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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(7): 18-20

www.thepharmajournal.com
Received: 11-05-2021
Accepted: 15-06-2021

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# Evaluation of efficacy of autoclave and microwave for sterilization of biomedical waste

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**Abstract**
This study investigated the efficacy of autoclave and microwave for treatment of biomedical waste. As an alternative to incineration technology, a steam autoclave and microwave was used to sterilize the biomedical waste. Biological indicator strips containing *Geobacillus Stearothermophilus* and *Bacillus atrophaeus* spores were used to test the efficacy of autoclave and microwave for sterilization of biomedical waste, respectively. It was reported that steam autoclave effectively treated the biomedical waste samples at 121 °C for 30 min and 121 °C for 45 min but at 121 °C for 15 min it showed ineffective sterilization. Microwave completely destroyed the all forms of microorganisms and provides effective sterilization at temperature of 70 °C to 100 °C, frequency of 2450 MHz and cycle of 30 min to 1 hour.

**Keywords:** Biomedical waste, autoclave, microwave, sterilization

## Introduction
The appropriate management of biomedical waste is extremely important due to its significant environmental and health hazards. Many attempts have been made to better manage the biomedical waste problem. If not properly handled, biomedical waste poses a great risk of infection through the spread of pathogens from health institutions into the environment (Mohee, 2005) [10]. Medical devices are now being manufactured for single use only, thus further increasing the amount of biomedical waste especially in developing countries. This will result in a rapid increase in biomedical waste amounts that should be disposed in a safe manner (Mbongwe *et al*., 2008) [11]. The terms infectious waste and biomedical waste are usually used for wastes that cannot be disposed of in a municipal solid waste landfill due to their pathogenic content. The safe disposal of biomedical wastes is of a great concern for the generators and the public. Different treatment methods can be applied in the treatment of biomedical waste. The main purpose in the treatment of biomedical wastes is to make it safe for human and environmental health. The process of sterilization is the treatment of biomedical wastes with steam at high temperature and pressure. If the temperature and contact time are sufficient, this process inactivates many types of microorganisms. Biomedical waste containers are placed in a closed chamber and sterilized with steam for a certain time at the required pressure and temperature. Biomedical wastes can be landfilled together with municipal solid wastes after steam treatment and size reduction. (Karagiannidis *et al*., 2010) [7]. Sterilization is the process of completely destroying all kinds of microbial life, including bacterial spores in biomedical wastes, by physical, chemical and mechanical methods, or reducing the level of these microorganisms by 99.9999%. Whether the biomedical wastes treated by sterilization are rendered harmless is tested using chemical and biological indicators. In the biological indicator test, the viability of the biological indicator is used to detect whether all potential infectious microorganisms have been destroyed in the sterilized waste (Yaman, 2020) [16].

## Materials and Methods
A total of 90 biomedical waste samples which included the animal tissues and body parts, blood and bodily fluids, infectious bedding of animal, catheters, needles, syringes, cotton and bandages used in dressing were collected from the various locations of Bikaner in sterilised biohazard bags and bins as per Bio-Medical Waste Management Rules, 2016.
In Group-I all the 45 samples were divided into three sub-groups containing 15 samples in each. For processing in autoclave the sample was kept in autoclave bag and a biological indicator (*Geobacillus Stearothermophilus*) was placed in the middle of the sample to check

the efficacy of sterilization cycle. First subgroup samples were processed in autoclave at 121 ºC, 15 psi for 15 minutes, second subgroup samples were processed in autoclave at 121 ºC, 15 psi for 30 minutes and third subgroup samples were processed in autoclave at 121 ºC, 15 psi for 45 minutes.

After autoclaving, total viable count of the treated samples were done by making elute with the help of dipping method as described above and the total viable count before the treatment and after treatment with autoclave was compared for cfu/gm of sample.

After autoclaving, biological indicator was dipped into the nutrient broth and incubated at 55 ºC-60 ºC for 7 days and the nutrient broth was checked for the development of turbidity. If there was no turbidity then it was considered as effectiveness of sterilization cycle.

In Group-II all the samples were processed in microwave. The samples were kept in bucket of microwave and a biological indicator (*Bacillus atrophaeus*) was placed in the glass beaker along with the samples to check the efficacy of sterilization cycle.

After microwaving, total viable count of the treated samples was done by making elute with the help of dipping method as described above and the total viable count before the treatment and after treatment with microwave was compared for cfu/gm of sample.

After microwave, biological indicator was dipped into the nutrient broth and incubated at 55 ºC-60 ºC for 7 days and the nutrient broth was checked for the development of turbidity. If there is no turbidity then it was considered as effectiveness of sterilization cycle.

## Results and Discussion

### Assessment of the efficacy of Steam Autoclave

In the subgroup-I there was presence of turbidity in the nutrient broth that show ineffective sterilization of the samples. In the sub group-II and sub-group-III the turbidity in the nutrient broth was absent that indicated the effective sterilization of the samples.

In sub-group-I there is turbidity in nutrient broth which showed inadequate sterilization of biomedical waste at 121 °C, 15 psi and 15 minutes which is not corroborated with study conducted by Le *et al.* (2005) who used a biological indicator containing *Geobacillus Stearothermophilus* (ATCC 7953) spores to determine the efficiency of autoclave and found that standard 121º C, 15 psi and 15 minutes dwell time are adequate for sterilization of biohazardous waste. Hossain *et al.* (2012) [6] reported that with increasing contact time and temperature, the number of surviving bacteria decreased and the optimum experimental conditions as measured by degree of inactivation of bacteria were 121 °C for 15 minutes for Gram negative bacteria, 121 °C and 131 °C for 60 and 30 min for Gram positive bacteria, respectively is substantiate with present findings. Ferdowsi *et al.* (2013) [3] evaluated the clinical samples from hospitals by using Class 6 TST (time, steam and temperature) sterilization indicator strips and spore tests and found that after autoclave process, spore tests were incubated and no change was observed in colour of tests after incubation and it is corroborated with present findings.

Panta *et al.* (2019) [12] used self-contained biological indicators, class-5 chemical indicators and autoclave indicator tape and results showed that 71.0% of the autoclave cycles were ineffective (i.e. showed positive results) when tested with biological indicators, 69.8% showed 'reject' results with class 5 chemical indicators and 13.5% of the sterilization cycles did not show a change in colour of the autoclave tape. Yaman (2020) [16] used biological indicator vials containing *Bacillus Stearothermophilus* and results showed that for efficient sterilization of biomedical wastes, the autoclave should be operated at a contact time of 45 min, a temperature of 150°C and at a steam pressure of 5 bar but the operating conditions is higher than present operated conditions.

In the present study bacterial colony was not found on nutrient agar after autoclaving which is substantiate with Saini *et al.* (2004) [13] who studied the efficacy of autoclave by calculating colony-forming units (CFU) and found that autoclaved infectious waste samples had no bacterial growth on culture. In present study the vegetative forms and spores of bacteria were completely killed at 121 ºC, 15 lbs for 30 minutes and 121 ºC, 15 lbs for 45 minutes that was shown by absence of turbidity in the nutrient broth containing the biological indicator organisms (*Bacillus Stearothermophilus*). The findings of present sudy were also supported by Hossain *et al.* (2012) [6] and Yaman (2020) [16]. According to Tonuci *et al.* (2008) [14], the differences in efficient sterilization at different operating conditions is due to nature of biomedical waste and variety of bacterial flora in biomedical waste.

### Assessment of the efficacy of Microwave

After microwaving, biological indicator was dipped into the nutrient broth and incubated at 55 °C-60 °C for 7 days and the nutrient broth was checked for the development of turbidity. The turbidity in the nutrient broth was absent that indicated the effective sterilization of the samples. After treatment in microwave the bacterial growth was not found on nutrient agar in the petri plates. So after treatment in microwave the total viable count of all the samples was zero. Hoffman and Hanley (1994) [5] processed bacterial and thermometric test pieces were passed through a microwave system and reported that after treatment, none of the bacterial test pieces yielded growth on culture which is similar with findings of present study. Devine *et al.* (2007) [2] processed *Bacillus atrophaeus* spore and Salmonella enterica vegetative cell samples in microwave and reported reduction in microbial load of Salmonella enterica vegetative cell and *Bacillus* spores in microwave system which is corroborated with present findings.

Tonuci *et al.* (2008) [14] processed inoculated pre-sterilized autoclaved public healthcare waste with $5x10^5$ *Escherichia coli* in microwave at power 100 W/kg and temperature 90°C and results showed that the operational condition of equipment are probably ineffective. Al-Hakami *et al.* (2013) [1] reported that under the effect of microwave irradiation, the *Escherichia coli* remove upto 98% from water.

Latimer and Matsen (1977) [8] studied the exposure of microwave irradiation at 2,450 MHz for different time periods on commonly encountered clinical bacterial pathogens, spore suspension, spore strips, clinical isolates (including *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis and Enterococcus* spp.) and contaminated petri plates and reported that sterilization brought about in 60 sec, 11 min, 5 min, 5min and 5 min, respectively is substantiate with present findings.

The findings of present study also corroborated with studied conducted by Gautam *et al.* (2019) [4] who found that 8 and 10 log reduction in *Staphylococcus aureus* and *Escherichia coli* was achieved in 5 and 10/20 min, respectively; for bacterial spores, the 6 and 8 log reduction was achieved in 5 min

exposure while 8 and 10 log reduction after 10 and 20 min in microwave (Opti Maser™), respectively at 70 °C and Woo *et al*. (2000) [15] who examined found that 5-log reduction of the viable count in *Escherichia coli* and *Bacillus subtilis* compared to the initial counts at 80 °C under the effect of microwave radiation. According to Tonuci *et al*. (2008) [14], the differences in efficient sterilization at different operating conditions is due to nature of biomedical waste and variety of bacterial flora in biomedical waste.

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