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Research Article – Basic and Applied Anatomy

Bacteria detected on surfaces of formalin fixed anatomy cadavers

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Summary

The purpose of this study is to determine if anatomy cadavers fixed in a formalin solution are a possible source of introduction of microorganisms into the anatomy laboratory. Routinely preserved cadavers were sampled for microbiological contaminates prior to examination and dissection by anatomy students. Regions sampled include the axilla, oral/nasal cavity, and inguinal/perineal region. Using conventional bacteriologic culture and identification methods our research group was able to successfully recover and identify a variety of organisms from all cadavers and in all regions tested.
The results indicate that cadavers processed with 10% buffered formalin have viable organisms on their surfaces that can be a source of contamination of laboratory equipment and clothing. Given the diversity of bacterial species cultured, preserved cadavers used for anatomy education as well as research must be considered a possible source for dissemination of bacterial organisms. This study underscores the importance of standard infection control protocols.

Key words

Cadavers; medical science education; research methods; contamination.

Introduction

Anatomic dissection of human cadavers is an invaluable teaching tool utilized by anatomists and medical educators. The process of embalming and preserving cadavers is necessary so that students are able to dissect and observe their subjects for an extended period of time without the risk of decay, tissue loss and pathogen transmission. Modern embalming practices involve the use of fixative agents, most commonly 10% buffered formalin with or without added glycerol, salts, disinfectants, and water. Formalin is a potent disinfectant that targets the amine functional groups in proteins, thereby denaturing them.

The cadavers used in this research study were embalmed following standard embalming practices. The procedure included tissue perfusion with a 10% formalin solution via a left femoral artery cannula, and also submersion in the same solution for six months. The cadavers were then wrapped in a 10% formalin-soaked cloth and enclosed in a vapor-proof body bag for shipping from Europe to our medical school.

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Earlier studies (De Craemer, 1994; Bayramoglu et. al, 2002; Kaufman, 2005) have shown that despite the use of fixative agents, several disease-causing agents may remain viable in preserved cadavers. Research into the efficacy of modern embalming processes in destroying pathogenic agents is ongoing and largely incomplete. The purpose of this study was to determine if any potentially pathogenic bacteria might be on the surfaces of cadavers prior to use in anatomic dissection. To this end, samples from the cadavers were obtained before the medical students had access to them, thus excluding any possible contamination of the cadaver from medical students' flora.

Materials and Methods

Samples were obtained using sterile saline swabs from the axilla, oronasal, and perineal regions from ten cadavers (three male and seven female). The Institutional Review Board has approved this study. The cadavers were fixed with a 10% buffered formalin solution with the following components: Na_2HPO_4 (70.4 g), $Na_2HPO_4.2H_20$ (49.3 g), formalin concentrated, i.e. 38% formaldehyde in double distilled water (900 ml), double distilled water (100 ml). After collection, each swab was used to inoculate 4 different media; commercially prepared Remel™ 5% sheep blood agar plates (BPA; Thermo Scientific, Waltham, MA), BBL™ Phenylethyl Alcohol Agar with 5% Sheep Blood (BPEA; Diagnostic Laboratory Services, Honolulu, HI) plates, locally prepared Mannitol Salt Agar (MSA) medium plates (Oxoid, Basingstoke, UK), and nutrient agar plates (Oxoid). Each plate was streaked for isolation of colonies using sterile disposable inoculating loops. Blood agar plates were incubated at 37°C in candle jars with low oxygen conditions; MSA, nutrient, and BPEA plates were incubated at 37°C under ambient oxygen conditions. Sterile saline swabs were inoculated as a negative control.

Once cultured, morphologic characteristics of isolated individual colonies were recorded. Slides of the individual colonies were Gram stained. These were used to confirm purity and to determine bacterial morphology. Once a pure culture was confirmed, the post isolation sample was tested for both catalase and coagulase activity (Remel Staphaurex Plus, Thermo Scientific).

Analytical Profile Index (API) strips are routinely used in microbiology settings to identify specific species of microorganisms based on a series of chemical reactions. Using the information collected from gross morphology, microscopic examination, catalase, and coagulation testing, the appropriate API strip (BioMérieux Clinical Diagnostics) was selected. Each API strip was inoculated with the appropriate sample and incubated. After incubation, the reactions were interpreted according to the instructions and tables provided by the BioMérieux test kits.

The final identification was made using the API Web computer software (BioMérieux). Acceptable identifications were made based on a confidence of 75% or greater and agreement with previously recorded laboratory test results; such as characteristic morphology, catalase, and coagulation reactions.

Results

The experiment demonstrated viable bacteria present within tested regions of all cadavers: each of the 10 cadavers exhibited bacterial contamination (10 of 10). Seven

cadavers demonstrated growth in the oronasal region, seven cadavers exhibited presence of microbes in the axilla, and eight cadavers had viable organisms in the perineum. The organisms identified in the individual sites are summarized in the Table 1. *Micrococcus* species and *Staphilococcus capitis* were identified on negative control plates. These organisms are therefore excluded from the presented data.

Table 1 – Bacteria cultured from the surfaces of formalin fixed anatomy cadavers (total # = 10)

Bacterial Species	# of cultures per body region		
	Axilla	Oronasal	Perineal
Aerococcus viridans	1	0	0
Cellulomonas	0	0	1
Corynebacterium propinquum	0	0	1
Corynebacterium striatum	0	0	1
Gardnerella vaginalis	1	0	0
Gemella haemolysans	0	0	1
Gemella morbillorum	2	3	0
Kocuria kristinae	0	1	2
Kocuria varians	0	1	0
Staph auricularis	2	0	0
Staph epidermidis	1	1	5
Staph haemolyticus	1	0	1
Staph hominis	2	1	1
Staph Lugdunensis	0	1	0
Staph warneri	0	1	0
Strep mitis	0	2	0
Total	10	11	13

Discussion

The goal of this study was to determine if bacteria could be recovered from cadavers used by medical students. The axillary, perineal and oronasal regions were chosen as sample sites because they are not usually contacted by people moving the cadavers from the formalin vats to the cadaver bags. In addition, pathogenic bacteria, such as *Staphylococcus aureus*, could potentially colonize these sites. These areas also are notable for the presence of skin folds that could potentially prevent direct contact between the formalin solution and the skin or mucosa during cadaveric preservation. The perineal region likely had the most growth because it is a site containing fecal matter. Most of the organisms found are classified as normal flora of the perineum, oronasal region, and skin.

Skin folds in the perineal region, acting as traps, were felt to contribute to the retention of viable bacteria post-preservation. Many of the organisms identified are

classified as normal flora of the perineum. Additionally, when swabbing this region, many specimens included fecal matter that may have contributed to the presence of some of the isolated bacteria.

The finding of *Gemella* species in the axilla is unusual considering these organisms are usually found in the oronasal region. The identification of *Gardenerella vaginalis* is the most unexpected finding because it was cultured from the axilla of a male cadaver. This organism is normally recovered from the female vagina.

The presence of *Cellulomonas* on one cadaver is noteworthy. This is a microorganism normally found in the soil and is most noted for having cellulase activity. It is unusual for *Cellulomonas* to be discovered on a human body.

All of the organisms identified on the cadavers can be opportunistic pathogens. Pathogenicity can occur if the microbes are found in an inappropriate body site, in an immunocompromised host, or with simple overgrowth. The number and variety of bacteria recovered suggests that if pathogenic organisms had been present pre-mortem they could survive the cadaver preparation process. The presence of *Gardnerella vaginalis* in the axilla of a male cadaver requires further discussion. This microbe is considered to be part of the normal flora of the female genitourinary tract and can occasionally be found in the nasopharynx. It is not normally found in males. This suggests the possibility of cross contamination between cadavers during cadaveric processing. Other unusual findings were the identification of *Gemella morbillorum* in the axilla of two separate cadavers, and *Gemella haemolysans* was cultured from the perineal region in one cadaver. Previous studies (Akiyama et al., 2001) have shown that the Gram positive cocci *Gemella morbillorum* and *Gemella haemolysans* are normally found in the oronasal and upper respiratory regions as well some portions of the upper gastrointestinal tract. *Gemella* has been implicated in a variety of human infections such as meningitis, septic arthritis, osteomyelitis, thoracic empyema with lung abscesses and endocarditis. These findings may indicate additional incidences of cross contamination.

In this research study, only the surfaces of the cadavers were examined for the presence of viable bacteria; however, internal organs could also harbor potentially harmful infectious agents from when the person was alive. As an example, Creutzfeldt-Jakob is a disease characterized by the presence of prions that have been detected post-embalming (Bayramoglu et al., 2002). It has previously been suggested that standard embalming methods used in cadaveric preservation are largely ineffectual in neutralizing prions. The students and faculty working with such cadavers are at risk of being exposed to prions and other infectious agents. Earlier reports (De Craemer, 1994) reveal with other pathogens, such as HIV, that the embalming process is usually sufficient to kill viruses; however, the process must be of appropriate duration.

In summary, there were viable bacteria on surfaces of all tested cadavers before medical students handled them. This is of concern because students and anatomists across the world may be exposed to potentially pathogenic organisms every time they work with a cadaver. It has been suggested that the examined preservation and disinfecting technique is inadequate to eradicate all microorganisms. Universal precautions to prevent dissemination of organisms from cadavers must be put in place in all anatomy laboratories. Cross contamination of cadavers by microbes may also occur during processing; protocols to decrease cross contamination should be instituted. Further studies could explore if bacteria obtained from the cadavers were present pre-mortem or if their presence is a result of contamination from other sources.

Our current findings raise the need for continued investigation of the role of anatomy cadavers in dissemination of pathogenic organisms. Evaluation of the persistence of pathogenic organisms in cadavers is important for developing protocols for the safe use of cadavers in medical and research institutions.

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References

Akiyama K., Taniyasu N., Hirota J., Iba Y., Maisawa K. (2001) Recurrent aortic valve endocarditis caused by Gemella morbillorum. Jpn. Circ. J. 65: 997-1000.

Bayramoglu A., Demiryurek D., Ustacelebi S. (2002) Infective agents in fixed human cadavers: a brief review and suggested guidelines. Anat. Rec. 269: 194-197.

De Craemer D. (1994) Postmortem viability of human immunodeficiency virus - implications for the teaching of anatomy. N. Engl. J. Med. 331: 1315.

Kaufman M. 2005. Dangerous dissections: the hazard from bodies supplied to Edinburgh anatomists, winter session, 1848-9. J. R. Coll. Physicians Edinb. 35: 268-274.