Journal of Animal Research: v.12 n.05, p. 721-727. October 2022

DOI: 10.30954/2277-940X.05.2022.14

Assessment of Comparative Efficacy of Disinfectants on the Microbial load of Sheep and Goat Slaughterhouses

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Received: 26 July, 2022 **Revised:** 04 Sept., 2022 **Accepted:** 10 Sept., 2022

ABSTRACT

The present investigation aimed to determining the efficacy of four different disinfectants on microbial load collected from the effluent of distinct areas like flaying, evisceration, exsanguination and running tap water of sheep and goat slaughterhouses at different localities in Tirupati, Andhra Pradesh, India. The disinfectant effectiveness at various concentrations was tested using the well diffusion method. The test disinfectant contains active compounds like sodium hypochlorite, calcium hypochlorite, benzalkonium chloride and carbolic acid at different concentrations. The results indicated that the efficacy of disinfectants generally increased by increasing the concentration from lower to higher level. However, some of the disinfectants were ineffective even at their highest recommended concentrations. Sodium hypochlorite and calcium hypochlorite at their recommended concentrations of 6 % and 5 % respectively were ineffective and disinfectant solution containing benzalkonium chloride 80 was highest at 2.5 % and also at 0.25 % concentration. This research concluded that the most effective disinfectant was carbolic acid at 10 % concentration among all selected disinfectants and it effectively decreased the microbial load on different areas of slaughter house.

HIGHLIGHTS

- Not only the type of disinfectant but also the concentration at which disinfection is used also plays a vicious role in disinfection.
- The efficacy of disinfectants generally increased by increasing the concentration from lower to higher level.
- The most effective disinfectant was carbolic acid at 10% concentration.

Keywords: Sheep and goat slaughter houses, disinfectants, comparative effect, well diffusion method

Cleaning and disinfection are essential steps in the slaughtering and also processing of meat in order to produce safe, high-quality products with long shelf life. Disinfection is the process that eliminates many or all pathogenic microorganisms except bacterial spores on inanimate objects (Mohapatra, 2017). Since different microorganisms are introduced into the slaughter house in large numbers, daily and complete disinfection is essentially required since biological waste contains high organic load (lipids, proteins and blood). In order to retain the nutritive value and natural state of the wholesome meat, it must be produced hygienically free from pathogenic microorganisms (Bhandare *et al*., 2007). The level of carcass contamination is ultimately higher if there is insufficient disinfectant treatment. So, cleaning and disinfection plays a crucial role in control of microbial contamination. Various factors such as environmental factors, including organic load, temperature and contact time are responsible for altering the kinetics of disinfection (Jang *et al*., 2017 and Pinto *et al.,* 2010).

How to cite this article: Bhargavi, K., Rishitha, D.J., Lakshmi Kavitha, K. and Bhaskar Reddy, G.V. (2022). Assessment of Comparative Efficacy of Disinfectants on the Microbial load of Sheep and Goat Slaughterhouses. *J. Anim. Res.,* **12**(05): 721-727.

Source of Support: None; **Conflict of Interest:** None

Sodium hypochlorite is a clear and slightly yellowish solution with a characteristic odour. In general, the household bleach contains 5-6% sodium hypochlorite. The appropriate concentration of sodium hypochlorite for disinfecting general biological waste is 5000 ppm *i.e.,* 0.5% (Girotti, 2015). The mechanism of action includes the release of hypochlorous acid (HOCl). Hypochlorous acid is divided into hydrochloric acid (HCl) and oxygen atom (O).

Calcium hypochlorite is a white granular solid having an odour of chlorine and contains 65% available chlorine and dissolves easily in water. It is usually recommended at 5% w/v concentration for domestic disinfection.

Benzalkonium chloride is a wide spectrum quaternary ammonium antibacterial agent and has long duration of action which is cationically charged and induces antibacterial action through attraction to the negatively charged bacterial membrane thereby inactivation of energy producing enzymes and denaturation of essential cell proteins (Merianos, 2001). Benzalkonium chloride (BAC, alternatively called alkyl dimethyl benzyl ammonium chloride/ADBAC) is a mixture of closely related QAC, varying in the length of the alkyl hydrophobic tail between eight and 18 carbon atoms (Al-Adham *et al.,* 2013). Its density (around 0.98) is close to water, meaning that in aqueous solutions, percent concentrations expressed as weight or volume BAC per weight or volume water (w/w, w/v or v/v) are nearly identical. Therefore, although reports vary in their concentration units, equivalence may cautiously be assumed between, say, '100 mg/L (or µg/mL)' in one reference and '0.01%' (units unspecified) in another.

Carbolic acid can be an effective disinfectant for toilets, stables, floors and drains. It is a type of organic compound which is colourless/white and has mild sugary odour and is a potent proteolytic agent by denaturing and coagulation of proteins and are general protoplasmic poisons and cause bacterial death by inactivation of essential enzyme systems and leakage of essential metabolites from the cell wall . It has good penetrating power into the organic matter and is mainly used for disinfection of equipment/organic materials that are to be destroyed. Recommended concentrations for bactericidal action were 1-2% and for bacteriostatic action was 0.1-1%. 5% was used to kill anthrax spores (Juszkiewicz *et al.,* 2019).

Presently there was a lack of information on efficacy of different disinfectants on microbial load of sheep slaughter houses. Hence the present study was undertaken to determine the efficacy of sodium hypochlorite, calcium hypochlorite, benzalkonium chloride and carbolic acid disinfectants on the microbial load of sheep and goat slaughter houses.

MATERIALS AND METHODS

Sample collection

A total of 20 swab samples were collected from different areas of five sheep and goat slaughter houses in and around Tirupati, Andhra Pradesh, India. Collected swab samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. Growth was observed by presence of turbidity then the samples were subjected for testing the efficacy of disinfectants by well-diffusion method.

Well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of disinfectants (Balouiri *et al*., 2016). The sterile nutrient agar plates were prepared and observed for sterility check. Lawn culture was made on these sterile nutrient agar plates and wells of 0.9 cm were made. A maximum of five wells and a minimum of three wells per plate were made so that the inhibition zones are not overlapped. Different concentrations of disinfectant were loaded into these wells and incubated at 37˚C for 24 hours and the inhibition zone was measured.

Disinfectants and their dilutions

Based on many preliminary trails, the following dilutions/concentrations (Table 1) of different disinfectants were used for determination of antimicrobial efficiency.

Table 1: Dilutions/concentrations of different disinfectants used in the study

Disinfectant	Dilutions/Concentrations
Sodium hypochlorite	40,000 ppm (4%)
Sodium hypochlorite	50,000 ppm (5%)
Sodium hypochlorite	60,000 ppm (6 %)

Calcium hypochlorite	5 %
Calcium hypochlorite	10 %
Benzalkonium chloride 80	2.5 %
Benzalkonium chloride 80	0.25 %
Benzalkonium chloride 80	0.05 %
Benzalkonium chloride 80	0.025 %
Carbolic acid	10 %
Carbolic acid	2 %
Carbolic acid	1 %

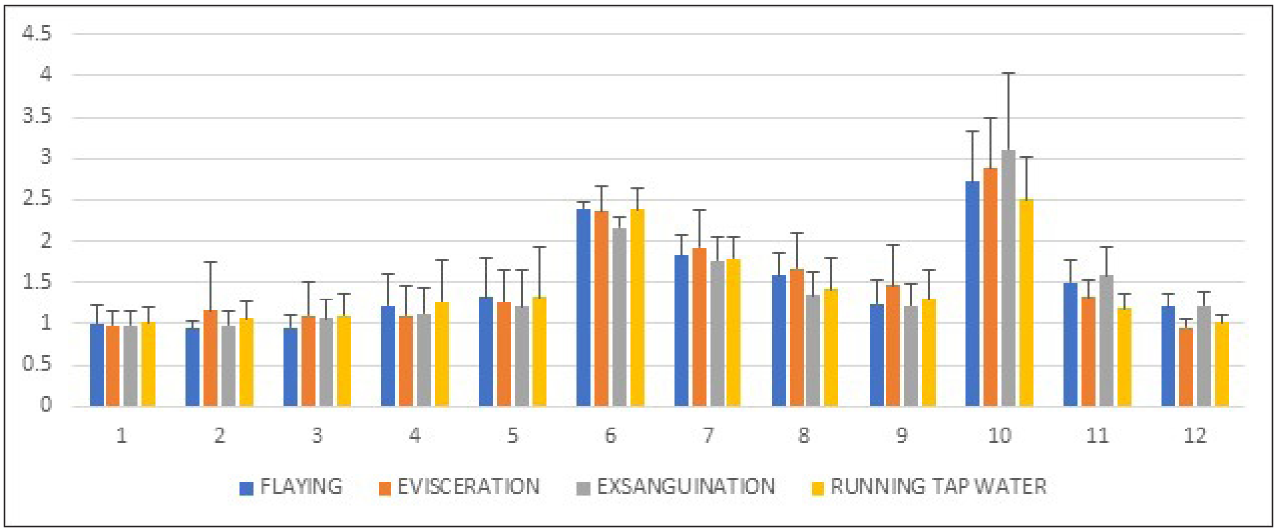
STATISTICAL ANALYSIS

The data (zone of inhibition) collected were subjected to one way ANOVA according to the general linear model procedure of statistical package for social sciences (SPSS) 22 version. A significance level of $P \leq 0.05$ was used in all tests. When analysis of variance indicated a significant treatment effect, Duncans multiple range test was used to compare the treatment and results were expressed as mean average value ± standard error.

RESULTS AND DISCUSSION

A total of 20 swabs were collected from five different slaughter houses and well diffusion test was conducted so as to determine the efficacy of four disinfectant agents used. The result of agar well diffusion test was shown in Fig. 1. Results indicated that the efficacy of disinfectant relied on the concentration of disinfectant and also on the microbial load present in the area of the test control point. Some disinfectants have wide efficacy against the microbial load while others were ineffective even at their higher recommended concentrations. When the sizes of zone of inhibition (cm) were taken under consideration, the tested disinfectants can be ordered from the foremost effective to the less effective one are as follows-Carbolic acid at 1:10(10%) dilution, Benzalkonium chloride at 2.5% and at 1:10 (0.25%) dilution, calcium hypochlorite at 10% concentration and Sodium hypochlorite at 6% (60,000 ppm) concentration.

Hypochlorites rapidly dissociates to form the highly reactive hypochlorous acid, which attacks the amino acids that make up proteins and so alter a protein's three-dimensional structure. A protein's structure is critical for a cell to function to function, so when they lose their shape cells no longer survive. This broad-spectrum attack makes it effective against wide range of bacteria. Sodium hypochlorite was tested from 100 ppm (0.01%) to 60,000 ppm (6%).



1. Sodium hypochlorite 40000 ppm (4%); 2. Sodium hypochlorite 50000 ppm (5%); 3. Sodium hypochlorite 60000 ppm (6%); 4. Calcium hypochlorite 5 % w/v; 5. Calcium hypochlorite 10 % w/v; 6. Benzalkonium chloride 80 2.5 %; 7. Benzalkonium chloride 80 0.25 %; 8. Benzalkonium chloride 80 0.05 %; 9. Benzalkonium chloride 80 0.025 %; 10. Carbolic acid 10 %; 11. Carbolic acid 2 %; 12. Carbolic acid 1 %.

Fig. 1: Efficacy of different disinfectants in different areas of sheep slaughter house

No inhibition zones are formed from 100 ppm (0.01 %) to 3,000 ppm (0.3 %) as shown in Fig. 2 and 3 whereas from 5000 ppm (0.5%) to 30,000 ppm (3%) a narrow zone of inhibitions were formed as shown in the Fig. 4. At 40,000 ppm (4%) to 60,000 ppm (6%) the mean inhibition zone range between 0.94 cm to 1.16 cm as shown in Fig. 4 and 5.

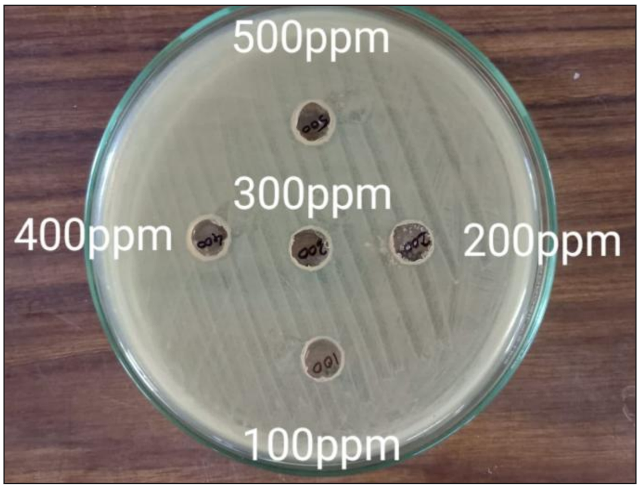


Fig. 2: Sodium hypochlorite at 100 ppm (0.01%), 200 ppm (0.02%), 300 ppm (0.03%), 400 ppm (0.04%), 500 ppm (0.05%) where no inhibition zones were found

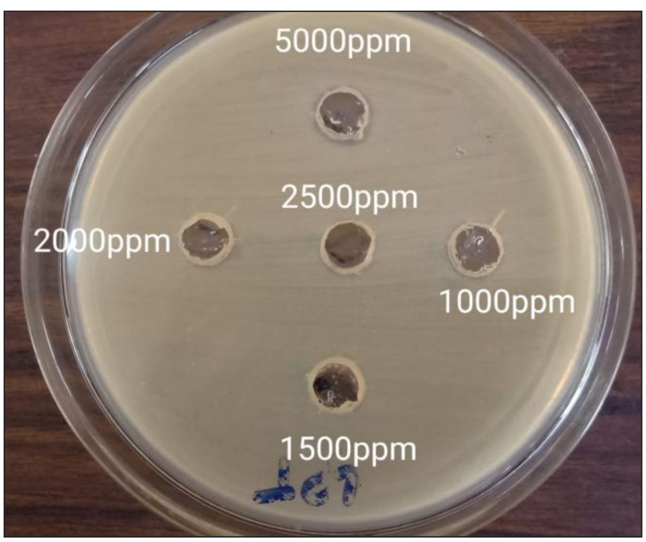
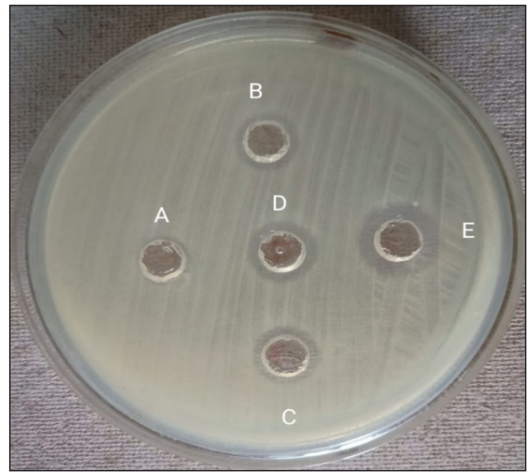


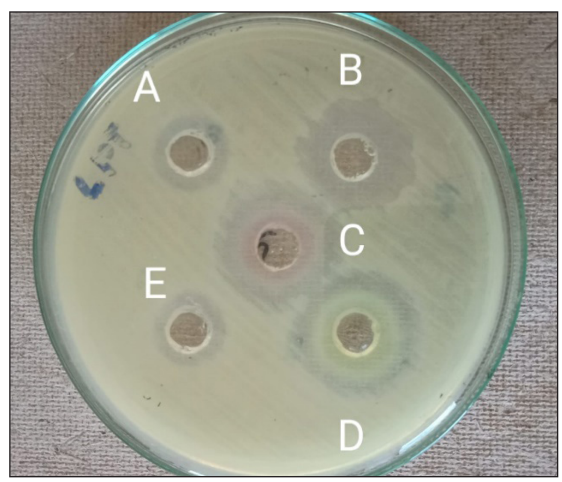
Fig. 3: Sodium hypochlorite- Small zone of inhibition is observed for 5000 ppm (0.5%)

Soliman *et al.,* (2016) also opined that sodium hypochlorite is not effective at the recommended concentration of 3 % but needed to use 8 % concentration. Calcium hypochlorite showed antimicrobial activity more efficiently at 10% than at recommended levels *i.e.,* 5 %. The zones of inhibition were within the range of 1.2 cm to 1.32 cm for 10 % dilution whereas it is in between 1.12 cm to 1.26 cm for 5% dilution.



Zone of inhibition A-5000 ppm (0.5%) - 1 cm; B-10000 ppm (1%) - 1.2 cm; C-20000 ppm (2%)- 1.3 cm; D-30000 ppm (3%)- 1.3 cm; E-40000 ppm (4 %)-1.4 cm.

Fig. 4: Flaying sample- Sodium hypochlorite

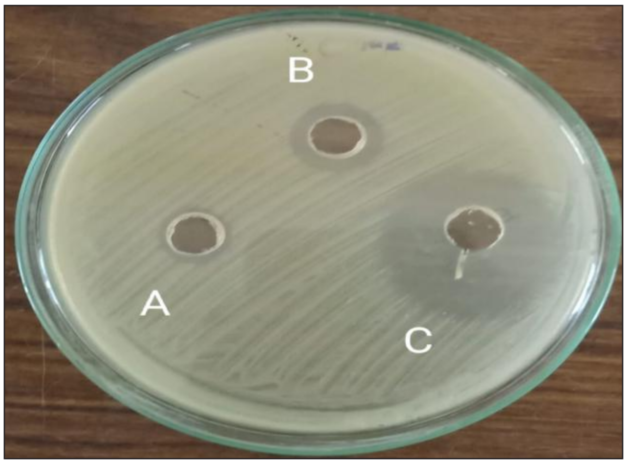


Zone of inhibition SH-A-50000 ppm (5%) – 1.4 cm; SH-B-60000 ppm (6 %) - 1.5 cm; BC-C-2.5 %- 2.2 cm; BC-D-0.25 %-2.1 cm; BC-E-0.05 %-1.3 cm

Fig. 5: Exsanguinations sample - Sodium hypochlorite (SH), Benzalkonium chloride (BC)

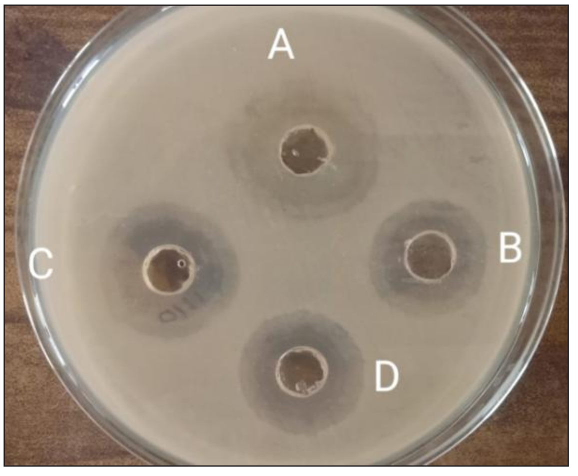
Calcium hypochlorite is relatively a stable compound with greater chlorine availability than sodium hypochlorite (Frazer *et al.,* 2013). Unmodified sodium hypochlorite solutions are alkaline (as a consequence of the formation of HOCl from OCl− and H_2O) and there is usually excess hydroxide ion in concentrated stock solutions, further raising the pH and improving chemical stability (OxyChem, 2014). Thus, hypochlorite ions predominate in concentrated solutions, which consequently show lower biocidal activity compared with more dilute solutions, of lower pH. The chlorine content of biocide solutions may be expressed as percent (w/v) available chlorine or percent NaOCl (these being approximately equivalent, as the molecular mass of NaOCl is similar to that of Cl_2), or as parts per million (ppm), where 10,000 ppm = 1% available chlorine (Anon, 2020). The mechanism(s) of chlorine's antibacterial effect at conventional disinfectant concentrations are not well understood, but disruption of bacterial nucleic acid and protein chemistries, and of membrane function, have all been described (Al-Adham *et al.,* 2013). As biocidal chlorine compounds in solution are highly reactive, the disinfectant activity of NaOCl is susceptible to quenching by reactions with organic soil (Al-Adham *et al.,* 2013). In research study by Gomez *et al.* (2020) demonstrated that similar antibacterial action was tested between the hypochlorites which are contrary to the obtained results were calcium hypochlorite is more effective than sodium hypochlorite.

Benzalkonium chloride 80 have mean inhibition zone diameters ranging from 2.16 cm to 2.4 cm when tested at 2.5% and 1.76 cm to 1.92 cm at 0.25%and was not effective at 0.05% and 0.025% and inhibition was between 1.22 cm to 1.66 cm. Benzalkonium chloride was effective at 1-10% concentration in accordance to (Mc Sharry *et al.,* 2021) and it has wide range of antimicrobial properties against bacteria, virus, fungi (Pereira and Tagkopoulos, 2019) and their stability for both long- and short-term usage. These chemicals are among the most common active disinfectant components (Tezel and Pavlostathis, 2012). The mean inhibition zone diameters of Carbolic acid at 1:10 (10%) dilution ranges from 2.5 cm to 3.1 cm. At dilutions 1:50 (2%) it varies from 1.18 cm to 1.58 cm and at 1:100 (1%) it is not much effective and the zone of inhibition ranges from 0.96 cm to 1.22 cm (well diameter is 0.9 cm). This disinfectant damages the permeability mechanisms and the repair is prevented by inactivating the enzymes. In concurrence with the above results Soliman *et al.* (2016) also observed the effect of carbolic acid at the recommended concentration of 6.5 % was not effective and found a 10 % concentration was suitable for cleaning and disinfection.



Zone of inhibition A-1 % concentration– 1.2 cm; B- 0.5 % concentration- 1.5 cm and C-10 % concentration- 3.2 cm

Fig. 6: Flaying sample –Carbolic acid



Zone of inhibition A-2.5 % concentration– 2.9 cm; B-0.25 % concentration- 2.5 cm; C-0.05 % concentration- 2.2 cm; D-0.025 % concentration-2 cm.

Fig. 7: Eviscerated sample –Benzalkonium chloride

When disinfectants were evaluated according to the active ingredient, the aromatic organic compound carbolic acid also known as phenol is more effective among the four tested disinfectants which ranges from 0.96 cm to 3.1 cm as shown in Figure 1 where the maximum inhibition diameter is obtained at 1:10 (10%) dilution as shown in Fig. 6. Then comes the next suitable disinfectant benzalkonium chloride which is a quaternary ammonium compound (QAC)in which wide inhibition zones was observed at 2.5% and at 0.25% as shown in Fig. 7. In hypochlorites, divalent cation containing calcium hypochlorite is effective than sodium hypochlorite containing monovalent cation as shown in Fig.1. According to statistical analysis and considering the mean values of different disinfectants the inhibition zone diameters of carbolic acid (10%) are far higher than when compared to other disinfectants. This research concluded that the most effective disinfectant was carbolic acid at 10% concentration among all selected disinfectants.

CONCLUSION

This study proves that routine cleaning and disinfection procedures performed at the slaughterhouse were not able to control microbial growth so, selection of appropriate disinfectants for disinfection procedures in slaughter house and meat plants is one of the most important points to get accurate sanitation. As for this, not only the type of disinfectant but also the concentration at which it is used also plays a vicious role in disinfection. Usual recommended concentrations failed to reach the expected efficacy and are no more suitable for sanitation purpose as the resistant bacteria are developed to such concentrations and disinfectants. So, appropriate disinfectant and then effective concentrations should be selected for maintaining hygienic practices in slaughter house and meat plants. Furthermore, application of green or nano disinfectants can be an option to control the microbial load in slaughter houses.

ACKNOWLEDGMENTS

The authors are highly thankful to Sri Venkateswara Veterinary University, Tirupati for providing the necessary facilities to carry out the work.

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