MATERIALS AND METHODOLOGY

**Source of Data:** Invitro study.

**Method of collection of data:**

The present randomized experimental study was carried out with the cooperation of Department of Microbiology, Rama Dental College, Kanpur, aiming at evaluating the disinfection effect of: sodium hypochlorite %0.525 (RANKEM), 2% Glutaraldehyde (CIDEX), 5% Phenol (LABSAFE), 1.75% Iodophor (D – 125), and Aloe vera (99.96% pure, Patanjali Ayurved) on the alginate impression material (NEOCOLLOID: Batch No. C–302205).

**Sampling Method:**

An appropriate mixture of water and alginate powder will be prepared in a bowl with a sterile spatula according to the manufacturers' instructions. Then the mixture will be poured into a 5 cc sterile syringe; after the required time for material setting, the impression material will be cut off and removed with a number 10 surgical blade from the end part of the syringe to different slices with 2 mm thickness. A total of 160 discs will be prepared. In order to assure that the discs were kept sterile during preparation, five discs will be selected as negative controls (blank) and will be incubated on TSB culture for 24 to 48 hours, after which the bacterial growth will be examined. And five more discs will be used as positive controls to check for any microbial pollution. To investigate the effect of different disinfectant materials, 50 discs will be used for each bacterial species. From 150 discs, 99.96% aloe vera will be used to disinfect 30 of them for 10 minutes. 30 discs will be disinfected with 2% glutaraldehyde for 10 minutes, 30 discs will be selected for disinfecting with 0.525 % sodium hypochlorite for 10 minutes, 30 discs will be selected for disinfecting
with 5% phenol for 10 minutes and 30 discs will be selected for disinfecting with 1.75% iodophor for 10 minutes.

**Preparation of Bacterial Suspension and Yeast**

For each type of susceptibility testing, a standard inoculum of bacteria must be used. The standard inoculums will be prepared to match the turbidity of $1.5 \times 10^8$ cfu/mL (equivalent to 0.5 McFarland) by transferring 1-2 colonies of 18–24 hours cultures to TSB medium and incubating at 35°C. For *Candida albicans* fungus, the samples will be taken from 48 hour Saborosa and Dextrose agar cultures.

**Contamination of Samples**

To evaluate the disinfection effect of above mentioned five substances, discs will be separately polluted with microbial solutions of *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), and *Candida albicans* fungus (PTCC5027). The discs will be kept in sterile test tubes separately with 1 cc of microbial suspension each and will be incubated at 35°C for one hour.

**Disinfection of Samples and Microbiological Surveys**

After contamination, all samples will be rinsed with sterile distilled water for 30 seconds. In order to disinfect all samples except controls, either 99.96% aloe vera, 2% glutaraldehyde, 0.525% sodium hypochlorite, 5% phenol or 1.75% iodophor will be used on each disc applying spray method for 10 puffs in 15 seconds. Then the discs will be kept into sterile plastic bags containing sterile humidified cotton to form a moisturized environment for 10 minutes.
Trypsin protease, which is able to isolate the microbes from contaminated environments, will be used. The recommended time and concentration for the effective use of trypsin is 60 minutes and 2%, respectively. This time concentration is based on the maximum microorganisms which can be isolated from the samples. After washing the discs with sterile distilled water for 30 seconds, they will be kept in trypsin 2% solution for 60 minutes. The suspensions of 1/2 and 1/4 trypsin solution will then be prepared. Using 100 micro liter sampler, these samples will be transferred to Mueller-Hinton agar for the *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Also Saborow Dextrose agar (SDA) medium will be used to investigate the presence of *Candida albicans* fungus. Using a Pasteur pipette bent with heat at 90 degrees, the samples will be spread on cultures. After 24 and 48 hours incubation, the grown bacterial colonies on culture will be counted. The grown fungus colonies of *Candida albicans* on SDA will be counted after 72 hours.

SPSS software will be used for data analysis edition 11.5, and Mann-Whitney test will be conducted to compare the efficacy of different disinfectant materials.