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# Determination of efficacy of different disinfectants on the microbial load of poultry slaughter houses

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**Abstract**
This study was aimed to investigate the efficacy of four commonly used disinfecting agents at different concentrations (sodium hypochlorite at 4, 5 and 6%, calcium hypochlorite at 5 and 10%, benzalkonium chloride 80 at 2.5, 0.25 and 0.05% and carbolic acid at 10, 2 and 1%) against the microbial load from different areas of operations in poultry slaughterhouses like flaying, evisceration and final discharge areas. The samples were collected at diverse vicinities in poultry slaughter houses in and around Tirupati, Andhra Pradesh, India. Well-diffusion technique was used for determining the efficacy of sodium hypochlorite, calcium hypochlorite, benzalkonium chloride and carbolic acid and was tested at different concentrations. The disinfectants were not effective in their given recommended concentrations. The most effective disinfectant is carbolic acid at 10% concentration and the least is sodium hypochlorite even at the highest recommended concentration of 6% v/v. Calcium hypochlorite and Benzalkonium chloride inhibited the growth of microbes with less extent. Based on the results, it can be concluded that, the test disinfectants can be ordered from the most effective to the least effective ones are as follows: carbolic acid at 10% concentration, calcium hypochlorite at 10% concentration, Benzalkonium chloride 80 at 2.5%, and Sodium hypochlorite at 6% concentration.

**Keywords:** Poultry slaughter house, disinfectants, antimicrobial efficacy, well-diffusion method

## 1. Introduction
Cleaning and disinfection are an integral part of slaughter houses and poultry processing plants to meat and meat products safety thus protect the consumer health and also extend the shelf life and quality during transportation, storage and distribution. Disinfection is the process that eliminates many or all pathogenic microorganisms except bacterial spores on inanimate objects (CDC, 2022) [1]. Proper cleaning and sanitation plays a crucial role in control of microbial contamination in food processing units and also prevent food poisoning. A daily and conscientious disinfection is essential to abate the contamination of carcasses as different microbes are introduced into slaughterhouse in a large number on daily basis. During slaughtering of poultry, primary contamination occurs due to contact of birds with faeces, secondary contamination by butcher knives and processing equipments etc. (FAO, 2022) [2]. A further source of contamination can be transport vehicle that has not been properly sanitized (Heyndrickx *et al.*, 2002) [3]. It is reported that if insufficient disinfection of poultry processing plants are applied, the level of carcass contamination is high and causes food borne infections (Rahkio *et al.*, 1996) [4]. Common bacterial genera found in poultry and poultry products are *Escherichia* sps, *Staphylococcus* sps, *Campylobacter* sps, *Salmonella* sps, *Enterobacter* sps, *Alcaligenes* sps, *Bacillus* sps, *Flavobacterium* sps, *Micrococcus* sps, *Proteus* sps, *Pseudomonas* sps, *Alteromonas* sps, *Acinetobacter-Moraxella sps and Corynebacterium sps* Mead GC, (2000) [5] and Conner *et al*., (2001) [6]. There are several chemical sanitizer formulations available for use in food industry. Chemical sanitizers can be formulated with cleaning agents to obtain cleanser-sanitizer compounds (Ayla Sener *et al.*, 2007) [7]. The effectiveness of disinfectant depends on various factors including chemical composition and concentration of disinfectant, exposure time and temperature, pH, presence of foreign substances (equipment clearness), water hardness, type, quantity and age of microorganism, and microbial attachment to a solid surface Marriott, (1989) [8]; Frank and Chmielewski, (1997) [9] and Gronholm *et al.*, (1999) [10].
Sodium hypochlorite (NaOCl) is a clear and slightly yellowish solution with a characteristic odor. Generally, household bleach contains 5-6% sodium hypochlorite and appropriate concentration of sodium hypochlorite for disinfecting general biological waste 5000 ppm (Girotti, 2015) [11].

The mechanism of action includes the release of hypochlorous acid (HOCl) which is divided into hydrochloric acid (HCl) and oxygen atom (O), the latter is a very strong oxidator. Calcium hypochlorite is a white granular solid having an odor of chlorine and contains 65% available chlorine and dissolves easily in water. It is usually recommended at 5% w/v concentration for domestic disinfection (Kylene Jones, 2022) [12].

Benzalkonium chloride is a wide spectrum quaternary ammonium compound, 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 (Idris Mohamed Mehdawi *et al.*, 2013) [13]. Carbolic acid also known as phenol can be an effective disinfectant for floor and walls and other sewerage drains. It is colorless/white and has a mild sugary odor and is a potent proteolytic agent by denaturing and coagulation of proteins and are general protoplasmic poisons (Drugbank, 2022) [14]. It has good penetrating power into the organic matter and is mainly used for the disinfection of equipment/organic materials that are to be destroyed (Mark L Wickstorm, MSD Veterinary Manual) [15]. Recommended concentrations for bactericidal action was 1-2% and for bacteriostatic action was 0.1-1%whereas, 5% was used to kill anthrax spores (Mark L Wickstorm, MSD Veterinary Manual) [15].

Presently there was a lack of information on efficacy of different disinfectants on microbial load of poultry 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 poultry slaughter houses.

## 2. Materials and Methods

**2.1 Sample collection:** A total of 15 swab samples were collected from different areas of five poultry 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.

**2.2 Disinfectants and their dilutions:** Based on many preliminary trails, the following dilutions/concentrations (Table 1) of different disinfectants were used for determination of anti microbial efficiency.

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

| S. No | Disinfectant | Dilutions/Concentrations |
|---|---|---|
| 1. | Sodium hypochlorite | 40,000 ppm (4%) |
| 2. | Sodium hypochlorite | 50,000 ppm (5%) |
| 3. | Sodium hypochlorite | 60,000 ppm (6%) |
| 4. | Calcium hypochlorite | 5% |
| 5. | Calcium hypochlorite | 10% |
| 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% |

**2.3 Well diffusion method:** Agar well diffusion method is widely used to evaluate the antimicrobial activity of disinfectants (Mounyr Balouiri *et al*., 2016) [16]. 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.

**2.4 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.

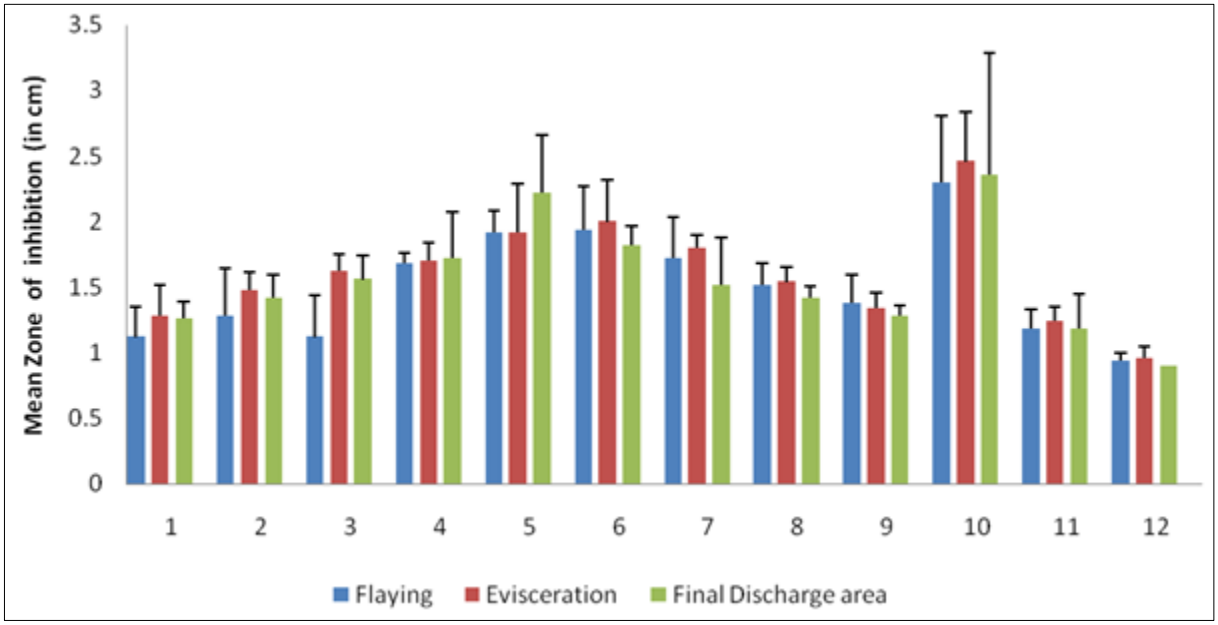
## 3. Results and Discussion

A total of 15 swabs were collected from five different slaughterhouses and were cultured. Well diffusion test was conducted in order to know the efficacy of the different concentrations of four disinfectant agents i.e. sodium hypochlorite, calcium hypochlorite, benzalkonium chloride and carbolic acid. The results of the agar well diffusion test were depicted in Figure.1 for different concentrations of the disinfectants. The results indicated that the efficacy of disinfectant depended on the concentration of disinfectant and the microbial load present in the area of the test control point of poultry slaughter house. Some disinfectants have wide efficacy against the microbial load while others were ineffective even at their higher recommended levels of concentrations. When the sizes of the zone of inhibition were taken into account, the test disinfectants can be ordered from the most effective to the least effective ones are as follows; Carbolic acid at 10% concentration, Calcium hypochlorite at 10% concentration, Benzalkonium chloride 80 at 2.5%, Sodium hypochlorite at 6% [60,000ppm] concentration, with some exceptions like benzalkonium chloride 80 is more effective than calcium hypochlorite of 10% on microbes present at flaying area and evisceration area of poultry slaughter house.

Sodium hypochlorite was tested from 100 ppm to 60,000 (6%) ppm. No inhibition zone was formed from 100 ppm to 3,000 ppm but from 5000 ppm to 30,000 ppm narrow zone of inhibitions was formed. At 40,000 ppm (4%) to 60,000 ppm (6%) showed the clear zone of inhibition and the diameters ranges between 1.12 and 1.56 cm (Figure 02). In hypochlorites, divalent cation containing calcium hypochlorite is effective than sodium hypochlorite containing monovalent cation which is differing with the work by Gomez *et al.*, (2020) [17] and quaternary ammonium compound [QAC] benzalkonium chloride is even more effective than sodium hypochlorite. Calcium hypochlorite showed antimicrobial activity more efficiently at 10% than at recommended levels *i.e*., 5%. The zone of inhibition (cm) were within the range of 1.92 to 2.22 cm for 10% dilution where as it is in between 1.68 cm to 1.72 cm for 5% dilution (Figure 01 and 02).

Benzalkonium chloride 80 have mean zone of inhibition (cm) diameter ranging from 1.82 to 2 cm at 2.5% concentration

(Figure 01 and 03). It was also tested at different diluted concentrations like 0.25%, 0.05% and 0.025% but the zone of inhibition were not effective and is in between the ranges of 1.28 to 1.8 cm (Figure 03). The efficacy of Quaternary ammonium compound, Benzalkonium chloride is comparatively more than sodium hypochlorite which is in accordance with Sener *et al.*, (2007) [7]. Benzalkonium chloride was effective at 1-10% concentration (Siobhan McSharry *et al*., 2021) [18].




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 poultry slaughter house.

The mean zone of inhibition diameters (cm) of carbolic acid at 10% dilution range from 2.3 to 2.46 cm. At dilutions 2% it varies from 1.18 cm to 1.24 cm and at 1% it is not much effective (Figure 04) and the zone of inhibition is not formed in some samples and it ranges from 0.9 to 0.96 cm (well diameter is 0.9 cm). In concurrence with the above results, Soliman *et al.*, (2016) [19] 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. When disinfectants were evaluated according to the active ingredient, the aromatic organic compound carbolic acid also known as phenol is more effective, but it is corrosive and volatile in nature and should be used cautiously as it causes blisters on skin (John W Downs *et al.*, 2022) [20]. Statistically, the zone of inhibition diameters in carbolic acid 1:10 dilution was more disperse and is having more standard deviation indicating

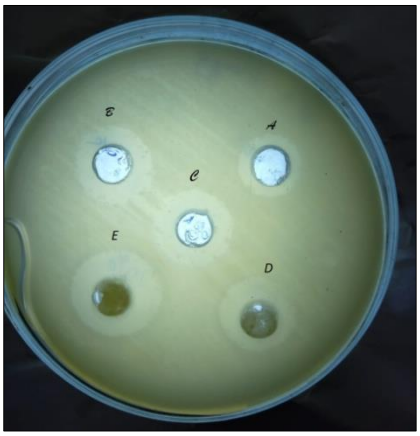



**Fig 2:** Sodium hypochlorite (A)-40000 ppm (4%), (B)-50000 ppm (5%), (C)-60000 ppm (6%) and Calcium hypochlorite (D)- 5% and (E)-10% concentrations.

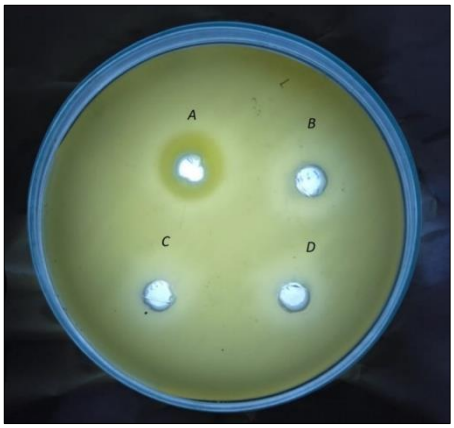



**Fig 3:** Benzalkonium chloride 80 (A)-2.5%, (B)- 0.25%, (C)-0.025% and (D)- 0.05% concentrations

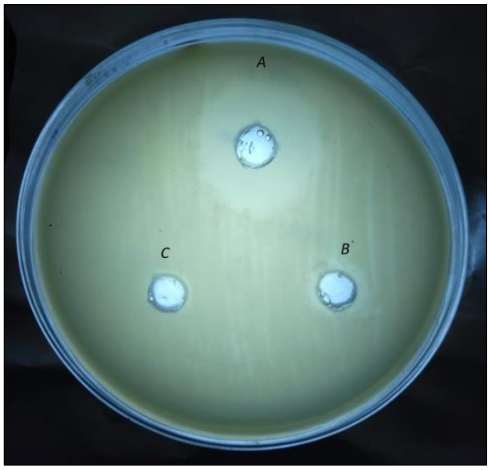



**Fig 4:** Carbolic acid (A) - 10%, (B) - 2% and (C) - 1% at concentrations.

That the diversity in action of carbolic acid on microbial load at different areas in slaughter house. Efficacy is more in carbolic acids action but uniformity is less. This might be because of the presence of more load of resistant bacteria in some slaughter houses and less in others. This is because of development of innate resistance in microbes to chemical sanitizers (CDC, Guidelines for disinfection and sterilization) [21]. But still among all the tested disinfectants phenol is more efficient. On assessing the efficacy of different disinfectants, Carbolic acid is found to be the best disinfectant to be used in different commercial poultry slaughter houses and also processing plants.

## 4. Conclusions

The present study reveals that the 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 vital role in disinfection. Usually recommended concentrations are not more suitable for sanitation purposes as the bacteria developed resistance to such concentrations and disinfectants. So, appropriate disinfectants and then effective concentrations should be selected for maintaining hygienic practices in poultry slaughter houses and meat plants.

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