

# Effectiveness of Sterilization and Disinfection of Extracted Human Teeth for Institutional Use: A Case Control Study conducted at a Tertiary Care Hospital

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## ABSTRACT

**Objectives:** To determine the most effective method of sterilization and disinfection of extracted human teeth for use in dental colleges..

**Study Design:** Case Control study

**Place and Duration of Study:** This study was conducted at OMFS & Microbiology Department, KMDC, Karachi from June 2013 to December 2013.

**Materials and Methods:** Freshly extracted human teeth (n=50) were obtained and sent for bacteriological processing. Teeth were dividing into 5 groups; samples were taken pre and post treatment. A platinum wire loop was flamed in red heat and cooled; sample was inoculated in enrichment media. Plates were placed in incubator at 37C for 48 hours. Colony count was noted to observe the quantity of microorganism, which determines the efficacy of the sterilizing method.

**Results:** The results of the study revealed that the autoclave, hot air oven had shown no growth. While 5 % sodium hypochlorite, hydrogen peroxide and normal saline had shown positive growth of microorganisms.

**Conclusion:** Autoclave and Hot air oven are effective methods of sterilization of extracted human being teeth for use in dental college in preclinical settings.

**Key Words:** Sterilization and Disinfection, Preclinical, Microbiology, Extracted teeth, Institution

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## INTRODUCTION

Extracted teeth used customarily in dentistry preclinical settings in dental colleges to teach and to build the technical and preclinical skills of the students. Some endodontic procedures can be teach using models, plastic blocks, and typodont tooth models for practicing in the beginning of the course and before entry to the clinical environment. However, there are instances when no substitute is available and students uses extracted teeth for examination, restorative and endodontic preparations or for research purpose.<sup>1</sup> Human extracted teeth used in dental institutes to teach the clinical methods for patient's treatment. More over these teeth were used for various purposes such as preparing ground section for histological study and for learning endodontic procedures such as cavity preparation, root canal treatment, developing and

testing various restorative materials, for crown preparation, and inlay.<sup>2</sup>

In recent years, Guidelines for the control of infection in dental institutes had revised due to likelihood of fractious infectivity from extracted teeth. Commands by American Dental Association (ADA) and Centre for Disease Control (CDC) called for removal of any organism capable of transmitting diseases from non-disposable items/instruments that comes in direct contact with blood stream and may transmit diseases like Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) used in patient care. The materials might be expose to blood or saliva. These body fluids were associated with extracted human teeth that are used in dentistry to setup system and skills.<sup>3</sup> In a recent study conducted at Institute of Dental Sciences, Uttar Pradesh, India, it was evident that formalin, hypochlorite, and autoclaving was the choice of sterilization of extracted human teeth for institutional use and extracted teeth are hazardous and should handled carefully.<sup>4</sup>

Extracted human teeth most commonly used in many institutes of Pakistan and after in depth search of data

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scant or no data available in Pakistan to determine the effectiveness of various methods of sterilization and disinfection on extracted teeth. Hence, this study was planned with the rationale that to determine the most effective method of sterilization and disinfection of extracted human teeth for institutional use.

## MATERIALS AND METHODS

This study was conducted at Department of Oral and Maxillofacial Surgery and Department of Microbiology, Karachi Medical and Dental College (KMDC) from June 2013 to December 2013. In this case control study the teeth of the patients i.e. cases and controls were recruited through non-probability, convenience-sampling method. The inclusion criterion was intact freshly extracted human permanent molars from both mandibular and maxillary arches. The exclusion criterion was third molars from both arches, teeth having amalgam filling and broken down roots or teeth and patients with known contagious disease.

Crudely extracted human teeth (n=50) were obtained from the Department of Oral and Maxillofacial surgery of Karachi Medical and Dental College (KMDC) and bacteriological process was carried out in the Department of Microbiology, Karachi Medical and Dental College and Abbassi Shaheed Hospital.: Total 50 teeth randomly divided into 5 groups each group having 10 teeth. Group 1 i.e. Autoclave is a control group while group 2 -5 are identified as Case groups. The teeth were kept in a container immersed in distilled water and then treated as follows.

Group – 1: Teeth were autoclaved at 121 degree Centigrade at 16 lbs pressure for 20 minutes duration as Control group.

Group – 2: Teeth were kept in hot air oven.

Group –3: Teeth were engrossed in 10ml of 5 % sodium hypochlorite in bottle for 7 days.

Group –4: Teeth were engrossed in vinegar for 7 days.

Group - 5: Teeth were engrossed in Normal Saline for 7 days.

A sample taken before treatment and after treatment from the container group wise. A platinum wire loop was flamed in red heat in burner and cooled. A loop full sample inoculated in simple, selective, enrichment media like Nutrient agar, Blood agar, Macokonkey agar. The plates placed in incubator at 37C for 48 hours. After 48 hours of incubation, growth in different types of colony, its size, consistency, lactose fermentation haemolysis etc observed carefully. Based on colony morphology, Grams and Biochemical reaction the organisms identified. The colony count noted to observe the quantity of microorganisms.

Efficacy of the sterilizing method was judged by assessing the colony count. No or minimal growth was considered as most effective method of sterilization.

## RESULTS

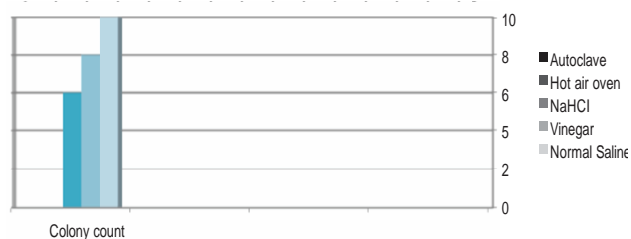
The results of the study revealed that the autoclave, hot air oven had shown no growth. Sodium hypochlorite 5%, hydrogen peroxide, and normal saline had shown positive growth. (Table- I and figure I).

**Table No. I: Showing colony count and growth of bacteria after using different disinfection and sterilization methods**

Type of Disinfection/ Sterilization	Duration	No. of Teeth	Efficacy of different sterilization methods and disinfectant solutions/ Colony count (10)	Growth of Bacteria
Autoclave at 121 C, 16lbs pressure(Control)	20 min	10	0	No growth
Hot air oven at 170 C	1hour	10	0	No growth
5 % Sodium hypochlorite	7 days	10	6	Growth positive
Vinegar	7 days	10	8	Growth positive
Normal Saline	7 days	10	10	Growth positive

**Table No.2: Infection control guidelines for use of extracted teeth in dental educational settings<sup>(18)</sup>**

- Extracted teeth used for the education of dental health care workers should be considered infective and classified as clinical specimens because they contain blood.
- All persons who collect, transport or manipulate extracted teeth should handle them with the same precautions as a specimen for biopsy.
- Before extracted teeth are manipulated in dental educational exercises, the teeth first would be cleaned of adherent patient material by scrubbing with detergent and water or by using an ultrasonic cleaner.
- Teeth should then be stored, immersed in a fresh solution of sodium hypochlorite (household bleach 1:10 with tap water) or any liquid chemical germicide for clinical specimen fixation.
- Persons handling extracted teeth should wear gloves. Gloves should be disposed off properly and hands washed after completion of work activities. Additional personal protective equipment e.g. face shield or surgical mask and protective eyewear should be worn if mucous membrane contact with debris or spatter is anticipated when the specimen is handled, cleaned or manipulated.
- Work surfaces and equipment should be cleaned and decontaminated with an appropriate liquid chemical germicide after completion of work activities.



**Figure No.1: colony count with different methods of disinfection and sterilization**

## DISCUSSION

Sterilization embrace absolute demolition of the entire forms of microbial existence together with bacteria viruses and spores on the surface of an entity or in a fluid to thwart disease spread linked with the use of that item. Whereas the disinfection process is proposed to considerably, diminish the quantity of pathogenic microbes on instruments by removing and/or killing them<sup>5</sup>. Extracted teeth serve for educational tool in teaching institute in particular to dental students. It was documented HIV, HBV, HCV, aerobic and anaerobic bacteria were present as pathogenic and non-pathogenic state in pulp, radicular and periradicular tissue of extracted human teeth.<sup>6,7</sup>

It was evident from the results of our study that sterilizing human extracted teeth within autoclave and hot air oven revealed no growth of microorganisms after sterilization. This is in consistent with the study, which showed autoclave as effective and recommended method of sterilization.<sup>8,9,10</sup> The use of autoclave is simple, willingly accessible, contemptible and appropriate technique of sterilization. It does not modify the “sense” and cutting characteristics of teeth. This finding supported by earlier studies on functional characteristics of extracted human teeth. Further, it could effectively destroy and kill all types of microorganisms. There is a apprehension regarding using it for sterilization of extracted teeth with amalgam restorations as it may liberate mercury fumes in the air through exhaust remaining mercury pollution of autoclave and hot air oven<sup>11,12</sup>. The thermal cycling may cause teeth with amalgam restorations to fracture due to their differences in co-efficient of thermal expansion. This is the reason we kept amalgam restorative teeth in exclusion criteria.

Sterilization with 5 % sodium hypochlorite showed little growth of microorganisms. This is not in consistent with the study conducted at India that revealed that sodium hypochlorite 5% could be efficiently used method for sterilization as it showed no growth of microorganism on culture media.<sup>10</sup> Another study conducted at University of Mosul revealed that NaOCl and autoclave prevented the growth completely in all types of the bacteria that were used to infect the teeth i.e. *Proteus* species, *Escherichia coli*, *Kelebsiella* species, *Staphylococcus aureus*, *Streptococcus mutans*

the type of bacteria that were inoculated inside the pulp chambers.<sup>10</sup>

In this study Vinegar has shown growth of microorganisms which is contrary to the study published in 2014 that showed 100% efficacy of vinegar in preventing the growth of microorganisms after immersing teeth in vinegar for 7 days.<sup>16</sup>

Normal saline though an isotonic solution, revealed growth of microorganisms which is in consistent with the study on extracted human teeth which showed that teeth engrossed in normal saline revealed ed positive development of microorganisms on culture media.<sup>15</sup>

The dissimilarity in efficacy of the methods experienced may possibly be due to:

- reduced infiltration of agents into the pulp space
- Inactivation of disinfectants by the macrobiotic substances present in the teeth.<sup>12</sup>

The advantage and important aspect of study is that institutional guidelines can be formulated and implemented regarding sterilization and disinfection of extracted teeth for preclinical students. In the light of study results cross infection control guidelines can also be implicated for preclinical students as autoclave and hot air oven are readily available, cost effective and less time consuming.

The limitations of the study were this is a single centre study thus findings cannot be generalized and small sample size.

## CONCLUSION

It has been concluded that autoclave and hot air oven are the proper methods of sterilization for preclinical student. Hence, the goal of the study is to develop an awareness in students and dentists to handle the extracted human teeth with care as non sterilized extracted teeth can be the source of life threatening cross infections.

**Recommendations:** It recommends that further research and longitudinal studies should be conducted to evaluate the effect of the use of autoclave, hot air oven, vinegar and sodium hypochlorite on the physical property of the teeth. It also recommends that students should be discouraged to use amalgam-filled teeth for preclinical use and if used these teeth should not be sterilized by hot air oven due to spillage of mercury vapors and contamination. It also recommends that students must follow Center for Disease Control and Prevention (CDC guidelines) for the routine handling of extracted teeth in dental institutions.<sup>18</sup> (Table – 2).

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**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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