

Identification of Bacterial and Fungal Species in Human Cadavers Used in Anatomy Teaching

Identificación de Especies Bacterianas y Fúngicas en Cadáveres Humanos Utilizados en la Enseñanza de la Anatomía

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MOLINA, C.; BERROCAL, L.; JOFRÉ, M. R.; ROSAS, C. & ROJAS, S. X. Identification of bacterial and fungal species in human cadavers used in anatomy teaching. *Int. J. Morphol.*, 32(2):473-476, 2019.

SUMMARY: Within the framework of undergraduate and postgraduate medical education, cadavers have been used to teach anatomy by dissection or by using prosected specimens. To accomplish this, an appropriated preservation process must guarantee that the cadaver is kept safe for harm, destruction, and decomposition. Embalming fluid contains fixatives, disinfectants, surfactants, buffers, salt, and water, making the cadaver safe for teaching anatomy. However, it remains unclear if there is any risk of dissemination of microorganisms during anatomy teaching, research, and dissection procedures on fixed cadavers. The purpose of this study is to identify bacterial and fungal species in fixed cadaveric material used in anatomy teaching. Samples of cadavers and anatomical sections were cultured and biochemical tests and molecular identification by polymerase chain reaction (PCR) were performed to identify the microorganisms. The results indicate that fixed cadaveric material has viable bacteria on its surfaces and almost all these correspond to gram-negative bacilli of the *Enterobacteriaceae* family. In conclusion, fixed cadavers could be a reservoir of bacteria. This study underscores the importance of generating safe manipulation protocols to avoid eventual contamination and disease.

KEY WORDS: Bacteria; Cadavers; Dissection; Embalming; Medical education.

INTRODUCTION

Since the Renaissance, the human body has been used for anatomy teaching. In fact, the old Greek roots of the word anatomy mean “cut or separate”. Cadaver use for anatomy teaching purposes and dissection gives students a wide and three-dimensional vision of anatomical structures, allowing them to observe real variability in the human body. From a teacher’s point of view, the use of anatomy cadavers allows students to guide their learning individually, integrate into a team and develop human values. For this reason, various authors have concluded that the use of cadaveric material for human anatomy teaching, clinical training, and surgical skill acquisition is an indispensable task (McLachlan *et al.*, 2004; Gupta *et al.*, 2013; Brenner, 2014).

Usually, cadavers for teaching, research, and dissection purposes come from donations. Once the person dies, the body is transferred to the university, and the corpse may be considered as infectious material (depending on the cause of death). For example, *Mycobacterium tuberculosis* can remain active for up to 36 days after the host’s

death. Bacillus transmission from cadavers to laboratory staff members has also been reported (Sterling *et al.*, 2000; Brenner; Correia *et al.*, 2014). Hepatitis B and C viruses have been detected in cadaver blood samples and tissue banks; virus transmission has even occurred through transplanted organs. Human immunodeficiency virus (HIV) has also been isolated from the bone, spleen, bone marrow, brain, and lymph nodes of a patient 6 days after death (Demiryürek *et al.*, 2002).

In some cases when a cadaveric material is destined for anatomy research, it is tested for these pathogens before delivery; nevertheless, this is not a standard procedure in all research centers and universities.

After the reception, cadaveric material for anatomy teaching must be fixed, for which a solution containing fixatives, disinfectants, surfactants, buffers, glycerol, salts, and water is used. Among the most often used agents which act as disinfectants and/or fixatives are included the following:

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Formalin: an aqueous solution of 37 % formaldehyde, which inactivates infectious agents like bacteria, viruses, and fungi by covalent bond formation between protein functional groups. Not effective against prions (Gupta *et al.*; Brenner; Osman *et al.*, 2014).

Ethanol: used for microbial growth control through lipid disassembly and protein denaturation. It is known to be effective against fungi but not endospores, naked viruses, or prions (Brenner).

Phenol: Exerts its activity by inactivating essential enzymes and disrupting membrane lipids and is effective against bacteria, viruses, and fungi. Not effective against prions (Brenner; Osman *et al.*).

One of the objectives of the cadaver preparation process for teaching is the prevention of the growth of microorganisms like bacteria and fungi. This can be achieved with the solutions used in this process. Most of the reports related to the presence of microorganisms in cadaveric material aboard cadavers before the fixation process, which seem to be more dangerous due to their cadaver origin and cause of death.

However, only a few studies have addressed the biological risk of fixed cadaver manipulation and the dissemination of pathogenic microorganisms during anatomy teaching, research, and dissection procedures (Osman *et al.*; Hayashi *et al.*, 2014). Tabaac *et al.* (2013), showed the presence of pathogenic bacteria in 10 fixed cadavers used in anatomy practices and teaching. By sampling the clothes of students who handled the cadavers, Kabadi *et al.* (2013) identified *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pyogenes*. The presence of fungal species like *Aspergillus*, *Trichophyton*, *Microsporum*, *Candida* and *Cryptococcus* in fixed cadavers has also been reported (Brenner; Osman *et al.*).

The purpose of this study is to identify bacterial and fungal species in fixed cadaveric material used in anatomy teaching.

MATERIAL AND METHOD

Cadavers and sampling. Six cadavers, three brains and three anatomical sections from the anatomy laboratory of the University of Chile, Santiago, Chile, were used for this study. All the have being used to anatomy teaching at least for 5 years and were previously fixed with formalin solution including concentrated formalin, glycerin, ethanol, and

phenol at different concentrations (two cadavers, one brain, one anatomical section at 10 % formalin, two cadavers, one brain, one anatomical section at 5 % formalin and two cadavers, one brain, one anatomical section at 50 % of formalin).

For each cadaver, we sampled sections of the oral, nasal, and abdominal cavities, the lung and surface of the skin using cotton swabs moistened with sterile physiological serum. Samples were first cultured on 84 blood-agar plates, and positive cultures were re-cultured on other agar media.

Culture conditions. After collection, each swab was used to inoculate different culture media such as blood, soy-blood, nutrient, chocolate, MacConkey, and Sabouraud agars previously prepared according to the manufacturer's instructions (LINSAN®). After inoculation, a total of 84 plates were incubated at 37 °C under oxygen conditions at different times. In some cases, plates were incubated in GasPak jars to create anaerobic conditions. Each sample was cultured and incubated in duplicate. Sterile saline swabs were also incubated as negative controls.

Morphological analysis and biochemical identification. After culture and incubation, morphologic characteristics of isolated colonies obtained were recorded using macroscopic analysis and Gram staining of slides with individual colonies. Once pure cultures were confirmed, biochemical identification was performed. The Analytical Profile Index 10S strips (API-10S strips) for the Enterobacteriaceae family of gram-negative bacteria were used to confirm the genus and species of bacteria founded in each positive sample. For this purpose, each strip was inoculated with the appropriate sample (using 0.5 McFarland as an indicator) and was incubated at 37 °C for 24 h. After incubation, the reactions were interpreted according to the manufacturer's instructions and tables provided by BioMérieux® test kits. Acceptable identifications were made based on a confidence of 75 % or higher and previous morphological characteristics.

PCR reaction for *Salmonella enterica* identification. Molecular identification of *Salmonella enterica* strains was performed with chromosomal DNA prepared as previously described (Santiviago *et al.*, 2003). PCR amplifications were performed using an Eppendorf thermal cycler and TaqDNA polymerase (Invitrogen). Reaction mixtures contained 1X PCR buffer, 1.5 mM MgCl₂, each dNTP (200 mM), primers (1 mM), 100 ng of template DNA, and 2 U polymerase. Standard conditions for amplification were 30 cycles at 96 °C for 40 s, 58 °C for 45 s, and 72 °C for 2 hours and 30 mins., followed by a final extension step at 72 °C for 10 min. The primers used for *Salmonella enterica* identification were E14D (AGCGACAACATGCACATCAT) and E14R

(CAGGGCCGTAGTTCTTGAT) (Fuentes *et al.*, 2008). Products generated by PCR amplifications were resolved in 0.8 % agarose gels.

RESULTS

In this study seven species of bacteria, six gram-negative (85.7 %) and one gram-positive (14.3 %), were identified from fixed cadavers belonging to the anatomy department of the Universidad de Chile, Santiago, Chile.

Different bacterial species were isolated from cadaveric samples or from anatomic regions of cadavers maintained in faculties of medicine from three different universities. Almost all these correspond to gram-negative bacilli of the *Enterobacteriaceae* family, such as *Salmonella enterica*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Proteus mirabilis*, *Serratia odorifera*, and the non-fermentative gram-negative bacillus *Xanthomonas maltophilia*.

In the 10 % fixed we found *Salmonella enterica* in the brain, nasal cavity, and skin sections. *Proteus mirabilis* was found in the lung and nasal cavity and *Xanthomonas maltophilia* in the brain and body sections. In the oral and abdominal cavities, we found a gram-positive coccus of the genus *Streptococcus*. In the 5 % fixed cadaver, we found *Enterobacter aerogenes* in all samples except for the skin surface, on which we found *Serratia odorifera*. In the 50 % fixed cadaver, we found *Citrobacter freundii* in a sample from the body section. None of the samples demonstrated growth of fungi; no colonies grew on the Sabouraud agar plates.

Finally, *Salmonella enterica* isolated was evaluated through PCR amplification to determine whether it corresponded to typhoidal or non-typhoidal serovars. In all cases, the products generated by PCR amplification coincided with non-typhoidal *Salmonella enterica* serovars (data not shown).

All these microorganisms were recovered and cultured from different cadaveric samples including skin; brain; lungs; and abdominal, nasal, and oral cavities

DISCUSSION

Previous studies have shown that fixed anatomy cadavers could be sources of different bacterial species, and the risks involved in this discovery are cause for concern (Demiryürek *et al.*; Tabaac *et al.*). In this study *Streptococcus* sp. were recovered from oral and abdominal cavities. The

presence of *Streptococcus* sp. in these cavities was expected due to the composition of the bacterial microbiota and to the role of species like *Streptococcus mutans* and *Streptococcus sobrinus* in biofilms and caries formation (Nishimura *et al.*, 2012).

Proteus mirabilis, *Xanthomonas maltophilia* and *Salmonella enterica* were also recovered from cadavers fixed with 10 % formalin. Unlike *Proteus*, which belongs to the human microbiota, *Salmonella enterica* was found in the brain, nasal cavity, and skin sections, uncommon places for the recovery of this gram-negative bacillus. The presence of *Salmonella enterica* serovar Typhi, the etiologic agent of typhoid fever (Wain *et al.*, 2014), was ruled out by a PCR identification from McConkey agar-cultured colonies (data not shown). Although the presence of *Salmonella enterica* in these cavities was not expected, the recovery of this bacterium can be explained by cross-contamination of the cadaver during its manipulation. The same method of inoculation can explain the finding of *Xanthomonas maltophilia* and *Serratia odorifera* from body sections of cadavers fixed at 10 % and 5 %, respectively.

Enterobacter aerogenes was recovered in all fixed cadaver samples from 5 % fixed cadaver. This finding was not uncommon, as *Enterobacter aerogenes* can be found in the gastrointestinal microbiota. On the other hand, its presence in all samples analyzed could have been due to cross-contamination of different cadaveric samples. *Citrobacter freundii* was the only species found in 50 % fixed cadaver, its presence can also be explained by dissemination through manipulation from the gastrointestinal tract of the sampled cadaver.

It is known that properly implemented cadaveric fixation processes using formalin, ethanol, and phenol eliminate the presence and growth of bacteria, even though some viruses such as hepatitis, HIV, and prions could remain active. Therefore, transmission of microorganisms from properly fixed corpses is extremely uncommon (Kabadi *et al.*). For this reason, the finding of different microorganisms indicates that there are differences in the fixation and/or maintenance processes in the analyzed centers, which could be due to the method of fixation, an ineffective fixation, or contamination by students and manipulators.

In summary, bacteria could be present in fixed cadavers and can eventually compromise the health of manipulators and students. These findings suggest that a maintenance protocol and microorganism assessment in human cadavers used for anatomy teaching should be implemented in order to generate safe manipulation and learning.

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RESUMEN: Dentro del *curriculum* de los programas de postgrado y pregrado de las carreras de la salud, los cadáveres han sido utilizados para la enseñanza de la anatomía mediante la disección o utilizando preparados anatómicos. Para poder llevar a cabo esto, el cadáver debe pasar por un adecuado proceso de preservación; en el que se utilizan fluidos que contienen fijadores, desinfectantes, surfactantes, buffers, sal y agua, los cuales lo protegen del deterioro y la descomposición. Las soluciones fijadoras y conservadoras contienen desinfectantes, surfactantes, fijadores, buffers, sal y agua, que hacen que el cadáver sea seguro para la enseñanza de la anatomía. Sin embargo, no está claro si existe algún riesgo de diseminación de microorganismos durante la enseñanza, investigación y/o disección en estos cadáveres. El propósito del estudio es identificar especies bacterianas y/o fúngicas en material cadavérico previamente fijado, usado en la enseñanza de la anatomía. Se realizaron cultivos y técnicas de identificación molecular mediante reacción en cadena de polimerasa de muestras tomadas desde material cadavérico para identificar los microorganismos encontrados. Los resultados indican que el material cadavérico previamente fijado posee bacterias en sus superficies, la mayoría corresponde a bacilos gram negativos de la familia de las Enterobacteriaceae. En conclusión, los cadáveres previamente fijados pueden ser reservorio de bacterias. Este estudio destaca la importancia de generar protocolos de manipulación con el fin de evitar una posible contaminación y enfermedad.

PALABRAS CLAVE Bacteria; Cadáveres; Disección; Embalsamar; Enseñanza médica.

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Received: 15-10-2018

Accepted: 07-01-2019