphology on SDCA at room temperature, at 37° C and 25° C in the BOD incubator was examined thoroughly. Macroscopic morphology of the fungal growth and colony appearance on SDCA along with microscopic morphology seen in lactophenol cotton blue (LPCB) mount helped in the identification and confirmation of the isolated fungi. Other media like Dermatophyte test medium, Potato dextrose agar, Cornmeal agar, CHROM agar Candida, etc. were used to facilitate the identification of the isolates. The disc diffusion method was used to evaluate the antifungal susceptibility of yeast isolates following CLSI'2024 guidelines. The sensitivity test was conducted using Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue.

All the media to be used were prepared in-house from dehydrated readymade media supplied by the Hi-Media, Mumbai. After their preparation, they were preserved in the refrigerator at 4°C.

The cadavers were then placed on a cadaver transferring trolley. 2 UV lights 72watt emitting ultraviolet irradiation-C (253.7 nm wavelength) were hung from the ceiling 5 feet above the cadaver. The previously mentioned areas were subsequently exposed to UV-C for 30 minutes each from ventral and dorsal aspects with utmost precautions. During irradiation, the corpse was not covered with any cloth. The samples from the sites were collected immediately after the UV radiation & processed following the same methodology. Thereafter, the cadavers were preserved in the cadaver tank for a further phase of sampling. The samples were collected following the same methodology from the irradiated site and cultured after 3 months.

Statistical Analysis

Data has been entered in Microsoft Excel. Then, the data were arranged in columns according to subject, site of sample, and type of sample.

Ethical Clearance

Ethical approval has been granted by the Institutional Ethics Committee, Diamond Harbour Government Medical College and Hospital, West Bengal, India under letter number- DHGMC/2024/471 dated 12 March 2024.

Results

Samples were collected from 6 sites of two embalmed cadavers along with a cadaver transferring trolley, Mackintosh gown used for dissections & cadaver tank fluid and processed for bacteriological and mycological culture by techniques mentioned above. A total number of incubations were done in 96 samples before & after UV-C exposure, for the growth of anaerobic, aerobic & mycological species.

Bacteriological processing of samples from cadavers 1 and 2 showing growth of *Klebsiella pneumoniae* before UV exposure from five different sites were depicted in Table 1.

Table 1-Bacteriological processing of samples from cadavers showing growth of Klebsiella pneumoniae before UV exposure

Sl no	Subject	Site	Sample
1	Cadaver 1	Lower lip	Swab stick
2	Cadaver 1	Axilla	Scrapings
3	Cadaver 2	Biceps brachii muscle	Swab stick
4	Cadaver1	Gluteal cleft	Swab stick
5	Cadaver 2	Liver surface	Scrapings

The bacteriological processing of samples from cadavers showing growth of *Pseudomonas aeruginosa* at five other sites is shown in Table 2.

Table 2-Bacteriological processing of samples from cadavers showing growth of Pseudomonas aeruginosa before UV exposure

Sl no	Subject	Site	Sample
1	Cadaver 1	Axilla	Scrapping
2	Cadaver 1	Lower back	Scrapings
3	Cadaver 1	Liver surface	Scrapings
4	Cadaver1	Trolley	Swab stick
5	Cadaver 1	Tank fluid	Swab stick

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One site each showed growth of *Methicillin-resistant Staphylococcus aureus* and *Proteus mirabilis* which has been presented in Table 3.

Table 3- Bacteriological processing of samples from cadavers showing growth of other microbes before UV exposure

Sl no	Subject	Site	Sample	Microbes
1	Cadaver 1	Tank fluid	Swab stick	*Methicillin resistant staphylococcus aureus*
2	Cadaver 1	Liver surface	Scrapings	*Proteus mirabilis*

Pseudomonas aeruginosa, Proteus mirabilis, and *Klebsiella pneumonia* were among the gram-negative bacteria that were found in scrapings taken from the underside of the livers of both cadavers. Additionally, the tank fluid was contaminated with *Pseudomonas aeruginosa* and *methicillin-resistant Staphylococcus aureus*. Among all the 32 samples, no bacterial growth was present in 22 samples. Colony counts of bacteria were, however, not possible due to logistical limitations. After exposure of both the cadavers to UV -C radiation for one and a half hours samples were collected. No bacteriological growth was found in any of the samples. Table 4 depicts the bacteriological processing of samples from cadavers showing the growth of organisms before UV exposure.

Table 4-Bacteriological processing of samples from cadavers showing growth of organisms before UV exposure

Organisms	Total no of sites where growth found
Klebsiella pneumonia	5
Pseudomonas aeruginosa	5
Methicillin-resistant Staphylococcus aureus	1
Proteus mirabilis	1

The sample was similarly collected from a total of 16 sites and sent for mycological examination. Culture revealed growth of *Trichosporon species* in 4 sites is shown in Table 5.

Table 5-Mycological processing of samples from cadavers showing growth of Trichosporon species before UV exposure

Sl no	Subject	Site	Sample
1	Cadaver 1	Oral Cavity	Swab stick
2	Cadaver 1	Axilla	Scrapings
3	Cadaver1	Ano-rectum	Swab stick
4	Cadaver 1	Liver surface	Scrapings

Table 6 represents the mycological processing of samples from cadavers showing growth of *Gliocladium species* before UV exposure at seven different sites including muscle, lower back, trolley, tank fluid, macintosh gown, liver surface, and muscles.

Table 6- Mycological processing of samples from cadavers showing growth of Gliocladium species before UV exposure

Sl no	Subject	Site	Sample
1	Cadaver 1	Muscle	Swab stick
2	Cadaver 1	Lower back	Scrapings
3	Cadaver 1	Trolley	Swab stick
4	Cadaver1	Tank fluid	Swab stick
5	Cadaver 2	Macintosh Gown	Swab stick
6	Cadaver 2	Liver surface	Scrapings
7	Cadaver 2	Muscle	Swab stick

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Aspergillus versicolor was found from one site and *Paecilomyces variotii* was found from two sites of cadaver 2, oral cavity and ano-rectum as shown in Table 7. No fungal growth from two sites -trolley and lower back of cadaver 2.

Table 7-Mycological processing of samples from cadavers showing growth of other fungi before UV exposure

Sl no	Subject	Site	Sample	Organisms
1	Cadaver 2	Axilla	Swab stick	*Aspergillus versicolor*
2	Cadaver 2	Oral cavity	Swab stick	*Paecilomyces variotii*
3	Cadaver 2	Ano-rectum	Swab stick	*Paecilomyces variotii*

After UV -C exposure for one and a half hours, mycological growth was reported from three sites represented in Table 8.

Table 8- Mycological processing of samples from cadavers showing growth after UV exposure

Sl no	Subject	Site	Organism
1	Cadaver 2	Axilla	*Aspergillus versicolor*
2	Cadaver 2	Lower lip	*Paecilomyces variotii*
3	Cadaver 2	Gluteal cleft	*Paecilomyces variotii*

Discussion

Dissecting human cadavers is the cornerstone of learning anatomy. Medical students, facilitators, and support staff handle embalmed cadavers. Fixative substances, most frequently 10% buffered formalin with or without glycerol, salts, disinfectants, and water, are used in modern embalming procedures. Formalin used as the principal agent of embalming causes disinfection and denatures the proteins causing a hardening effect. The purpose of embalming is to stop the human body from decomposing, but its main goal is to lessen or get rid of any pathogens that could make handling cadavers more dangerous.

So, we chose to study the presence or absence of microbiota and if growth is found to identify the organism by taking samples from different sites of the cadaver and dissection hall equipment. Bacteriological analysis showed culture of *K. pneumoniae, P. aeruginosa, P. mirabilis,* and *Methicillin-resistant staphylococcus aureus.*

In a post-mortem bacteriological study conducted by Diac I et al., lung tissue/pleural fluid cultures tested positive for Enterobacteriaceae, including *Klebsiella pneumoniae*, followed by other pathogens related to healthcare, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, regardless of the postmortem interval [8]. In our study, *K. pneumoniae* and *P. aeruginosa* were found from five different sites each. These are potential pathogens and steps should be taken to prevent their spread.

Usually affecting susceptible patients in intensive care units, *K. pneumoniae* is linked to a significant percentage of ventilator-acquired pneumonia and hospital-acquired pneumonia [9]. It has been demonstrated that this bacteria can be acquired in hospitals through a variety of routes, such as direct interpersonal contact and contaminated surfaces and equipment [10]. *K. pneumoniae* was demonstrated by Centeleghe I et al. to be able to form a DSB and endure in a dehydrated state for four weeks [11].

Staphylococcus aureus/epidermidis could potentially colonize the axillary, perineal, and oronasal regions as shown by Tabaac B et al [12]. In our study, *methicillin-resistant staphylococcus aureus* was found in the tank fluid. Not many studies are available to corroborate our findings. Contamination during sampling procedures may be characterized by the presence of *Staphylococcal species*. Moreover, bacterial translocation from the gastrointestinal tract may result in colonization of the Enterobacteriaceae family like *Klebsiella species*.

In our study mycological examination revealed growth of *Trichosporon species* in 4 sites, *Gliocladium species* at 7 sites, and *Aspergillus versicolor* and *Paecilomyces variotii* was found from one site each. There is a paucity of literature to corroborate our findings.

The necessity for further research into the function of anatomical cadavers in the spread of harmful germs is highlighted by our recent findings. Developing procedures for the safe use of cadavers in medical and research facilities requires an assessment of the persistence of pathogenic organisms in cadavers. So, we adopted exposure of the cadaver to UV-C radiation and took samples from different sites one and a half hours after exposure. According to a study by Muller et al., UV-C radiation is frequently employed in cell culture facilities and industries to eradicate bacteria and fungi. No resistance formed and there is no environmental pollution because UV-C irradiation causes physical destruction. It is also a cheap and easy method to reduce microbial growth [3]. In our study, no bacterial growth was found after UV-C exposure but fungal growth of *Aspergillus versicolor* and *Paecilomyces variotii* was

found from the axilla, lower lip, and gluteal cleft suggesting improper exposure at those sites.

Conclusion

The study concluded that before being handled by medical students, cadavers had live bacteria and fungi on the body surfaces of cadavers and at different sites of the anatomy dissection room. Anatomists and students around the world may come into contact with these potentially harmful drug-resistant virulent pathogens each time they handle a cadaver, which is concerning. Proper composition of the embalming fluid and UV-C radiation exposure may be taken as combined measures to eradicate the growth of pathogens from cadavers. All anatomy labs must implement it as a universal protocol to limit the contamination of corpse microbes.

Limitations

The limitation of the study was that the colony count of bacteria and fungi could not be done in our setup which is a limitation of our study.

Recommendations

This study recommends integrating routine UV-C radiation treatment into disinfection protocols for embalmed cadavers and dissection materials to reduce microbiota growth. Additionally, it suggests further research on the efficacy of non-toxic chemical disinfectants as a potential intervention to enhance safety in anatomical education environments.

Acknowledgment

We are grateful and would like to thank those who generously donated their bodies for education and research at Diamond Harbour Government Medical College, and without their contribution, we wouldn't have been able to conduct this study. We are indebted to the Respected Prof (Dr) Utpal Dan, Principal of Diamond Harbour Government Medical College, for providing all sorts of arrangements and support.

List of Abbreviations

UV- Ultraviolet
KOH- Potassium Hydroxide
SDCA- sabouraud's dextrose agar with chloramphenicol
BOD- Biological Oxygen Demand
LPCB- lactophenol cotton blue
CHROM- chromogenic culture media

Funding

There was no separate fund for this study. Arrangement and supply of all types of required culture media, UV-C light, swab stick, Petri dish, and other required materials were arranged by the respective investigator.

Conflict of interest

No conflict of interest

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PUBLISHER DETAILS:

Student's Journal of Health Research (SJHR)
(ISSN 2709-9997) Online
(ISSN 3006-1059) Print
Category: Non-Governmental & Non-profit Organization
Email: studentsjournal2020@gmail.com
WhatsApp: +256 775 434 261
Location: Scholar's Summit Nakigalala, P. O. Box 701432, Entebbe Uganda, East Africa