Identification of *Oligella ureolytica* from Formalin-Embalmed Cadavers

Amin A. Bakir¹, Pinar K. Kudhur¹*, Lajan Q. Rahman¹

¹- Department of Microbiology, College of Medicine, Hawler Medical University, Erbil, Kurdistan Region, Iraq.

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*Corresponding Author:*
Pinar K. Kudhur  
Pinar.khudur@med.hmu.edu.krd

**ABSTRACT**

Formalin is a widely used fixative for embalming of cadavers with a well recognized antimicrobial activity. However, with improper preservation techniques some human pathogens such as *Oligella ureolytica* can overcome this feature of formalin. The current study was performed to detect this microorganism and consequently prevent its risks to medical students and embalmers. Swab and tissue samples were collected from lesions, intact area, and surrounding surfaces of three formalin embalmed cadavers from tutorial anatomy lab in the college of medicine - HMU. Samples were subsequently transferred to nutrient, MacConkey, and blood agar then, purified colonies were subjected to biochemical tests and microscopic observation. Accordingly, advanced automated instrument was applied to confirm identification. The isolated microorganism was then inoculated under increasing formalin concentrations. The genus *Oligella ureolytica* was identified which appeared as Gram negative, rod shape with singular arrangement under microscopic observation. The biochemical tests revealed positive for urease, oxidase, catalase, and motility with no evidence for hemolysis. The growth of *Oligella ureolytica* was significant at 1% and 2% and low at 5% and 10% formalin concentration but no growth at higher concentrations. There are many reasons to choose formalin as fixative for embalming. However; it is not totally able to protect cadavers from decomposition by microorganisms like *Oligella ureolytica*. Thus, possible risk of contamination for medical students and embalmers exists when a fixative of low concentration was used.

1. INTRODUCTION

Embalming in most modern cultures is the art of temporarily preserving human remains from forestall decomposition to make it suitable for display at a funeral. It is also, a discipline on preserving human body for anatomical study (Coleman and Kogan, 1998). The process of embalming is performed with a mixture of chemicals called an arterial solution which is a combination of formaldehyde and/or glutaraldehyde. Formalin refers specifically to 40% aqueous formaldehyde used in the preservation of anatomical specimens (Bajracharya and Magar, 2006) and it is indeed, an ancient companion of embalmers throughout centuries (Bedino, 2004). The first documented human cadaver to be embalmed with formaldehyde has been reported in 1899.
Later, over a century, only a minor amendment has been occurred to the basic chemistry or technique of preserving human cadavers with formaldehyde (Bedino, 2003). As a colorless irritating gas, formaldehyde is a potent lachrimal and the most copious airborne aldehyde (Cikmaz et al., 2010). It has the capacity of explosion on exposure to fire as such it is a potentially human carcinogen (Ongwandee et al., 2009). Formaldehyde is used in embalming in regard to its disinfection and fixation properties. Basically, there are three competing reaction scenarios in formaldehyde fixation: First, rapid reaction and coagulation with reversible adducts. Second, stabilized bridging but susceptible to acid-hydrolysis and reversal. Third, a considerable endpoint bridging is possible through resistance from high acid-hydrolysis that ends with a stable fixation. The outcomes of endpoint protein fixation can be seen as inter and intra molecular cross linkages that create insolubility, widespread hydrophobicity, trapping of a range of macromolecules in the stabilized matrix of cross linked polypeptides, and dehydration of microbiological interaction and chemical attack (Bedino, 2003) However, some microorganisms can overcome these properties of formaldehyde particularly at its lower concentrations (Sterling et al., 2000). Among the most resistant bacteria species against formaldehyde effects is the genus of Oligella.

Oligella ureolytica (formerly CDC group IVe) is a non-fermentative Gram-negative bacillus harboring urease enzyme capable to induce infection in the urinary tract of human. In vitro, colonies of Oligella ureolytica are first seen as slow-growing on blood agar medium. They are white, opaque, entire, and non-hemolytic colonies that phenotypically resemble asaccharolytic Achromobacter, species such as Bordetella bronchiseptica, and Cupriavidus pauculus in that they are non saccharolytic, oxidase-positive, and motile by means of peritrichous flagella. Most isolates have been obtained from human urine, often in patients with long-term indwelling catheters also cases of bacteriemia in patients with obstructive uropathy, AIDS, and pneumonia (Winn et al., 2006). There is hardly any study on the effect of fixatives on Oligella species. It was recognized in our anatomy lab that it can interfere with the preservation of human cadavers leading to decomposition tissues even in the presence of formalin. Our research team tried to tackle this problem through the study of lesions occurred over cadavers embalmed with formaldehyde fixative.

Thus, the current research is a scientific investigation with microbiological approach primarily to identify the causative agent decomposing embalmed cadavers. Moreover, it is important to estimate the proper protective concentration of fixative against the microorganism.

2. MATERIALS AND METHODS

2.1. Study Design

A prospective investigating study was done that involved microbiological experiments. Seven samples were collected using sterile swabs from three cadavers with apparent lesions preserved in anatomy lab in the college of medicine, Hawler Medical University:

Cadaver No.1 lesion of knee, intact body parts, surface of basin, and cover plate of basin.

Cadaver No 2 lesion of shoulder and surface of basin.

Cadaver No.3 lesions on face

2.2. Requirements and Preparations

To achieve the best results three different media were used with the following processing: 1- Nutrient agar (7g nutrient agar powder dissolved in 250ml distilled water). 2- Blood agar (10g blood agar powder mixed in 250ml distilled water). 3- MacConkey agar (13g MacConkey agar powder mixed in 250ml distilled water)
2.3. Cultivation

Each sample was inoculated into blood agar, MacConkey agar, and nutrient agar and incubated at 37°C overnight. An additional nutrient agar culture was used for anaerobic incubation in anaerobic jar at 37°C for 24 hours. Purification of microorganism is performed on blood agar the day after. Accordingly, colonies of microorganism were taken for microbiological and biochemical tests including urease test oxidase test, catalase test, and motility test.

2.4. Identification of Microorganism

Two methods were used for identification of microorganism in which manual tests for primary identification and subsequently confirmation of species was done using Phoenix instrument to verify results attained from manual procedures.

2.5. Minimal Inhibition Dose

A series of gradually increasing concentrations of formalin were made in distilled water as 1%, 2%, 5%, 10%, 15%, 20%, 25%, and 30% with studied microorganism being mixed in them. Consequently, each suspension was inoculated into blood agar to define the inhibitory effect of each concentration.

2.6. Data Analysis

Descriptive and inferential statistics were applied to the obtained data using Statistical Package for Social Science (SPSS v.18.0). The data were graphed using GraphPad Prism v.6.1.

3. RESULTS

3.1. Cultivation of Samples

The inoculation of samples showed growth of microorganisms on nutrient agar, MacConkey Agar and blood agar (Figure 1).

![Figure 1](image1.png)

Figure 1 Inoculation of samples generated colonies. A) Spread and pinpoint colonies on nutrient agar under aerobic condition. B) Large, flat and mucoid colonies on nutrient agar under anaerobic condition. C) Small, pinkish, convex and round colonies on Mac Conkey agar. D) Pinpoint, convex, white-grey colonies with no hemolysis on blood agar.

3.2 Sub-culturing and purification of bacteria

Singular colonies of bacteria were transferred to new Mac Conkey agar. There was growth of bacteria under aerobic and anaerobic conditions (Figure 2)
3.3 Microscopic Observation
Using the purified colonies of bacteria after gram’s staining the microscopic characteristics were: rod-shaped, singular, gram negative microorganisms (Image is not available).

3.4 Biochemical Tests
A number of biochemical tests were performed in order to characterize the isolated microorganism (Table 1)

| Biochemical Tests | Results                     |
|-------------------|-----------------------------|
| Catalase          | Slightly Positive           |
| Oxidase           | Positive                    |
| Motility          | Positive                    |
| Urease            | Positive                    |
| Hemagglutination  | (α-hemolysis)               |

3.5. Identification and Confirmation of Isolated Species
The characterized microorganism was investigated for verification for which an automated microbiology system for microbial identification (BD Phoenix, USA) was used. The analysis revealed a species of bacteria as: Oligella ureolytica.

3.6. Growth on Diluted Formalin
The isolated Oligella ureolytica colonies were treated with increasing concentration of formalin (see 2.2.4) then inoculated into nutrient agar. The results from colony formation units (CFU) counts demonstrated growth of bacteria at lower concentrations (i.e. 1%, 2%, and 5%), a poor growth at 10% and absolutely no growth at 15%, 20%, 25%, and 30% formalin (Figure 3).

Figure 2 Bacterial colonies after re-inoculation. A). Pinpoint, convex, smooth, grey-white colonies on MacConkey agar under aerobic condition B) Mucoid, colorless, spread, overlapping and odorous colonies on MacConkey agar under anaerobic condition.

Figure 3 Isolated Oligella ureolytica was found capable to grow under low concentration of formalin. There is a significant decrease in CFU count with increasing the concentration. Error bars: Min and max values (n= 5)

4. Discussion
In this prospective study samples were taken from suspected parts of embalmed cadaver that were undergoing decomposition. This can rarely occur and unexpected as long as the entire body has been embalmed using 10% formalin. Thus, our samples were taken from different sections of cadaver's body in addition to surrounding area to cover all
possibilities for detection of causative factor of decomposition. Given that, sampling was performed at one time with the exactly same processing for each sample, there is very few chance of variation if any for collected samples to give non-related outcomes.

The inoculation of samples (Figure 1) and sub-culturing of colonies (Figure 2) at the earliest stages of this investigation made it evident that a microorganism can most probably be the responsible factor of corrosion. Since, other potential factors as higher temperature, improper embalming procedures, and even physical factors were not involved or at least not effective to induce deterioration of preserved bodies.

The biochemical tests (Table 1) and microscopic observations provided clues to identification of bacteria called Oligella ureolytica. In order to further confirm the results, the purified colonies of bacteria was submitted to the reference laboratory where our findings verified by Phoenix microbiology analysis system which offers an advanced technology specific for identification of bacterial species.

Despite the fact that formalin fixative was applied to all cadavers, it was not surprising to detect Oligella species from dead body specimens. Hence, supporting literature imply the view that this genus of bacteria are able to grow over dead tissue. There are numerous reports from burn units indicating for common presence of Oligella ureolytica contaminating burned skins particularly among those patients suffering from low hygiene condition and lack of proper decontamination procedures (Singleton and Sainsbury, 2002). Though, Oligella species are mostly attributed to urinary tract infection; they are potential human pathogens capable of inducing diseases especially among those with suppressed immune system namely AIDS patients (Bedino, 2003). So that, in the absence of immune surveillance this genus of bacteria can grow over the body surfaces and deteriorate the skin which can frequently occur in burns. However, in the presence of formalin there is limited opportunity of growth for microorganisms, as it's been revealed in samples of cadavers taken from deep formalin rinsed parts. These findings create enquiries that there is possible link between low concentration of formalin and consequent growth of microorganisms leading to deterioration of body surfaces. It can be perfectly proven by our findings (Figure 3), that there is a significant association between reduced formalin concentrations with increased CFU counts. In this regard, the consistency of results was assured by applying minimum of five replicates per concentration in order to be statistically representative. In view of the fact that colony formation units only represent those microorganisms that have grown on media, it should be mentioned that lack of CFU counts would not necessarily and may not exactly mean that the bacteria are killed. Nevertheless, CFU counts are the main procedures that have been universally used for measuring the growth of bacteria (Kairo et al. 1999).

It is well known that formalin has a strong anti-microbial activity used for prevention of fungal and bacterial growth (Sterling et al., 2000) meanwhile; our results implied a defect in such function of formalin. The problem with formalin fixation that has been known since 1902 is the reversibility and susceptibility to acid hydrolysis of the coagulated protein. In many instances, there is significant formalin wash-off, i.e. non – reacted or reversed formaldehyde found in post-treatment buffer wash. The amount of formalin that does not wash out is reacted in a dehydration reaction. Reversing of fixation and acid hydrolysis has been known to be possible since the early 1960’s by acid catalyzed hydrolysis, water
immersion or heat, or a combination of above. It seems, in general that weaker and reversible links are generated during fixation results in a significant amount of acid – resistant linkages. As a side note, surprisingly and counter-intuitively, fixation does not alter the secondary structure of proteins. The more complex tertiary structures are however, probably seriously affected by coagulation and fixation (Singleton and Sainsbury, 2002). In this regard, bacteria with potential effects on protein deformation can overcome the function of fixative among them Oligella Ureolytica which is pathogen of human urinary tract. The bacteria possess urease enzyme that can block formaldehyde function (George and Garrity, 2005). However, when proper concentration of formalin is applied as displayed in this study, the microorganisms will remain harmless subsequently then, safer ground will be provided for medical students and embalmers.

5. CONCLUSIONS

There is always a variety of risks to medical students and staffs. This study addressed one of such risks that can occur in spite of routine precautions and application of known antimicrobial agents such as formalin. In view of that, isolation of Oligella ureolytica which is a known human pathogen from formalin fixed cadavers could be hazardous upon any improper fixation or a low concentration of formalin.

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Conflict of interest

This study was performed and funded by the authors. There is no conflict of interest

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