Research Article

Assessing Cleaning and Disinfection Regime in a Slaughterhouse against Carcasses Contamination

Essam Samir Soliman[1*], Sherif Abdel-Rahman Moawed[2], Ahmed Mohamed garhy Ziaan[3]

[1]Department of Animal Hygiene, Zoonosis and Animal Behavior; [2]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt-41522; [3]Slaughterhouses Inspection Department, Directorate of Veterinary Medicine, Ismailia, Egypt, 41513

Abstract | Cleaning and disinfection regime effectiveness in a slaughterhouse was evaluated against the recovery of environmentally-robust zoonotic enteric pathogens such as *E. coli* and *Salmonella.* About 64%, 59% of carcass samples (Muscles, Lymph nodes, Organs as Liver and Spleen), and 55%, 52% of environmental samples (Wall, Floor swabs and Water) were tested positive to *E. coli* and *Salmonella* spp.; respectively after applying routine disinfection procedures in the slaughterhouse. Two experimental designs (dry and wet models) were used in an attempt to mimic the conditions of disinfection regime used in the slaughterhouse. Carbolic acid was tested at the concentrations 5%, 6.5% (recommended conc.) and 10%, while crude carbolic acid was tested at the concentrations 3% (recommended cons.); 5%; 8% and 10%. Carbolic acid wet models: 6.5% achieved 100% ($P < 0.01$) efficacy after 4 h; 10% achieved 100% ($P > 0.05$) efficacy after 2 h. Carbolic acid dry models: 6.5%; 10% achieved 97% ($P < 0.01$); 100% ($P > 0.05$) efficacy after 4 h; respectively. Sodium hypochlorite wet model: 3% achieved 47.5% ($P > 0.05$) efficacy after 4 h; 5%; 8% and 10% achieved 78% ($P < 0.01$); 94% ($P < 0.01$), and 100% ($P > 0.05$) efficacy after 4 h; respectively. Meanwhile; Sodium hypochlorite dry models: 3%; 5%; 8% and 10% achieved 46%; 73%; 86% and 100% ($P < 0.01$) efficacy at 4 h, respectively. In conclusion; recommended carbolic acid (6.5%) and sodium hypochlorite (3%) concentrations failed to achieve the required efficacy; thus correction of concentrations up to 10%; 8%; respectively for 4 hours contact is required. Application of green disinfectants should be considered.

Keywords | Disinfection, Cleaning, Effectiveness, Salmonella, *E. coli*, Slaughterhouse

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***Correspondence** | Essam S Soliman, Department of Animal Hygiene, Zoonosis and Animal Behavior, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt-41522; **Email:** soliman.essam@vet.suez.edu.eg
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INTRODUCTION

A well-planned, well-executed and controlled cleaning and sanitation program inside a slaughterhouse is very important to achieve the required hygienic standard. Cleaning and sanitation alone, however, will not assure the required hygienic standard; where process disinfection is important factor. Lack of efficient sanitation and improper disinfection program in a slaughterhouse can contributes bacterial contamination of carcasses (Dixon et al., 1991).

Microbial contamination of animal carcasses during slaughtering is an unavoidable condition (Dickson and Anderson, 1991). Carcass dressing and evisceration processes constitute critical points in the microbial contamination of muscle; for which corrective measures need to be implemented (Gill et al., 1999; Bacon et al., 2000; Abdalla et al., 2009a; Abdalla et al., 2009b). Fecal matter was a major source of contamination and could reach carcasses through direct deposition, as well as indirect contact with contaminated equipment, workers, installations and air (Borch and Arinder, 2002).

Poor sanitation procedures have been linked to sustained

bacterial levels (Pepper et al., 1993; Soliman et al., 2009), thus disinfectants that are effective against bacterial suspensions may have a reduced efficacy especially against bacteria that adhere to surfaces (Mosteller and Bishop, 1993). The effective use of reliable disinfectants is of fundamental importance to many control measures, particularly in all-in-all-out systems (MacLaren et al., 2001); as potential disinfectants act on microorganisms at several target sites resulting in membrane disruption, metabolic inhibition, and lysis of the bacterial cell (Maillard, 2002). Disinfectant efficacy is often tested against laboratory bacterial suspensions (Bloomfield et al., 1991). However, this approach may not always prove to mimic commercial; slaughtering and processing conditions, thus, making it difficult to determine the true and actual effectiveness of the disinfectant.

The objectives of this study was to evaluate cleaning and disinfection regime in a slaughterhouse (Abo-Khalifa abattoir- Ismailia- Egypt) against recovery of environmentally-robust zoonotic enteric pathogens such as *Salmonella* spp. and *E. coli* spp., as well as conducting an experimental designs for laboratory evaluation of the used chemical disinfectants' effectiveness against these pathogens.

MATERIAL AND METHODS

Study Design

The study design was conducted in two pathways, the first pathway was a field assessment of cleaning and disinfection regime in a slaughterhouse, and the second pathway was conducting a laboratory evaluation of the used chemical disinfectants. Regular Visits on a weekly basis for a period of six months (from April 2015 to September 2015) were assigned to a slaughterhouse (Abo-Khalifa abattoir-Ismailia- Egypt). Routine cleaning and disinfection procedures were investigated (water under pressure with soap, followed by sodium hypochlorite solution 3%, and finally carbolic acid 6.5%) for evaluation.

Sampling

A total of 480 samples (180 environmental and 300 carcass samples) were collected during the study period. Environmental swab samples (60 wall swabs and 60 floor swabs) were collected using sterile swabs previously moistened in peptone buffered water (PBW) before and after slaughtering procedures. Each swab sample was obtained by swabbing five points of 25 cm × 25 cm. Sixty (60) water samples were collected before and after slaughtering procedures from the main water sources inside the slaughterhouse using plastic bottles; they were thoroughly washed, rinsed with deionized water and soaked for 48 h in 50% HNO_3, then rinsed thoroughly with deionized water and air-dried.

The carcass samples, including 120 muscle samples (right and left shoulder muscles, right and left colata muscles), 120 lymph node samples (right and left pre-scapular lymph nodes, right and left pre-femoral lymph nodes) and 60 organ samples (liver and spleen) were collected. All samples were preserved in ice box and transferred to the laboratory. Environmental samples (wall, floor swabs and water) were kept in refrigerator at 4 °C, while non-environmental samples (muscles, lymph nodes and organs) were kept frozen at -20 °C until used for examination.

Sample Preparation

Environmental and carcass samples were prepared according to the technique recommended by APHA (2001). Frozen carcass samples were thawed by placing them in the refrigerator overnight, the meat package (organs, lymph nodes and muscles) were opened in biological safety cabinet, then 25 g from each sample were transferred aseptically to a sterile polyethylene bags containing 225 mL of 0.1% sterile buffered peptone water. The content of the bag were homogenized using stomacher (Lab. Blender 400, Seward Lab., and London) to have a dilution of 10^{-1}. One mL of the original dilution of all samples (wall and floor swabs, water samples, muscles, lymph nodes and organs) was transferred aseptically to a test tube containing 9 mL sterile 0.1% buffered peptone water (w/v) to prepare a dilution of 10^{-2}. Tenfold decimal serial dilution up to 10^{-6} were prepared to cover the expected range of samples contamination which could be easily counted.

Bacteriological Examination

Aerobic Bacterial (TBC) and Enterobacteriaceae Count (TEC): Bacterial counts (total bacterial count TBC and total enterobacteriaceae count TEC) were applied using drop plate technique (Zelver et al., 1999; Herigstad et al., 2001). Total aerobic Bacterial Count (TBC) was performed using standard plate count agar at 37°C for 24 - 48 h. On the other hand, Total Enterobacteriaceae Count (TEC) was conducted using Eosine Methylene Blue Agar (EMB) at 37 °C for 24 - 48 h revealing the growth of typical metallic green colonies. Counting plates showed 30 - 300 CFU per plates (Cruickshank et al., 1975, 1980). Five typical colonies (metallic green colonies) were selected and cultured onto MacConkey agar plates, and incubated at 37°C for 24 h, pure colonies on MacConkey agar plates were inoculated onto nutrient slant and incubated at 37°C for 24 h and kept for further identification.

Isolation of *Salmonella* and *E.coli* spp.

All samples were pre enriched in peptone buffer water and incubated at 37°C for 8±2 h. 0.1 mL of pre-enriched samples was transferred to 10 mL pre-warmed tetrathionate broth, incubated at 42°C for 24±3 h. A loop from tetrathionate broth was streaked onto CHROMagar plates

Table 1: Log Total Bacterial Count (TBC) and Total Enterobactereacie Count (TEC) in carcasses samples' slaughtered in the examined slaughterhouse

Sample	Sample level	Log TBC mean ±SE	Log TEC mean ±SE
Muscles	R. Colata Ms	4.51336[a] ± 0.15438	3.00529[a] ± 0.09501
	L. Colata Ms	4.63806[a] ± 0.15853	3.05745[a] ± 0.10994
	R. Shoulder Ms	4.68878[a] ± 0.14458	2.99325[a] ± 0.09810
	L. Shoulder Ms	4.53089[a] ± 0.17871	3.01761[a] ± 0.13067
	P value	**0.840**	**0.711**
Lymph Nodes	R. Prefemoral L.n.	4.39252[a] ± 0.16721	2.75361[a] ± 0.10420
	L. Prefemoral L.n.	4.66924[a] ± 0.18649	2.97011[a] ± 0.10075
	R. Prescapular L.n.	4.80139[a] ± 0.15277	2.96378[a] ± 0.09548
	L. Prescapular L.n.	4.54119[a] ± 0.17058	3.16341[a] ± 0.11645
	P value	**0.368**	**0.058**
Internal Organs	Liver	4.64887[a] ± 0.18027	3.14379[a] ± 0.10705
	Spleen	4.50044[a] ± 0.16903	3.06634[a] ± 0.08002
	P value	**0.357**	**0.449**

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$); Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$)

and incubated at 37°C for 24 h. Typical *Salmonella* spp. colonies showed pink colour, while *E. coli* colonies showed blue colour. Five typical colonies were streaked on the surface of nutrient agar and incubated at 37 °C ± 1 for 24±3 h. The growing colonies were picked and kept for biochemical confirmation using traditional biochemical set including indole test, Methyl Red, Voges Proskauer, Cimmon Citrate (IMVIC) and TSI (Triple sugar iron agar) / LIA (Lysine Iron Agar) reactions.

Laboratory Evaluation of in-use Disinfectants

Preparation of Dry and Wet Models of Bacterial Suspensions: *E.coli* and *Salmonella* spp. that were stored onto nutrient slopes were resuscitated on tubes of tetrathionate broth and incubated at 42°C for 24 h, then plated onto CHROMagar and incubated at 37°C for 24 h, typical colonies of *E. coli* and *Salmonella* spp. were counted and ten-fold serial dilution was prepared. A concentration of (8×10^3 CFU) was used for preparing two models: dry and wet models (dry model was prepared on a stainless steel carrier and wet model was prepared on buffered peptone water), one mL of wet model, as well as carriers of dry models were added to 4.5 mL of 5% yeast suspension (a source of organic matter, dispensed into tubes, sterilized by autoclaving at 121 °C / 20 min, subjected to pre-enrichment in buffered peptone water followed by selective culture, to demonstrate freedom from *E.coli* and *Salmonella* contamination) to simulate the conditions of slaughterhouse.

Preparation of Disinfectant Concentrations: The disinfectants were used in the slaughterhouse, Carbolic Acid 6.5% and Sodium Hypochlorite 3% were prepared from original stocks with additional concentrations (above and below the recommended concentration by manufacturer). Carbolic Acid was tested at the concentrations 5%, 6.5% and 10%. Sodium Hypochlorite was tested at the concentrations 3%, 5%, 8% and 10%.

Testing of Disinfectant Concentrations: One mL of bacterial suspension (wet model), as well as ten carriers (dry model) were added / immersed to four replicates of 9 mL disinfectant concentration: carbolic acid (5%, 6.5% and 10%), sodium hypochlorite (3%, 5%, 8% and 10%). Combinations were mixed, allowed to stand at cool room temperature and shaken briefly. After 0.25, 0.5, 1, 2 and 4 h contact time points, 0.1 mL aliquots were mixed with 10 mL disinfectant neutralizer (tween 80 3%). One mL of the combination in neutralizer was transferred to a resuscitation tube of 9 mL nutrient broth. The inoculated resuscitation tubes were incubated overnight, then plated onto CHROMagar and incubated at 37 °C for 24 h. The development of typical colonies of both *E.coli* and *Salmonella* was detected and counted.

Statistical Analysis

The data obtained were assessed using SPSS 10.01 (version 20). Differences between the treatments and groups were determined with the one-way analysis of variance (ANOVA) test with Duncan's posttest for some parameters, while the nonparametric Kruskal–Wallis was used to detect the mean rank and frequencies of both *E. coli* and *Salmonella*. Univariate analysis of variance was used to compare the influence of the tested disinfectant concentrations against pathogens in relation to exposure time. The data were expressed as mean ± standard error of the mean (SEM). Differences were considered significant at

P ≤ 0.05 and P < 0.01 (Levesque, 2007). Bacterial count logarithmic transformation were done before analysis. The correlation co-efficient was calculated to compare the influence of each measured parameter mean values on each other (Fulekar, 2009).

RESULTS

A uniform pattern of carcass contamination from the slaughterhouse's floors and walls was reflected on carcasses samples' bacteriological examination. A high log total bacterial and enterobacteriaceae counts with no significant differences (P > 0.05) between the different types of carcass samples including muscles, lymph nodes and internal organs as a result of insufficient cleaning and disinfection routine in the slaughterhouse (Table 1). On another view, the collected floor; wall swabs and water samples from the slaughterhouse revealed a highly significant increase (P<0.01) as revealed in (Table 2) in log total bacterial and enterobacteriaceae counts in after slaughtering samples when compared to after disinfection samples.

Total bacterial count in carcass and environmental samples (Table 3) revealed a highly significant (P < 0.01) strong positive (r = 0.819), significant (P ≤ 0.05) intermediate positive (r = 0.441) and a highly significant (P < 0.01) strong positive (r = 0.719) correlations between muscles with lymph nodes, liver and spleen, respectively. Significant (P ≤ 0.05) weak positive (r = 0.391), a highly significant (P < 0.01) strong positive (r = 0.736) correlation between lymph nodes with liver and spleen, respectively. A highly significant (P < 0.01) strong intermediate (r = 0.589) correlation between liver and spleen. A predominant non-significant (P > 0.05) weak (positive / negative) correlations was revealed between carcass and environmental samples in total bacterial count (Table 3).

Total enterobacteriaceae count in carcass and environmental samples (Table 3), revealed a highly significant (P<0.01) strong positive (r=0.805), significant (P≤0.05) intermediate positive (r=0.436) and a highly significant intermediate positive (r=0.511) correlations between muscles with lymph nodes, liver and spleen, respectively. Non-significant (P>0.05) weak positive (r=0.320), strong, significant (P≤0.05) intermediate positive (r=0.443) correlation between lymph nodes with liver and spleen, respectively.

Table 2: Log Total Bacterial Count (TBC) and Total Enterobactereacie Count (TEC) in environmental samples from in the examined slaughterhouse

Sample	Sample level	Log TBC mean ± SE	Log TEC mean ± SE
Floor Swabs	After Disinfection	1.65596^{b} ± 0.20401	0.75655^{b} ± 0.17424
	After Slaughtering	5.46737^{a} ± 0.09120	3.75536^{a} ± 0.04249
	P value	0.001	0.001
Wall Swabs	After Disinfection	0.74618^{b} ± 0.17050	0.91647^{b} ± 0.18322
	After Slaughtering	4.51728^{a} ± 0.09374	3.34518^{a} ± 0.03697
	P value	0.001	0.001
Water samples	After Disinfection	2.50222^{b} ± 0.56055	1.60020^{b} ± 0.13621
	After Slaughtering	4.78108^{a} ± 0.09901	3.77128^{a} ± 0.08028
	P value	0.001	0.001

Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01); Means carrying the same superscripts in the same column are non-significantly different at (P > 0.05)

Table 3: Log Total Bacterial Count (TBC) correlations (Above Diagonal) and Log Total Enterobactereacie Count (TEC) correlations (Below Diagonal) between carcasses and environmental samples from the examined slaughterhouse

Bacterial Count r	Muscle	Lymph Nodes	Liver	Spleen	Floor Swabs	Wall Swabs	Water
Muscle	**1**	0.819**	0.441*	0.719**	0.087	0.056	0.042
Lymph Nodes	0.805**	**1**	0.391*	0.736**	-0.015	0.060	0.269
Liver	0.436*	0.320	**1**	0.589**	0.208	0.258	0.093
Spleen	0.511**	0.443*	0.432*	**1**	0.140	0.189	0.193
Floor Swabs	0.307	0.223	0.221	0.084	**1**	0.319	-0.060
Wall Swabs	0.162	0.278	-0.003	0.218	0.282	**1**	-0.209
Water	-0.275	-0.197	-0.416*	-0.310	-0.395*	-0.240	**1**

****:** Correlation is highly significant (P < 0.01); ***:** Correlation is significant (P ≤ 0.05); **NS:** Correlation is non-significant (P > 0.05); **r:** 0.1 – 0.39 represent weak correlation; **r:** 0.40 – 0.69 represent intermediate correlation; **r:** 0.70 – 1.00 represent strong correlation (Fulekar, 2009)

Table 4: Intensity and frequencies of E. coli infection in carcasses and environmental samp

Type/level of Samples			Mean Rank	Frequency		
				Positive	Negative	Total
Carcass Samples	Muscle	R. Colata Ms	62.50[a]	22[a]	8[a]	30
		L. Colata Ms	62.50[a]	22[a]	8[a]	30
		R. Shoulder Ms	64.50[a]	23[a]	7[a]	30
		L. Shoulder Ms	52.50[a]	17[a]	13[a]	30
		P value	0.326	**P**=0.321	χ^2=3.492	
	Lymph nodes	R. Prefemoral L.n.	63.50[a]	25[a]	5[a]	30
		L. Prefemoral L.n.	59.50[a]	23[a]	7[a]	30
		R. Prescapular L.n.	59.50[a]	23[a]	7[a]	30
		L. Prescapular L.n.	59.50[a]	23[a]	7[a]	30
		P value	0.900	**P**=0.898	χ^2=0.589	
	Internal Organs	Liver	32.00[a]	19[a]	11[a]	30
		Spleen	29.00[a]	16[a]	14[a]	30
		P value	0.436	**P**=0.432	χ^2=0.617	
Environmental Samples	Floor Swabs	After Disinfection	26.50[b]	12[b]	18[a]	30
		After Slaughtering	34.50[a]	20[a]	10[b]	30
		P value	0.040	**P**=0.038	χ^2=4.286	
	Wall Swabs	After Disinfection	26.50[b]	14[b]	16[a]	30
		After Slaughtering	34.50[a]	22[a]	8[b]	30
		P value	0.037	**P**=0.035	χ^2=4.444	
	Water	After Disinfection	28.50[a]	25[a]	5[a]	30
		After Slaughtering	32.50[a]	29[a]	1[a]	30
		P value	0.088	**P**=0.085	χ^2=2.963	

Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01); Means carrying the same superscripts in the same column are non-significantly different at (P > 0.05)

Significant (P ≤ 0.05) intermediate positive (r = 0.432) correlation between liver and spleen. A predominant non-significant (P > 0.05) weak (positive / negative) correlations was revealed between carcass and environmental samples in total enterobacteriaceae count (Table 3).

E. coli and *Salmonella* isolation (Table 4 and 5) from carcass samples revealed no significant differences (P > 0.05) in frequencies of isolation and nearly similar mean rank (60%) among the 300 carcass samples. On the other hand the frequencies of isolation among the environmental samples (20% - 40%) revealed a highly significant increase (P<0.01) in floor, wall swabs and water samples collected after slaughtering compared to samples collected after disinfection, in contra verse of a non-significant differences (P>0.05) in log TEC from water samples collected after the two stages of sampling.

Carbolic acid wet model 6.5% (Table 6) achieved a highly significant (P<0.01) 100% killing efficacy after 4 h exposure, compared to carbolic acid wet models 5%, 10% that achieved non-significant (P>0.05) 51% killing efficacy after 4 h contact, non-significant (P>0.05) 100% killing efficacy after only 2 h exposure, respectively. Carbolic acid dry models 5%, 6.5% and 10% (Table 6) achieved highly significant (P<0.01) 60%, 97%, and non-significant (P>0.05) 100% killing efficacy after 4 h contact, respectively.

Sodium hypochlorite wet model 3% (Table 7) achieved non-significant (P>0.05) 47.5% killing efficacy after 4 h exposure, compared to Sodium hypochlorite wet model 5%, 8% and 10% achieved highly significant (P<0.01) 78%, 94% and non-significant (P>0.05) 100% killing efficacy after 4 h contact, respectively. On the other hand, Sodium hypochlorite dry models 3%, 5%, 8% and 10% revealed highly significant (P<0.01) 46%, 73%, 86% and 100% killing efficacy after 4 h exposure, respectively.

DISCUSSION

At the slaughterhouse, standard strict measures were pointed as a proper control measure to prevent the transmission of the micro-organisms to and from animal carcasses and slaughterhouse environment. Meanwhile, even when cleaning procedures were classified as satisfactory and a strong disinfectants were used.

Table 5: Intensity and frequencies of Salmonella infection in carcasses and environmental samples

Type/level of Samples			Mean Rank	Frequency		
				Positive	Negative	Total
Carcass Samples	Muscle	R. Colata Ms	58.50[a]	17[a]	13[a]	30
		L. Colata Ms	54.50[a]	15[a]	15[a]	30
		R. Shoulder Ms	64.50[a]	20[a]	10[a]	30
		L. Shoulder Ms	64.50[a]	20[a]	10[a]	30
		P value	0.479	**P**=0.475	χ^2=2.500	
	Lymph nodes	R. Prefemoral L.n.	59.50[a]	20[a]	10[a]	30
		L. Prefemoral L.n.	65.50[a]	23[a]	7[a]	30
		R. Prescapular L.n.	61.50[a]	21[a]	9[a]	30
		L. Prescapular L.n.	55.50[a]	18[a]	12[a]	30
		P value	0.575	**P**=0.571	χ^2=2.003	
	Internal Organs	Liver	30.50[a]	14[a]	16[a]	30
		Spleen	30.50[a]	14[a]	16[a]	30
		P value	1.0	**P**=1.000	χ^2=0.001	
Environmental Samples	1. Floor Swabs	After Disinfection	22.00[b]	6[b]	24[a]	30
		After Slaughtering	39.00[a]	23[a]	7[b]	30
		P value	0.001	**P**=0.001	χ^2=19.288	
	Wall Swabs	After Disinfection	23.50[b]	6[b]	24[a]	30
		After Slaughtering	37.50[a]	20[a]	10[b]	30
		P value	0.001	**P**=0.001	χ^2=13.303	
	Water	After Disinfection	20.00[b]	3[b]	27[a]	30
		After Slaughtering	41.00[a]	24[a]	6[b]	30
		P value	0.001	**P**=0.001	χ^2=29.697	

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$); Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$)

Table 6: Laboratory efficacy of Carbolic acid (5%, 6.5%, 10%) at different times of exposure against bacterial mixture in wet model and dry model

Contact Time/hr	Model	Concentration			
		5%	6.5%*	10%	Total
0.25	Dry	3.1[e] ± 1.0825	9.0[e] ± 2.8584	22.1[d] ± 3.7974	11.4[e] ± 2.8154
	Wet	3.1[e] ± 1.0825	6.2[e] ± 2.2243	20.9[e] ± 4.3712	10.1[d] ± 2.7885
0.5	Dry	16.8[d] ± 3.1663	23.1[d] ± 1.6535	45.6[c] ± 2.1949	28.5[d] ± 3.9313
	Wet	17.5[b] ± 2.4473	32.8[d] ± 2.1875	60.3[b] ± 5.3125	36.8[c] ± 5.6627
1	Dry	35.3[c] ± 1.9348	75.0[c] ± 0.5103	85.4[b] ± 1.5598	65.2[c] ± 6.5584
	Wet	45.0[a] ± 2.1040	84.0[c] ± 1.3858	99.3[a] ± 0.3865	76.1[b] ± 6.9421
2	Dry	49.6[b] ± 0.7864	92.3[b] ± 0.7447	98.7[a] ± 0.2570	80.2[b] ± 6.5786
	Wet	50.0[a] ± 1.0206	91.3[b] ± 2.0999	100.0[a] ± 0.000	80.4[a] ± 6.6169
4	Dry	60.0[a] ± 1.3501	97.9[a] ± 0.2991	100.0[a] ± 0.000	85.9[a] ± 5.5622
	Wet	51.2[a] ± 0.5103	100.0[a] ± 0.000	100.0[a] ± 0.000	83.7[a] ± 6.9307
Total	**Dry**	**33.0[c] ± 4.8313**	**59.5[b] ± 8.4001**	**70.4[a] ± 7.1907**	**54.3 ± 4.4512**
	Wet	**33.3[c] ± 4.5152**	**62.8[b] ± 8.4579**	**76.1[a] ± 7.3393**	**57.4 ± 4.5892**

*Recommended concentration by manufacture to be used in field; Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$); Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$)

Table 7: Laboratory efficacy of Sodium Hypochlorite (2%, 5%, 8%, 10%) at different times of exposure against bacterial mixture in wet model and dry model

Contact Time/hr	Model	Concentration				
		3%*	5%	8%	10%	Total
0.25	Dry	$3.2^{e} \pm 1.0825$	$16.8^{e} \pm 2.7243$	$18.1^{e} \pm 1.0825$	$22.1^{e} \pm 1.3858$	$15.0^{e} \pm 2.0041$
	Wet	$7.5^{d} \pm 2.1040$	$19.6^{e} \pm 3.2425$	$19.6^{e} \pm 1.8663$	$27.5^{d} \pm 1.3501$	$18.5^{e} \pm 2.1036$
0.5	Dry	$11.8^{d} \pm 0.8068$	$30.6^{d} \pm 2.5259$	$37.5^{d} \pm 0.5103$	$37.5^{d} \pm 2.4473$	$29.3^{d} \pm 2.8275$
	Wet	$21.8^{c} \pm 3.0830$	$36.8^{d} \pm 1.3010$	$39.3^{d} \pm 1.6535$	$57.8^{c} \pm 3.1612$	$38.9^{d} \pm 3.4727$
1	Dry	$25.6^{c} \pm 1.9432$	$50.0^{c} \pm 1.0206$	$58.1^{c} \pm 0.8068$	$71.2^{c} \pm 2.6516$	$51.2^{c} \pm 4.3645$
	Wet	$35.9^{b} \pm 1.6437$	$50.6^{c} \pm 2.0728$	$71.5^{c} \pm 2.4138$	$80.6^{b} \pm 1.9432$	$59.6^{c} \pm 4.6104$
2	Dry	$34.6^{b} \pm 1.6437$	$60.0^{b} \pm 1.3501$	$73.1^{b} \pm 1.5728$	$92.9^{b} \pm 0.4824$	$65.1^{b} \pm 5.4927$
	Wet	$49.0^{a} \pm 0.5983$	$69.3^{b} \pm 1.4878$	$85.6^{b} \pm 1.1967$	$99.4^{a} \pm 0.3321$	$75.8^{b} \pm 4.8737$
4	Dry	$46.2^{a} \pm 1.6137$	$73.1^{a} \pm 1.6535$	$86.7^{a} \pm 0.6929$	$100.0^{a} \pm 0.000$	$76.5^{a} \pm 5.1649$
	Wet	$47.5^{a} \pm 1.3501$	$78.4^{a} \pm 2.2462$	$94.2^{a} \pm 1.0325$	$100.0^{a} \pm 0.000$	$80.0^{a} \pm 5.2988$
Total	Dry	$24.3^{d} \pm 3.5937$	$46.1^{c} \pm 4.6910$	$54.7^{b} \pm 5.6429$	$64.7^{a} \pm 7.0166$	47.4 ± 3.1289
	Wet	$32.3^{d} \pm 3.7068$	$51.0^{c} \pm 4.9650$	$62.0^{b} \pm 6.5159$	$73.0^{a} \pm 6.3542$	54.6 ± 3.1861

*Recommended concentration by manufacture to be used in field; Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$); Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$)

Some micro-organisms as *E.coli* and *Salmonella* was still able to survive and detected in environmental samples of the slaughterhouse and in carcass samples (Carrique-Mas and Davies, 2008).

It has been described that holes in floors and walls make it difficult for the penetration of disinfectant solutions and what is more, the biofilms created by *Salmonella* can make the action of the disinfectants more difficult (Marin et al., 2009). The average intermediate degree of correlations between total bacterial counts, total enterobacteriaceae counts with the different environmental and carcass samples ensured the deficient access of disinfectant to some areas in the slaughterhouse contributing a definite contamination of the carcass directly or indirectly through the workers, equipment and air.

It was cleared from our results that carbolic acid recommended concentration (6.5%) achieved the 100% killing efficacy only after 4 h in wet model, on the contrary carbolic acid 6.5% dry model achieved only 97% at the same time of contact and under the same experimental conditions. The same distinguish in killing efficacy was noticed in sodium hypochlorite dry model 3 % (46% after 4 h contact) compared to sodium hypochlorite wet model 3% (47.5% after 4 h contact). The differences in disinfectant performance between the two models (dry, wet) are probably a result of several factors. Physiological status of the micro-organism especially *E.coli* and *Salmonella* in the dried versus the wet preparations; adaptive responses by the micro-organism in conditions of low water activity (Russell, 2004; Fraise et al., 2008) and reduced nutrient availability (Hoff and Akin, 1986). It has been observed that susceptibility of members of the enterobacteriaceae to certain antiseptics and disinfectants, may increase or decrease depending on cell density, growth rate and the limiting nutrient (Brown et al., 1990; Bjergbæk et al., 2008).

The reduction of the microbial contamination (Rahkio and Korkeala, 1996) depends on the enforcement of hygienic practice such as regular disinfection of working tools and worker hands are important in reducing the microbiological contamination of carcasses. The used routine disinfection in the slaughterhouse depends on the usage of disinfectants that are effective and efficient against wide variety of micro-organisms and doesn't tent the meat and its marketability (Sander et al., 2002)

CONCLUSION & RECOMMENDATION

Current research prove that routinely cleaning and disinfection procedures performed at the slaughterhouse were not able to control microbial growth, and subsequently carcass contamination with some zoonotic enteric pathogens such as *E. coli* and *Salmonella* spp. using the recommended concentrations of in-use disinfectants. Although the high germicidal power of carbolic acid, it is not recommended to be used inside the slaughterhouses. Recommended carbolic acid (6.5%) and sodium hypochlorite (3%) concentrations failed to achieve the expected efficacy. Correction of carbolic acid and sodium hypochlorite concentrations up to 10%, 8%, respectively is required.

A new line and strategies have to be taken in consideration to enhance the cleaning and disinfection procedures in a slaughterhouse, such as considering the usage of green

chemistry disinfectants that might have a higher germicidal potential, long term effectiveness and safe to be used inside the slaughterhouses. Others sources of contamination have to be considered during evaluation of the routine cleaning and disinfection program in the slaughterhouse, and in disease control strategies as workers; equipment; air and containers used for transportation of animals to the slaughterhouse.

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AUTHORS' CONTRIBUTION

All authors contributed equally in samples collection, reviewing and improving of the manuscript.

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