MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

The failure on the effectiveness of formalin on cadaver disinfection and alternative methods

Ozgur YANILMAZ[1], Mehmet Mucahit GUNCU[2], Mehmet Burak AKSU[3], Mazhar OZKAN[4], Umit Suleyman SEHIRLI[4]

[1] Medical Microbiology Laboratory, Marmara University, Pendik Training and Research Hospital, Pendik, Istanbul, Turkey.
[2] Department of Microbiology and Medical Microbiology, Institute of Health Sciences, Marmara University, Maltepe, Istanbul, Turkey.
[3] Department of Medical Microbiology, School of Medicine, Marmara University, Maltepe, Istanbul, Turkey.
[4] Department of Anatomy, School of Medicine, Marmara University, Maltepe, Istanbul, Turkey.

Corresponding Author: Ozgur YANILMAZ
E-mail: dryanilmaz@gmail.com

Submitted: 23.05.2022 **Accepted:** 17.10.2022

ABSTRACT

Objective: It was aimed to identify the contaminant and determine the alternative disinfectant detection in the microbial growth observed in various parts of the cadaver stored in the formalin tank in the dissection laboratory of Marmara University Anatomy Department. We also performed a literature review of this unusual pathogen.

Materials and Methods: Swab samples were inoculated on agar mediums. After incubation, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis was used to identify the isolate from the detected uniform colonies. Sample solution from the cadaver tank, freshly prepared 5% formalin and 0.55% ortho-phthalaldehyde were used to determine the disinfectant sensitivity of the isolate.

Results: According to 16s rDNA sequence analysis, it was concluded as *Skermanella aerolata* with 99% similarity. In the disinfectant susceptibility test, it was observed that *S. aerolata* and control bacteria could grow in 5% formalin taken from the cadaver tank. No growth was detected in other disinfectants.

Conclusion: To prevent cadaver contamination in anatomy laboratories, the quality control of the embalming solutions and indoor air filtration of the dissection rooms should be checked at regular intervals. Members of *Skermanella* genus have been identified as environmental organisms in several studies, however, recent researches reported this bacterium as a human pathogen.

Keywords: *Skermanella aerolata*, Cadaver, Anatomy, Formalin.

1. INTRODUCTION

Human cadavers are essential educational and research materials for students and anatomists in medical anatomy education.

The very first proofs for use of human cadavers to examine the human body can be traced back to ancient Egypt [1].

Today, several studies have concluded that the use of cadavers is a valuable tool for anatomy education, clinical training, and development of surgical skills [2, 3].

Embalming and preservation process of cadavers used for dissection studies is essential to prevent tissue loss, decomposition and pathogen contamination. Nowadays, the main approach for embalming applications involve the use of fixative agents including formalin, phenol, thymol and glycerin [4, 5].

Among these chemicals, formalin is considered to be the most commonly used agent in embalming solutions in anatomy departments worldwide [6].

Formalin exerts its effects on tissue proteins by cross-linking the amine groups; by the way tissue becomes resistant to microbial contamination and decaying process. This chemical is bactericidal, sporicidal and virucidal; it has been reported that formalin-embalmed cadavers could be used for over a 12-months period without tissue decay [7].

Several studies have shown that despite the implementation of fixative solutions, bacterial, fungal and viral agents may contaminate the preserved cadavers [8, 9]. The detected organisms include a broad range of pathogens and non-pathogens

How to cite this article: Yanilmaz O, Guncu MM, Aksu BM, Ozkan M, Sehirli US. The failure on the effectiveness of formalin on cadaver disinfection and alternative methods. Marmara Med J 2023: 36(1):14-17. doi: 10.5472/marumj.1244383

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1244383
Marmara Med J 2023;36(1): 14-17

such as *Mycobacterium tuberculosis*, Enterobacterales members, hepatitis B and C viruses, and HIV; *Bacillus* spp., *Streptomyces* spp., *Aspergillus flavus, Geotrichum candidum, Penicillium* spp. [10, 11].

The Anatomy Laboratory is set up on the basement floor of the School of Medicine. Dissection laboratory contained formalin-fixed human cadavers for study and research purposes. The smooth, sticky, whitish substance indicating microbial growth was detected on the soft tissues and open spongy ends of the bones of the male cadaver stored in the formalin tank.

The aims of this study are to find out the cause of failure of formalin disinfection on anatomy cadaver, to determine the effectiveness of various disinfectants for the decontamination process and to detect the microbial contaminant.

2. MATERIALS and METHODS

Sampling

Embalming solution samples (20 mL) were collected from the cadaver pool to determine the effectiveness of formalin against bacteria. The affected male cadaver stored in 5% formalin had white-yellow tissue deterioration on the facial soft tissue surfaces and the spongy parts of the facial bones (Figure 1). Ten swab samples were taken from these sites with suspicion of microbial colonization.

Figure 1. *Presentation of contamination on the superficial soft tissues and spongy parts of bone tissue of the anatomy cadaver. Arrows indicate whitish-colored substance on cadaver superficial tissues.*

Microbial Isolation and Identification

Swab samples taken from cadaver surface were immediately inoculated on 5% sheep blood agar media (BioMérieux, France) and incubated at 35℃ in an aerobic environment. After 72 hours of incubation, visible uniform colonies were detected on cultured media. Gram staining performed and the isolate was determined as Gram-negative bacillus. Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis (Vitek-MS, BioMérieux, France) was used to identify the isolate [12].

However, no matching organism could be detected within the peptide profile library of the system, and microbial identification could not be made.

Genomic deoxyribonucleic acid (DNA) of the isolate was extracted by boiling method. Bacterial 16s rDNA was amplified by Polymerase Chain Reaction (PCR) using universal primers [13].

PCR product was sequenced and the Basic Local Alignment Search Tool (BLAST) was applied on resulting 16s rDNA sequence for bacterial identification [14].

Disinfectant Sensitivity Determination

In order to determine the disinfectant activity, sample solution taken from the cadaver tank, freshly prepared 5% formalin (Merck, Germany) and 0.55% ortho-phthalaldehyde (OPA, Nuova Farmec, Italy) as an alternative disinfectant were used in the disinfectant sensitivity testing.

Escherichia coli ATCC 25922 was used as the control strain. Bacterial suspensions were prepared at a density of 10^8 cfu/ml by using spectrophotometry. The bacterial suspensions and disinfectant samples were mixed at a ratio of 1/1 (v/v) and incubated at room temperature. At the 15th minute and 8th hour of the incubation, 100 µl samples were taken from the mixtures and inoculated on MacConkey agar medium (BioMérieux, France). The colonies grown on media were evaluated after 24-72 hours of incubation under aerobic conditions at 35℃ [15].

Statistical Analysis

All tests were two-tailed; p values of <0.05 were considered statistically significant. Statistical analyses were performed by using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Ethical Approval

This study was approved by Marmara University, School of Medicine Ethics Committee with the following date and numbers: 07.01.2022, 09.2022.88.

3. RESULTS

Microbial Identification

Samples taken from the facial tissues of the cadaver revealed round, convex, pink-colored colonies on 5% sheep blood agar medium (Figure 2).

http://doi.org/10.5472/marumj.1244383
Marmara Med J 2023;36(1): 14-17

Table I. *Disinfectant sensitivity test results.*

Bacteria	PBS*		Cadaver Tank		Formalin 5%		OPA** 0.55%	
	15 m	8 h	15 m	8 h	15 m	8 h	15 m	8 h
E. coli ATCC 25922	Growth	Growth	Growth	No-growth	No-growth	No-growth	No-growth	No-growth
S.aerolata isolate	Growth	Growth	Growth	No-growth	No-growth	No-growth	No-growth	No-growth

** Phosphate-buffered saline; ** Ortho-phthalaldehyde; p<0.05 (compared to formalin 5% and OPA 0.55%)*

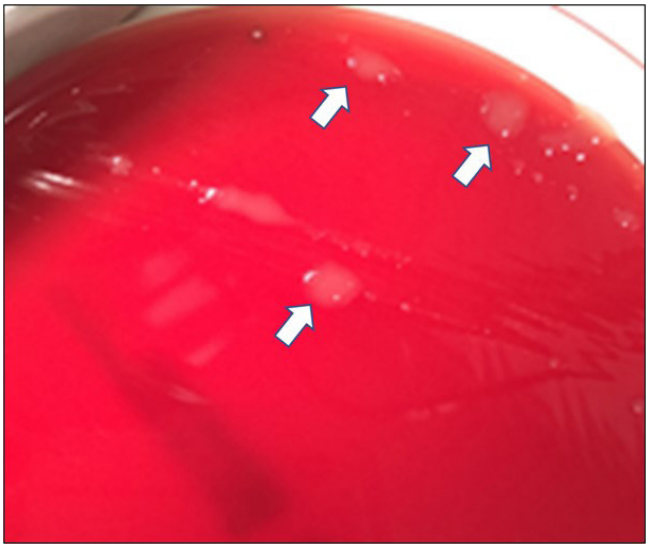

Figure 2. *Colony morphology of S. aerolata has grown on 5% sheep blood agar plate. Arrows indicate pink-colored S. aerolata colonies.*

Microscopic examination of the Gram stained smears prepared from colonies revealed Gram negative bacilli. MALDI-TOF MS analysis resulted with no match with any microorganism.

The result of the BLAST search conducted on 16s rDNA sequence of the isolate was *Skermanella aerolata* with 99% similarity with Genbank deposited reference sequence (Accession No. JX841089).

Disinfectant Sensitivity

In the disinfectant sensitivity test, it was observed that both *S. aerolata* and *E. coli* ATCC 25922 strains had grown in the cultures prepared at the 15th minute of incubation of bacteria with 5% formalin taken from the cadaver tank. Bacteria did not grow in the cultures of the same sample after 8 hours of incubation. Comparison with other substances, freshly prepared 5% formalin, and 0.55% OPA resulted with no growth of either bacterial strains after 15 minutes and 8 hours of incubation (Table I).

4. DISCUSSION

The human cadaver has been identified as a distinct educational material with unique features, such as a three-dimensional model presenting with a low health hazard and high quality of experience, and moderate cost. In a comparison of medical educational materials, human cadavers are classified as unique teaching tools without viable alternatives [3, 16].

Preservation of the cadaver is among the most important principles for the use of the human body as a teaching tool. The process of preservation is accepted as adequate if the cadaver is kept safe from contamination, destruction or decomposition. This is maintained by the cadaver treatment with embalming solutions such as formalin [3].

However, environmental conditions (dryness, humidity, etc.), errors in preparation of embalming solutions, and storage problems may cause microbial contamination of cadavers.

Microorganisms identified on the cadavers can be endogenous (related with body microbiota members) or exogenous.

In this study, we tested the disinfectant sensitivity of the *S. aerolata* isolate and *E. coli* ATCC25922 to embalming solution (5% formalin) from cadaver tank in anatomy laboratory, freshly prepared formalin (5%) and OPA 0.55% which is commonly used as a high-level disinfectant for medical instruments. Both bacterial strains were found to be resistant to embalming solution from cadaver tank in 15 min exposure time. These findings suggest that there is a problem with the embalming solution sampled from the cadaver tank, such as an error in preparation or activity loss due to environmental conditions.

In addition, the bacterial agent obtained from cadaver's superficial tissues was identified as *S. aerolata.* The genus *Skermanella* was first described and classified in the genus *Conglomeromonas* in 1983 by Skerman et al.[17].

Later, this bacterium was determined to be a new genus and was named in honor of Skerman who made the first identification [18].

Skermanella aerolata was first isolated from air samples in South Korea in 2007 by Weon et al. The bacteria has Gram-negative bacillus morphology, obligate aerobe with polar or subpolar flagella. Colonies are light pink in color, convex, rounded shape and have open margins. It can express high salt tolerance (up to 5%) and can grow at low temperatures (down to 5°C) [19].

Skermanella aerolata was accepted as an environmental microorganism till it was isolated from a human breast milk specimen [20].

In the second report, *S. aerolata* was detected as the cause of necrotizing fasciitis on the lower extremity of the patient [21].

In conclusion, we reported that a potentially pathogenic bacterium present in nature may cause cadaver contamination. Effective measures such as quality control of embalming solutions, indoor air filtration of dissection halls should be implied to prevent

http://doi.org/10.5472/marumj.1244383
Marmara Med J 2023;36(1): 14-17

contamination of cadavers in anatomy laboratories which in turn may threat students and anatomists studying with cadaver.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Marmara University, School of Medicine Ethics Committee.

Financial Support: The authors have no relevant financial information to disclose.

Conflict of interest: The authors have no conflicts of interest to declare.

Authors' Contributions: OY and BA: Designed the study, OY, MMG, and MO: Performed the laboratory work and analysed the data, BA and MMG: performed the statistical analysis, OY, BA, MMG and USS: wrote the manuscript. All authors approved the final manuscript.

5. REFERENCES

[1] Loukas M, Hanna M, Alsaiegh N, Shoja M M, Tubbs R S. Clinical anatomy as practiced by ancient Egyptians. Clin Anat 2011; 24: 409-15. doi: 10.1002/ca.21155

[2] Gupta J, Chaturvedi M, Patil M. Embalmed cadavers – are they safe to handle, a study to see the microbial flora present in the embalmed cadavers. Int J Pharma Bio Sci 2013; 4:383-6.

[3] Brenner E. Human body preservation – old and new techniques. J Anat 2014; 224:316-44. doi: 10.1111/joa.12160.

[4] Kalanjati VP, Prasetiowati L, Alimsardjono H. The use of lower formalin-containing embalming solution for anatomy cadaver preparation. Med J Indones 2012; 21:203-7.

[5] Ruhsar E, Demiraslan Y. Kadavra hazırlamada kullanılan solüsyonlar ve güncel yaklaşımlar. Dicle Üniversitesi Veteriner Fakültesi Dergisi 2018; 11:105-8.

[6] Benkhadra M, Gerard J, Genelot D, et al. Is Thiel's embalming method widely known? A world survey about its use. Surg Radiol Anat 2011; 33: 359-63. doi: 10.1007/s00276.010.0705-6

[7] Balta J Y, Cronin M, Cryan J F, O'Mahony S M. Human preservation techniques in anatomy: a 21st century medical education perspective. Clin Anat 2015; 28:725-34. doi: 10.1002/ca.22585

[8] Demiryurek D, Bayramoglu A, Ustacelebi S. Infective agents in fixed human cadavers: a brief review and suggested guidelines. Anat Rec 2002; 269: 194-7. doi: 10.1002/ar.10143

[9] Kaufman M. Dangerous dissections: the hazard from bodies supplied to Edinburgh anatomists, winter session, 1848-9. J. R. Coll Physicians Edinb 2005; 35: 268-74.

[10] Sri-Indrasutdhi V, Ueapattanakit J, Sommatas A. Investigation of airborne fungi and their ability to grow on formalin-fixed human cadavers. Mycosphere 2015; 6:729-36. doi:10.5943/mycosphere/6/6/8

[11] Faghani L M, Farzanegan A. Decrease of microbial contamination by application of routinely glycerol and phenol solution on human cadavers. J Curr Biomed Rep 2021; 2:74-8.

[12] Dworzanski J P, Snyder A P. Classification and identification of bacteria using mass spectrometry-based proteomics. Expert Review of Proteomics 2005; 2:863-78. doi: 10.1586/14789450.2.6.863

[13] Weisburg W G, Barns S M, Pelletier D A, Lane D J. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991; 173:697-703. doi: 10.1128/jb.173.2.697.703.1991

[14] Altschul S F, Gish W, Miller W, Myers E W and Lipman D J. Basic local alignment search tool. J Mol Biol 1990; 215:403-10. doi: 10.1016/S0022-2836(05)80360-2

[15] Akamatsu T, Minemoto M, Uyeda M. Evaluation of the antimicrobial activity and materials compatibility of orthophthalaldehyde as a high-level disinfectant. J Int Med Res 2005;33:178-87. doi: 10.1177/147.323.000503300205.

[16] Brenner E, Maurer H, Moriggl B and Pomaroli A. The human cadaver as an educational tool – classification and comparison with other educational tools. Ann Anat 2003; 185: 229-30.

[17] Skerman V B D, Sly L I, Williamson M L. *Conglomeromonas largomobilis* gen. nov., sp. nov., a sodium-sensitive, mixed-flagellated organism from fresh waters. Int J Syst Bacteriol 1983; 33:300-8.

[18] Sly L I, Stackebrandt E. Description of *Skermanella parooensis* gen. nov., sp. nov. to accommodate *Conglomeromonas largomobilis subsp. parooensis* following the transfer of *Conglomeromonas largomobilis subsp. largomobilis* to the genus *Azospirillum*. International Journal of Systematic and Evolutionary Microbiology 1999; 49:541-4.

[19] Weon H Y, Kim B Y, Hong S B, et al. *Skermanella aerolata sp. nov*., isolated from air, and emended description of the genus *Skermanella*. Int J Syst Evol Microbiol 2007 ;57:1539-42. doi: 10.1099/ijs.0.64676-0.

[20] Onori R, Marín M, Rodríguez-Sánchez B, et al. First isolation of *Skermanella aerolata* from a human sample. Rev Esp Quimioter 2018; 31:552-3.

[21] Heo S T, Kwon K T, Yoo J R, Choi J Y, Lee K H, Ko K S. First case of necrotizing fasciitis caused by *Skermanella aerolata* infection mimicking vibrio sepsis. Ann Lab Med 2018;38:604-6. doi: 10.3343/alm.2018.38.6.604.