Title: Fixed Cadavers as Pathogen Carriers or Educational Tools

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Abstract

Background: Cadavers are important tools for the field of human anatomy education. However, there are controversies over the necessity of using fixed cadavers for teaching purposes. Despite the antimicrobial function of the fixative agents, some reports reflected the presence of some pathogens in embalmed cadavers; these data indicate the presence of resistant bacteria. Thus, this study aimed to collect and identify bacteria species from different parts of the cadavers maintained without formalin for 6 months. **Methods:** In this study, sampling was conducted in the dissection room of Iran University of Medical Sciences. The required samples were collected from 16 different body parts of 5 cadavers by a sterile swab. Next, they were transferred to a transport media for further analysis. Then, biochemical experiments were conducted for bacterial identification. **Results:** Overall, among 80 samples collected from 16 different parts of five cadavers, 24 were positive for microbial infection. Microbial positive cultures were from various parts, including interdigital space (n=5), cubital fossa (n=4), nostrils (n=4), the inner angle of the eyes (n=3), skin over mastoid area (n=3), perineum (n=2), external acoustic meatus (n=1), inguinal area (n=1), and small intestine (n=1).

Conclusion: In conclusion, the cadavers could have detrimental viable organisms which can be a source of contamination. Therefore, following Laboratory Safety Rules and Guidelines is strongly suggested. Such a recommendation is due to the potential infection of cadavers to protect the laboratory staff.

Introduction

Anatomy is considered as the basic element of medical sciences, which has dramatically changed from 1600 BC. The cadaver was considered an essential tool for educational purposes in the field of anatomy. The anatomists fixed cadavers with formalin 10% for optimal use in the field of teaching human anatomy since the Renaissance (1). However, several controversies exist over the necessity of using embalmed cadavers to teach human anatomy (2). Nevertheless, fixed cadavers are globally applied in numerous universities for educational purposes.

Cadavers are fixed for optimal use and prevention from decay. Thus, they can be used by students for observing different body parts and learning how to dissect. Fixative agents, including formalin 10%, glycerol, ethanol, and phenol are employed for embalming cadavers. Each agent presents a specific effect on a cadaver. Formalin is a mixture of formaldehyde and water with antifungal, antibacterial, and antiviral properties [st1]. However, it provides no effects on prions (3, 4). Glycerol is a humectant, i.e., not considered as an antiseptic agent; however, it can lead to the aggregation of the antiseptic properties of formalin. Like formalin, phenol has antifungal, antibacterial, and antiviral properties. This is because of their ability the destruction of bacterial cell walls and saturation and the sedimentation of proteins and protein derivatives. However, this compound can alter the color of the tissue. Ethanol is an alcohol solvent and antiseptic agent which do not affect the spores (1, 3). Despite the antimicrobial properties of fixative agents, several reports considered the presence of resistant bacteria in various parts of fixed cadaver [1](5). Some reports have indicated that Mycobacterium tuberculosis (Mtb) remains viable 24-48 hours after embalming (6). On the other hand, some scholars suggested the possibility of the transmission of viral agents, such as viral hepatitis and Human Immunodeficiency Virus (HIV) as well as prions (3). Accordingly, cadavers could be considered as a pathogen source that can threaten the health of students and professors. Some anatomists believe that the microorganisms cannot grow on the cadavers and the corpse is completely safe. The current study aimed to investigate the odds of microorganism's growth on a cadaver.

Materials and methods

The required samples were collected from 5 male cadavers maintained in a dissection room at Iran University of Medical Sciences, Tehran, Iran. Cadavers were donated by the Forensics department and had no specified identification code. The fixation method was similar for all 5 cadavers for which 15 liters of fixation solution, containing formalin 38% (2 liters), alcohol 96% (7 liters), phenol (2 Kg), glycerin (3 liters), and distilled water (3 liters) was used. Cadavers were preserved in a formalin tank for ≥6 months and were re-transferred to a formalin tank after each educational semester for two months. The necessary samples were collected using sterile swabs from 16 different body parts. These parts included the base of the skull, popliteal fossa, oral cavity, cubital fossa, the skin covering in mastoidal area, axillary space, navel, interdigital fold, nostrils, the inner angle of the eyes, perineum, external acoustic meatus, inguinal area, small intestine, and the skin covering the popliteal fossa. Furthermore, a sample was obtained from the bed on which the cadaver was located. A sample was also collected from each part of further Ziehl–Neelsen stains. Swabs were immediately inoculated on media, including thioglycolate broth, brain-heart infusion agar, 5% sheep blood agar, and chocolate agar. Plates were incubated at 37°C for 24 hours. A sterile swab was streaked on each media and the plates were further incubated at 37°C as negative controls. Bacteria that had growth in each media were differentiated by gram staining and morphologically identified under a microscope. Gram-positive cocci were further identified by biochemical tests. These evaluations included catalase, oxidase, coagulase, urease, and alkaline phosphatase. This measure was followed by culturing bacteria in media containing blood agar, mannitol salt agar, and DNase agar to identify the potential fermentation of maltose, sucrose, and mannose. Accordingly, the gram-positive cocci were considered in the cases where biochemical tests and fermentation were positive. Mueller-Hinton agar was used for determining the zone of inhibition for antibiotics, such as Bacitracin

and Novobiocin. To determine the gram-positive bacilli, the following tests were conducted: spore staining, catalase, acid production from D-mannitol, indole test, urea hydrolysis test (urease), nitrate disk test, motility (SIM), and hemolysis on blood agar.

Results

Among 80 samples collected from 16 different parts of 5 cadavers, 24 cultured samples of the following parts were positive concerning bacteria: interdigital folds (n=5), cubital fossa (n=4), nostrils (n=4), the inner angle of the eye (n=3), the skin covering in the mastoidal area (n=3), perineum (n=2), external acoustic meatus (n=1), inguinal area (n=1), and small intestines (n=1).

Twelve bacterial species were identified by microbial tests on 24 positive samples. Accordingly, *S. aureus* (n=5) and Nocardia (n=5) were the most prevalent bacterial species (Table 1). Other identified bacteria included Staphylococcus saprophyticus (n=2), Bacillus subtilis (n=2), Corynebacterium minitissimum (n=2), Staphylococcus hominis (n=1), Clostridium perfringenes (n=1), Staphylococcus cohnii (n=1), Bacillus lentiformis (n=1), Corynebacterium jeikum (n=1), Rothia (n=1), and Micrococcus (n=1) (Table 1).

The results of Ziehl–Neelsen staining revealed that all 80 samples were negative for acid-fast bacteria, including mycobacteria.

Discussion

Anatomists use fixative agents for the preservation of cadavers. Resistance to antimicrobial agents has been reported; this is because the microorganisms can adapt to environmental and chemical conditions. Formalin is a fixative and antiseptic agent which can expire microbial agents in cadavers [2]. Phenol can inactivate bacterial and fungi enzymes as well as denature viruses, bacteria, and fungi protein. Alcohol is another group of materials with antibacterial, antifungal, and antimycosal effects [3]. Currently, phenol, alcohol, and formalin are among the most commonly-used fixatives in cadaver embalming. Numerous studies reported the

toxic effect of embalming fluid; however, there are some documentary reports in association with cadaver pathogenicity [4, 5]. This study aimed to clarify the status of the microbial population of embalmed cadavers, as data in this regard are scarce. Subsequently, the presence of bacteria was identified in different parts of the cadavers out of the formalin tank for 6 months. Such measures were performed based on culture and biochemical tests. These body parts included interdigital folds, cubital fossa, nostrils, the inner angle of the eyes, the skin covering the mastoidal area, perineum, external acoustic meatus, inguinal area, and small intestine.

Various agents, including *Myocardia* spp and *S aureus*, might threaten individuals who work with cadavers. More than 60% of human nocardiosis occurs in individuals with a suppressed immune system; males are more susceptible than females in this respect (a ratio of 3:1) [6]. Nocardia is often considered as an opportunistic pathogen that causes systemic or localized infections in humans and animals. Nocardia is mostly transmitted through respiration (respiratory nocardiosis, pulmonary abscess, & cavitation) or skin injuries (cutaneous nocardiosis). Besides, the infection can be disseminated to the brain, kidneys, joints, heart, eyes, and skeletons [7, 8]. Although nocardiosis typically occurs in patients with cell-mediated immunosuppressive conditions, the infection may occasionally develop in immunocompetent patients. Unfortunately, In this study, 5 samples were positive for Nocardia spp., i.e., mostly obtained from interdigital folds (3 isolates). Other parts were positive for Nocardia spp. included cubital fossa and the angle of the orbits. It is critical to note that a single cadaver, i.e., mostly used for teaching was positive for *Nocardia* spp in three different parts.

Moreover, *S. Aureus* was another microbial agent, i.e., often obtained from external sources. These bacteria can be inoculated by open wounds and cause infection. Most human hosts are infected by bacterial strains, which can colonize on the skin or mucosal surfaces [2, 9]. Five

samples were positive for *S. Aureus*, i.e., mostly isolated from the mastoidal skin (2 isolates). Additionally, *S. aureus* was isolated from nostrils, oculus, and the auditory canal (one isolated from each part). One of the cadavers was positive for this bacterium from three different parts. This prevalence pattern could be related to the fact that most individuals carry this bacterium and transfer that in different body parts. Such parts consist of nostrils, throat, perineum, skin, and intestine, which could be the source of transmission to the cadaver. Furthermore, *S. aureus* has been associated with various community and hospital-acquired infections. Such contaminations include bloodstream, pneumonia, prosthetic device, and surgical site infections, which may result in serious and severe consequences.

All the isolated bacteria in this study were opportunistic pathogens, microorganisms with the ability to cause infections. *Clostridium perforin genes* are the causative agent of food poisoning and gas gangrene [10]; *Bacillus lichen forms* can cause gastroenteritis and septicemia [11], and *Corynebacterium minutissimum* can induce erythrasma [12]. *Corynebacterium cohnii* is also a highly opportunistic pathogen that can lead to upper respiratory tract infections [13]. Additionally, *Bacillus subtilis* could rarely generate food poisoning [14]; *Micrococci* are associated with endocarditis [15], and *Corynebacterium jeikum* leads to pneumonia, osteomyelitis, as well as wound and soft tissue infection [16]. Moreover, *Staphylococcus saprophyticus* and *Staphylococcus hominis* can cause infection in the urinary tract [17, 18]. Besides, *Rothia* spp. can be the causative agent of endocarditis in individuals with suppressed immune systems [19].

In a study by Tabaac et al. in 2013, to reduce the possibility of contamination, sampling was conducted on embalmed cadavers before exposure to medical students. However, bacteria were isolated from the axilla, perineum, or nasal swabs (23). The study samples were collected after fixation from the parts that students are less in contact with; therefore, the odds of the incapability of fixative agents arisen in killing microorganisms during fixation. Due to the lack of access to fixed

materials in those areas, novel methods may be required for this issue (1). However, other methods should be studied for the growth of microorganisms. Gupta et al. (2013), collected samples from peritoneal cavities, perineum covering skin, subcutaneous tissue, and the muscle compartments of fixed cadavers outside the formalin tank to explore bacterial and fungal growth. In this study, samples collected from cadavers i.e., outside the formalin tank for 15 days were negative for bacterial and fungal infection. Although growth while the next sampling (after day 15) was positive for S. aureus and Aspergillus on the skin (24). In our study, a diverse bacterial population was observed in different parts of the cadaver due to the duration of being outside the tank (6 months). The current study samples were not tested for the presence of fungi. However, Sri-indrasutdhi found various fungal mycelium on two formalin-fixed cadavers. The colony on the fingers of the right foot and the colony on the throat and chin were purified and detected using Polymerase Chain Reaction (PCR) by their 16srRNA genes; they were reported as Penicillium oxalicum and Cladosporium colocasia, respectively (25). However, in this study, a suitable condition was prepared for fungal growth on embalmed cadavers. This measure was according to specific conditions, including the absence of a ventilation system for 70 days.

Conclusion

According to the diversity of the cultured bacteria from different parts of the cadaver, the cadaver can be a pathogen source apart from being a tool for educational purposes. We demonstrated that all the isolated bacteria from cadavers were opportunistic. Accordingly, they could induce infection in individuals with a suppressed immune system. We suggest laboratory personnel use protective equipment, such as gloves, gowns, and masks to prevent the transmission of pathogens while working with embalmed cadavers. It is important to note that cadavers can play a deniable role in medical education. Further studies are required to understand the transmission route of these microorganisms and design a guideline to protect individuals from pathogens agents.

	External acoustic Meatus	Mastoidal area	Nustrile	Inguinal space	Small intestine	Fingers space	Cubital Cavity	Perineum	Medial angle of eye	Total
Nocardia						3	1		1	5
Clostridium perfringenes						1				1
Corynebacterium minitisimaum						1	1			2
Staphylococcus cohnii			1							1
Bacillus Licheni formis			1							1
Bacillus subtilis					1			1		2
Corynebacterium jeikeum				1						1
Micrococcus nishinomyensis								1		1
Staphylococcus aureus	1	2	1						1	5
Rothia							1			1
Staphylococcus saprohyticuse		1							1	2
Staphylococcus hominis			1				1			2
Total	1	3	4	1	1	5	4	2	3	24

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