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Impact of Microbial Load of Slaughterhouse Environment on the Degree of Broiler Chicken Carcass Contamination, with a Focus on *Campylobacter* Prevalence

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Abstract

A poultry abattoir's environment is the primary source of potential cross-contamination and bacterial contamination. Three automatic poultry slaughterhouses were selected in these governorates: Giza (1), Menoufeya (2), and Sharkeya (3). This study aimed to determine whether the microorganism load in the abattoir environment (TBC, TCC, and *Campylobacter* count) is associated with carcass contamination. Additionally, we wanted to investigate the effects of adding chlorine at different levels during the processing of carcasses on the microbial load. There were 15 air samples collected, as well as 30 swabs taken from the walls, floors, and processing equipment, from the three abattoirs (Reception, Bleeding and Plucking, Processing, Packing, and Refrigeration) in each abattoir, plus roughly five samples collected prior to and after carcass immersion from the scald tank, chill tank, and pre-chiller tank. In addition, approximately 12 broiler carcasses were randomly selected midday from each slaughterhouse's process line. All three slaughterhouses showed significant differences in microbial counts (TBC and TCC); the most significant differences were found on the walls and floors. A significant difference exists between the different abattoir halls. The lowest count was found in air samples at the refrigeration room (TBC and TCC recorded 0.14 and 0.12 $\log_{10}$ CFU, respectively). Three slaughterhouses, 1, 2, and 3, had varying *Campylobacter* prevalence rates: 8 (22.8%), 15 (50%), and 6 (20%), respectively. By ANOVA, it was discovered that there was a significant positive correlation (r = 0.88, 0.89, and 0.95) between the rates of contamination of the floor with equipment, the floor with carcass rinse, and the equipment with carcass rinse. Chlorine added to chilled water in concentrations ranging from 20 to 100 ppm led to a further reduction in microbes on the skin's surface. The effectiveness of the sanitation standard as well as the use of chlorine in chilled tanks should be checked to prevent carcass contamination. The proliferation of bacteria, particularly *Campylobacter*, and the contamination of broiler carcasses by the bacteria found in the intestinal material during processing could lead to monitoring hygienic status.

KEYWORDS
Slaughterhouse, Carcass rinses, TBC, *Campylobacter*, Chlorine

INTRODUCTION

Poultry slaughter is an important factor that might have an impact on the hygienic condition of the chicken meat. The environment in chicken abattoirs is the main risk factor for bacterial contamination and cross-infection of broiler carcasses, which can result in substantial economic losses for processors and damage poultry products (Lytou *et al*., 2020). Proper slaughterhouse operations help stop the spread of dangerous environmental bacteria in poultry slaughterhouses. However, some environmental elements continue to impact the health of poultry carcasses. Controlling the proliferation of microorganisms is therefore crucial, particularly during commercial poultry processing (Babacan *et al*., 2020). Abattoirs vary in their capacities, arrangements, technology, equipment design, staff hygiene training, management motivation, ecological issues, etc., which results in the abattoirs' varying hygienic performances (EFSA, 2012; Habib, *et al*., 2012; Djekic *et al*., 2016; Alvseike *et al*., 2019). Due to the risk of infection from handlers, water, packing, utensils, and the gastrointestinal tract, special caution must be used when processing poultry meat (Bryan, 2001; Nwachukwu, 2013). The level of contamination of live birds and contamination and cross-contamination between carcasses, processing equipment, and the processing environment (Gill *et al*., 2006; Pacholewicz *et al*., 2015; Althaus *et al*., 2017) are two variables that affect the microbiological status of broiler carcasses. Cegar *et al*. (2022) claimed that the pre-abattoir circumstances surrounding chilled carcasses could have an important effect on the presence of infections in these carcasses. The degree of indication for frozen carcasses largely depends on the how hygienic process of the slaughter. Rasschaert *et al*. (2008) speculated that several carcasses could be infected by one sick broiler that arrived at the processing facility. Salmonella spp., *Campylobacter* spp., and thermotolerant coliforms are a few pathogens that can be harmful to human health and are found in the intestines of chickens (Scallan *et al*., 2011). In Europe in 2019, there were 220,682 and 87,923 reported cases of *Campylobacter* and Salmonella, respectively, which are the most common foodborne illnesses that could be spread from animals to humans. The incidence rates per 100,000 people were 59.7 and 20.0, respectively (EFSA/ECD, 2021).

Bacteria can be reduced or eliminated during the processing of poultry using several techniques, including heat, water,

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chemicals, and machinery. even though it is challenging to apply sanitation practices and HACCP can reduce the level of contamination on poultry carcasses without eliminating it (Mbata, 2005). Understanding how the microbial composition changes during processing is crucial for the creation of cleaning and disinfection techniques that would lower pathogens in the finished product (Kim *et al*., 2017). Chlorinated water has been found to reduce cross-contamination and microbiological contamination in equipment sprays, immersion chillers, and carcass cabinet washers, according to Northcutt *et al*. (2003) and Bashor *et al*. (2004).

The effect of microbial communities in poultry slaughterhouses has previously been studied. However, the effects of microbial community transfer on the contamination of carcasses have not received much attention in the past. Thus, the goal of the current investigation was to determine the association between the number of microbes (TBC, TCC, and *Campylobacter* count) present in the abattoir environment and the level of carcass contamination. Another goal was to research how adding chlorine at various levels during the processing of carcasses impacts microbial sources. This provides a helpful scientific basis upon which managers can further enhance the quality of the product.

MATERIALS AND METHODS

The examined slaughterhouses

Three automatic poultry slaughterhouses in three governorates in Egypt; Giza (1), Menoufeya (2), and Sharkeya (3) were chosen; from May to October 2021; slaughterhouses 1 and 3 had a production capacity of 6,000 birds per hour, while slaughterhouse 2 had a capacity of 3000 birds per hour. The chicken passes through hand slaughter, bleeding out, a scalding tank, a plucking machine, and mechanical evisceration on an automated processing line at each abattoir. To lower the interior flesh temperature, carcasses are then washed in water, pre-chilled, and immersed in cold water.

Collection of samples

All samples from each abattoir were collected at the same day.

Environmental samples

Air sampling

A total of fifteen air samples were collected from the five abattoir halls (Reception, Bleeding and Plucking, Processing, Packing and Refrigeration) during slaughtering day. Samples were collected using the settling plate technique. Using 10 cm-diameter plates containing plate count agar and Macconkey, that were dispersed throughout various abattoir halls where total bacterial counts and coliform counts were measured; and CCDA (*Campylobacter* blood-free selective agar with specific supplement) to determine the prevalence of *Campylobacter* spp. After sampling, plates were sealed and rapidly delivered to the lab in an ice box.

Surfaces sampling

Six swabs per hall were used to sample the walls, floors, and processing equipment in each of the five halls of each abattoir, totaling around 30 swabs. Using sterile cotton swabs moistened with sterile normal saline solution, an area measuring about 10x10 cm from each surface was swabbed. The samples were then placed into sterile cotton-plugged test tubes, each of which contained 5 ml of sterile normal saline solution and transported to a lab for microbiological analysis.

Water sampling

Each abattoir had roughly 5 samples taken from the scald tank, chill tank, and pre-chiller tank before and after the carcass immersion. Using sterile plastic 250-ml cups connected to a dipping rope, water samples were obtained. According to Cavani *et al*. (2010), physical analysis was utilized for the on-the-spot assessment of pH and temperature using digital equipment. According to Hecer *et al*. (2007) a microbiological investigation was used to determine the most probable number of coliforms (MPN/100 ml) and *Campylobacter* spp. Counts, samples from chilled tank which contain chlorine was taken in 10 mL of Dey-Engley neutralizing broth.

Carcasses rinses

Approximately 12 broiler carcasses from each slaughterhouse's process line were selected at random in the middle of the working day for the purpose of sampling the carcass surface. evisceration, washing, pre-freezing, and chilling processes, each carcass was placed separately in a sterile plastic bag and cleaned with 400 ml of buffered peptone water (Johnson, 2010). Following completion of sampling, carcasses were removed while wearing sterile gloves, and rinsed and then were aseptically transferred to smaller sterile bags before being sent to the laboratory at the Department of Veterinary Hygiene and Management, Cairo university to be used for microbiological examination.

Microbiological examination of the collected samples

Under strictly aseptic circumstances, tenfold serial dilutions of the original samples of surface swabs, water, and carcass rinses in 0.1% peptone were made in the lab. After plate counting, the original samples were infected onto plate count, MacConkey, and CCDA agar plates with 0.1 ml of each dilution using the spread plate technique. All samples, including air, were placed on plates, and they were subsequently incubated in accordance with ISO (2006) Plate count and McConkey agar plates were kept at 37°C for 24–48 hours, whereas CCDA plates were kept at 41.5°C for 40–48 hours in a microaerobic environment. The counts for air, swabs, water, and carcass rinses were then calculated as CFU/Ø/5 minutes, CFU/cm^2, and CFU/ml, respectively.

Statistical analysis

The use of SPSS version 16.0 was used for data analysis. Initially, $\log_{10}$ was applied to all counts. To determine whether there were statistically significant variations in diversity indices between samples, one-way ANOVA tests were run. A $P < 0.05$ was used to define statistical significance. To evaluate the relationship between overall bacterial counts from the abattoir environment and carcass washing, Spearman's rank correlation coefficient was utilized. Excel 2010 was used to create the graphs.

RESULTS

Table 1 presents the microbiological analysis of the environmental samples collected from various processing rooms. Overall, there is a significant difference in microbial count (TBC and TCC) between the three slaughterhouses; the most significant

samples were taken from walls and floors (P<0.05 in all processing halls), whereas samples taken from the air have a constant count except for TBC from the plucking and packaging rooms have the highest count with a mean log_{10} (3.59 and 2.62 CFU/Ø/Min respectively). Surface samples from the equipment reveal notable variations between the evisceration and processing halls. The findings in Fig. 1 demonstrate a significant distinction between the reception, plucking, processing, packaging, and refrigeration processes. The lowest count was found in air samples at the refrigeration room (TBC and TCC recorded 0.14 and 0.12 log_{10} CFU, respectively). The highest bacterial count was found on equipment surfaces in the reception room with means of 6.66

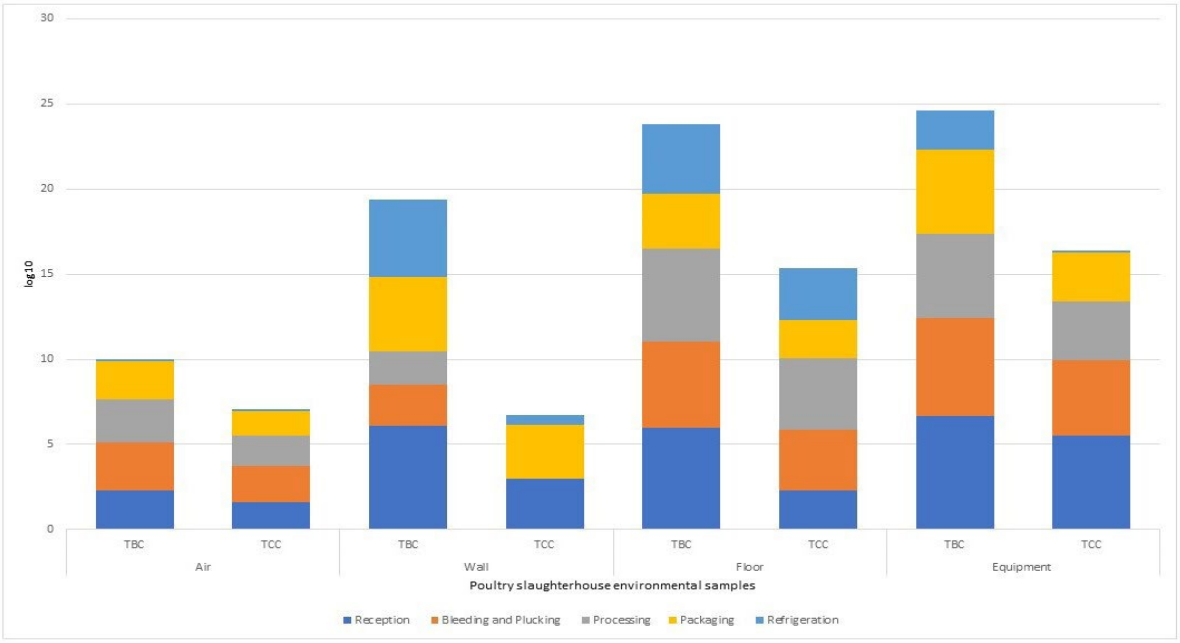

Fig 1. Log 10 of TBC, TCC recovered from different surfaces of different slaughterhouses processing halls.

Table 1. log 10 of average total bacterial and coliform counts recovered from air samples (Cfu/ Ø/Min.) and surface swabs (Cfu/ cm2) of the examined poultry processing plants during different processing steps.

Environmental samples	Slaughterhouse	Reception		Bleeding and Plucking		Processing		Packaging		Refrigeration		*P* value	
		TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC	TBC	TCC
Air	1	2.45±.26	1.62±0.33	1.79±0.34^{b}	1.68±.20	2.21±.14	1.89±.18	1.59±.177^{b}	1.20±.058^{b}	.00±.00	.00±.00		
	2	1.85±.37	1.43±.23	3.22±.23^{a}	2.25±.38	2.80±.20	1.77±.18	2.62±.13^{a}	1.823±.09^{a}	.00±.00	.00±.00		
	3	2.53±.32	1.76±.26	3.59±.22^{a}	2.51±.24	2.58±.37	1.69±.12	2.36±.100^{a}	1.24±.06^{b}	.43±.43	.36±.36		
	P value	0.33	0.71	0.01	0.19	0.17	0.7	0.01	0.00	0.42	0.42		
	Mean± SE-	2.27±.19^{A}	1.60±.14DE	2.87±.30^{A}	2.15±.18^{D}	2.53±.13^{A}	1.78±.08DE	2.19±.17^{A}	1.42±.10^{E}	.14±.14^{B}	.12±.12^{F}	0	0
Wall	1	5.24±.49^{b}	.00±.00^{c}	1.13±.098^{c}	.00±.00	.00±.00^{b}	.00±.00	4.71+.15^{a}	3.91+.07^{a}	3.50+.15^{b}	.00±.00^{b}		
	2	7.49±.09^{a}	5.76±.16^{a}	3.86±.10^{a}	.00±.00	5.79+.13^{a}	.00±.00	4.40+.10^{ab}	2.27+.09^{c}	5.15+.04^{a}	1.73+.31^{a}		
	3	5.56±.17^{b}	3.28±.14^{b}	2.29±.39^{b}	.00±.00	.00±.00^{b}	.00±.00	4.07+.13^{b}	3.23+.19^{b}	4.99+.12^{a}	.00±.00^{b}		
	P valve	0.00	0	0.00		0		0.03	0	0	0.00		
	Mean± SE	6.09±.38^{A}	3.01±.83^{D}	2.42±.41^{BC}	.00±.00^{E}	1.93±.96^{C}	.00±.00^{E}	4.39±.11^{AB}	3.14±.24^{D}	4.55±.27^{A}	.58±.30^{E}	0	0
Floor	1	6.06±.54	.00±.00^{b}	5.54±.59^{a}	4.92±.33^{a}	5.74±.42	4.56±.29^{a}	1.39±.20^{a}	.00±.00^{c}	4.38±.25^{b}	3.64±.07^{a}		
	2	6.68±.36	6.96±.03^{a}	3.59±.22^{b}	2.25±.38^{b}	4.58+.43	3.22±.19^{b}	6.08±.19^{a}	5.01±.06^{a}	2.49±.058^{c}	1.52±.12^{b}		
	3	5.22±.46	.00±.00^{b}	5.98±.13^{a}	3.42±.11^{b}	6.13±.54	4.92±.56^{a}	2.21±.06^{b}	1.69±.35^{b}	5.33±.088^{a}	3.95±.10^{a}		
	P value	0.16	0	0.01	0.00	0.13	0.01	0	0	0	0		
	Mean± SE	5.98±.31^{A}	2.32±.1.16	5.03±.41^{ABC}	3.53±.41	5.48±.33^{AB}	4.23±.29	3.23±.72^{C}	2.23±.73	4.07±.42^{BC}	3.04±.38	0.00	0.21
Equipment	1	6.52±.39	5.58±.77	6.27±.10^{a}	4.86±.09^{b}	6.41±.24^{a}	4.45±.49^{a}	6.11±.21	4.86+.09^{b}	-	-		
	2	7.33±.68	5.91±.18	3.59±.22^{b}	2.51±.24^{c}	4.86±.09^{b}	3.59±.22^{b}	5.11±.12	3.59+.22^{c}	-	-		
	3	6.11±.21	5.11±.12	7.33±.39^{a}	5.91±.18^{a}	3.59±.22^{c}	2.25±.38^{b}	5.91±.18	5.91+.18^{a}	-	-		
	P value	0.1	0.5	0	0	0	0.02	0.1	0				
	Mean± SE	6.66±.24^{A}	5.53±.25^{D}	5.73±.57^{A}	4.43±.51DE	4.95±.42^{A}	3.43±.37^{E}	4.95±.39^{A}	2.87±.55^{E}	2.32±.75^{B}	0.11±.11^{F}	0	0

Data are calculated as mean log 10 CFU ± SE)
Cfu=Colony Forming Unit; Ø=diameter of plate (5cm); SE= Standard Error; TBC=Total Bacterial Count; TCC = Total Coliform Count
^{a,b,c} different letters in columns refer to statistical difference between all slaughterhouses for the same environmental sample (c < b< a) (p value ≤0.05)
$^{A, B, C}$ different letters in rows refer to statistical difference between TBC in different slaughterhouse processing halls (p value ≤0.05)
$^{D, E, F}$ different letters in rows refer to statistical difference between TCC in different slaughterhouse processing halls (p value ≤0.05)

log_{10} CFU of TBC and 5.53 CFU of TCC. Fig. 2 displays the microbiological status of environmental samples taken from the three slaughterhouses. Samples from the second slaughterhouse contained the highest TBC and TCC mean log values, with a TBC average reaching up to 5 log_{10} CFU and a TCC average reaching up to 3 log_{10} CFU. The microbial counts in samples from other slaughterhouses, especially the first, were noticeably lower. The occurrence of thermotolerant *Campylobacter* in the environment of three slaughterhouses yielded isolation of 29 *Campylobacter* spp., as shown in Table 2. In slaughterhouses 1, 2, and 3, the prevalence rates were 8 (22.8%), 15 (50%), and 6 (20%), respectively. All environmental samples were taken from slaughterhouse 1 except those from the air and water containing *Campylobacter* spp. In the second, all the samples contained *Campylobacter* spp. While it is not found in the third slaughterhouse's air samples. Conclusions from physical water analysis in all slaughterhouses collected during various processing steps is shown in Figure 3; the pH was nearly constant at 7:8.1 on average. The temperature (°C) fluctuated; it was 58 at the point of scalding water and reached 22 at the point of pre-chilling; it then fell to 4 before chilling and rose to 16 after chilling.

The microbiological status of water used during different processing steps was shown in Table 3. The MPN of the water before scalding was ranged < 2 to 46 CFU/ 100 ml in all 3 slaughterhouses, and the mean of TBC (1.56 CFU/ml), but scalding water after recorded > 1800 CFU/ 100 ml in all abattoirs. the same in pre-chiller water, but after chilling the MPN reach to <2 in 1st slaughterhouse, and still >1800 CFU/100ml in 2nd and 3rd slaughterhouse, with significance difference $p<0.05$. In Table 4, sodium hypochlorite is added at 20 to 100 parts per million to chlorinat-

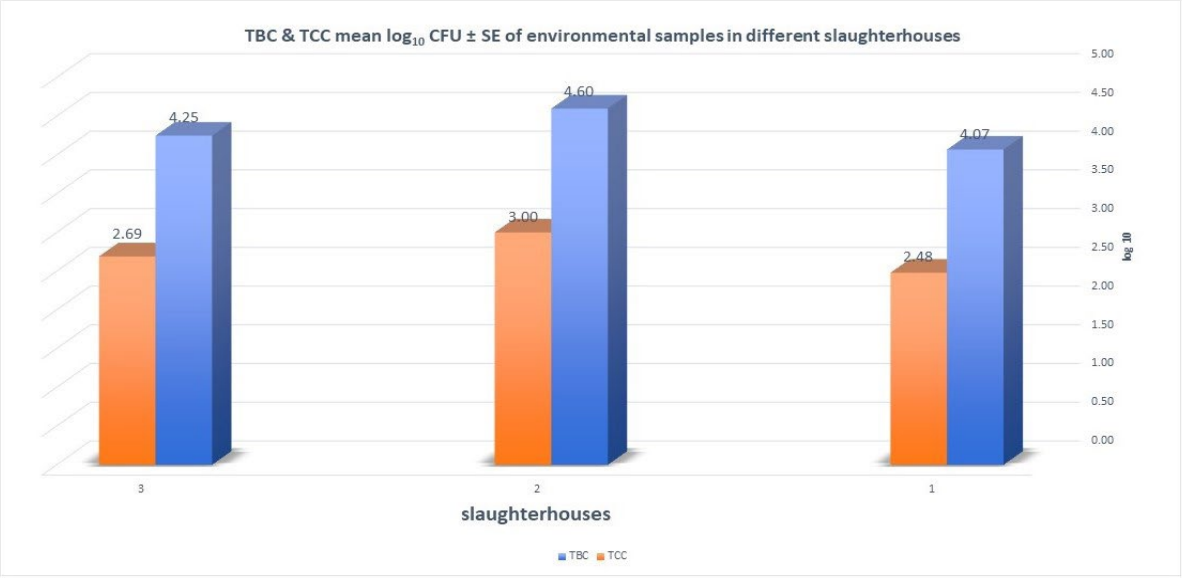

Fig 2. Microbiological results (TBC&TCC) of collected environmental samples from different poultry slaughterhouses.

Table 2. Prevalence of *Campylobacter* spp. In the environmental samples collected from the three slaughterhouses.

slaughterhouse	Environmental samples	Number of samples	Number of Positive Samples	Prevalence of *Campylobacter* spp. (%)
Slaughterhouse 1	Air	5	N	0%
	wall	5	2	40%
	Floor	5	3	60%
	Equipment	15	3	20%
	Water samples	5	0	0%
	Total	35	8	22.86%
Slaughterhouse 2	Air	5	1	20%
	wall	5	3	60
	Floor	5	3	60%
	Equipment	10	6	60
	Water samples	5	2	40%
	Total	30	15	50%
Slaughterhouse 3	Air	5	N	0
	wall	5	1	20
	Floor	5	1	20
	Equipment	10	3	30
	Water samples	5	1	20%
	Total	30	6	20

N: *Campylobacter* negative

ed chilling water in various poultry slaughterhouses. As a result of using chlorine, the number of microbes on the skin's surface decreased further. Following 100 ppm chlorination, microbial counts decreased in 1st slaughterhouse; TBC is 99.84%, TCC is 95%, and *Campylobacter* counts are 100%. At 50 ppm, chlorine treatment successfully reduced counts (100% for TBC and *Campylobacter* and reaching 99.5% for TCC). At low levels of 20 ppm chlorine reduces TBC, TCC, and *Campylobacter* counts by 99%, 99%, and 100%, respectively. According to Table 5 and Figure 4, the contamination rate of the carcass rinses was significantly correlated with samples from the slaughterhouse environment. ANOVA revealed a significant positive correlation (r = 0.88, 0.89, and 0.95) between the contamination rates of the floor with equipment, the floor with carcass rinse, and the equipment with carcass rinse during various processing steps. While air floors and walls had negative correlations (r = -0.39), floors had a correlation of 0.88. Additionally, a strong correlation (R2 = 0.92) was found using a linear regression analysis between the microbial count of carcass rinses and various environmental samples (floor, air, wall, and equipment).

DISCUSSION

Poultry is slaughtered in large-scale slaughterhouses using a highly automated process. Despite scientific progress, there are still plenty of chances for bacterial contamination and spread during slaughter (Wages, 2020). While safe slaughterhouse practices are used to prevent the growth of dangerous environmental microorganisms, some environmental factors still have an impact on poultry carcass safety (Song *et al*., 2021).

Table 1 indicates a statistically significant difference in microbial counts at various processing stages between the three slaughterhouses. Evisceration and plucking of the carcass are necessary steps in preventing microbial contamination of the muscle that will have an impact on carcass contamination (Bacon *et al*., 2000; Abdalla *et al*., 2009). During slaughter, contaminated feces escape from the cloaca, resulting in higher contamination levels in broiler carcasses (Berrang *et al*., 2001). There was a significant difference ($P < 0.05$) among the various processing steps in this study. The equipment had the highest mean log TBC reading (4.92 CFU), followed by the floor (4.76 CFU), the wall (3.88 CFU), and the air (2.00 CFU). Medium air, regarded as a crucial quality standard for foods that encounter air during procedures like cooling and freezing, is vital for microbial contamination (Tükel and Doan 2000). Ünlütürk and Turantaş (1999) indicated that the maximum number of bacteria in the atmosphere shouldn't be more than 10^3 CFU/m^3. In the air and walls, the highest coliform counts were found (1.42 and 1.34 CFU, respectively), followed by equipment and flooring (3.27 and 3.07). Ghougal *et al*., (2021) suggested that contaminated knives, cutters, and other tools and equipment may spread pathogenic bacteria. The results showed that there are significant variations in the procedures used for receiving, evisceration, processing, packaging, and refrigeration (see Fig. 1). The equipment surfaces in the receiving room produced the highest TBC counts of 6.66 $\log_{10}$ CFU and 5.53 $\log_{10}$ TCC, whereas air samples from the refrigeration room produced the lowest counts of.14 $\log_{10}$ CFU and 12.12 $\log_{10}$ CFU, respec-

Table 3. Microbiological status of water used in the examined slaughterhouses during different processing steps.

	Slaughterhouse	MPN Mean $\log_{10}$ CFU/100 ml	TBC Mean $\log_{10}$ CFU/ml	*Campylobacter* mean $\log_{10}$ CFU/ml
Scalding water before carcass immersion	1	$< 2^{c}$	$.0^{c}$	0
	2	46^{a}	2.69^{a}	0
	3	32^{b}	2^{b}	0
	P Value	0	0	
	Mean of all samples	26.66 ± 6.60^{C}	$1.56\pm.40^{B}$	$.00\pm.00^{B}$
Scalding water after carcass immersion	1	>1800	5.47^{a}	$.0^{c}$
	2	>1800	4.46^{c}	3.3^{a}
	3	>1800	5.1^{b}	2^{b}
	P Value	1	0	0
	Mean of all samples	1866.66 ± 16.67^{A}	$5.023\pm.15^{A}$	$1.77\pm.48^{A}$
Pre-Chiller water after carcass immersion	1	>1800	5.36^{b}	0.0c
	2	>1800	6.7a	5.3a
	3	>1800	4.3c	1.35b
	P Value	1	0	0
	Mean of all samples	1866.67 ± 16.67^{A}	$5.48\pm.35^{A}$	$2.24\pm.799^{A}$
Chilling water before carcass immersion	1 (100 ppm chlorine)	<2c	0c	0
	2(50 ppm chlorine)	140a	2.47b	0
	3(20 ppm chlorine)	90b	3.45a	0
	P Value	0	0	
	Mean of all samples	77.22 ± 20.30^{C}	$1.94\pm.51^{B}$	$00\pm.00^{B}$
Chilling water after carcass immersion	1 (100 ppm chlorine)	<2b	4.34c	0
	2(50 ppm chlorine)	>1800a	5.23b	0
	3(20 ppm chlorine)	>1800a	6.50a	0
	P Value	0	0.01	
	Mean of all samples	1245.00 ± 311.13^{B}	$4.65\pm.12^{A}$	$00\pm.00^{B}$
P Value		0	0	0

Cfu=Colony Forming Unit; SE= Standard Error; MPN=most probable number cfu/100 ml; TCC = Total Coliform Count

^{a,b,c} different letters in columns refer to statistical difference between all slaughterhouses for same environmental sampling ($c < b < a$) (p value ≤ 0.05).

^{A,B,C} different letters in columns refer to statistical difference the microbial counts of water recovered at different processing steps (p value ≤ 0.05).

tively, for both TBC and TCC (P <0.05). according to Guastalli *et al*. (2016) High coliform levels suggest contamination from post-processing or inadequate sanitization.

Indicator microorganisms are recognized in the scientific literature as an effective tool for testing abattoir process hygiene (EFSA, 2012; Schaffner and Smith-Simpson, 2014; Alvseike *et al*., 2019). Our analysis, as shown in Fig. 2, indicated that the microbial load in the second slaughterhouse is significantly higher than that in the first and third slaughterhouses. This could be due to our investigations, which revealed that abattoirs 1 and 3 had updated equipment while abattoir 2 had outdated equipment. In general, the first and third abattoirs could be considered lower risk. In contrast, the second abattoir was considered to have a higher risk for the three groups of indicator microorganisms. According to Collobert *et al*. (2002), it appeared that the general lack of hygiene and the effectiveness of sanitary interventions did not meet the needs of this infrastructure. Floors and drains, on the other hand, can provide a favorable environment for microbial growth.

The prevalence of *Campylobacter* in the three slaughterhouses is shown in Table 2. There were 22.8%, 50%, and 20% for abattoirs 1, 2, and 3, respectively, more cases of *Campylobacter* at the second sample site than at the first and third. The air samples collected from the slaughterhouses did not contain *Campylobacter*, but it was present in the other samples. This implies that the handling and execution of chicken carcasses resulted in contamination. Our findings are in line with those of Allen *et al*. (2000), who discovered cross-contamination while processing chickens that tested negative for *Campylobacter* in the abattoir. This supports earlier studies' findings that *Campylobacter* concentrations in various abattoirs may vary depending on the number of birds present, the time of slaughter, and the cleanliness of the equipment (Newell *et al*., 2001; Whyte *et al*., 2004).

In slaughterhouses, the water used for cleaning and pro-

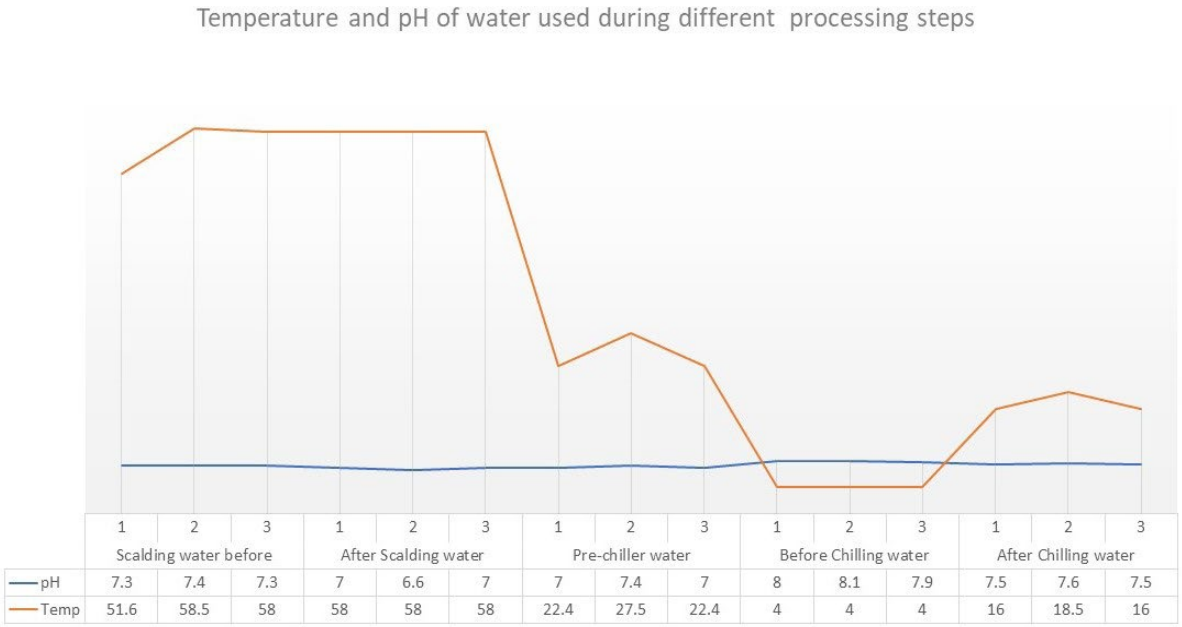

Fig 3: Physical parameters (Temperature and pH) of water samples collected from the examined slaughterhouses at different processing steps.

Table 4. log 10 of the average total bacterial, coliform, and *Campylobacter* counts (cfu/ml) recovered from carcass rinses in different poultry processing plants using different concentrations of chlorine in chilling tank water.

Processing steps	Slaughterhouse	TBC mean ± SE	R%	TCC mean ± SE	R%	*Campylobacter* count mean ± SE	R%
Before wash	1	7.69 ±0.06^{c}	-	7.25 ±0.146^{b}	-	6.54±0.073^{b}	-
	2	8.59 ±0.035^{b}	-	8.17 ±0.066^{a}	-	6.49±0.0436^{b}	-
	3	8.71 ±0.014^{a}	-	8.23 ±0.082^{a}	-	8.07 ±0.062^{a}	-
	P value	0		0.00		0	
After wash	1	6.59±0.066^{b}	92%	6.47±0.034^{c}	83.33%	6.11±0.07^{b}	72.5
	2	6.47±0.037^{b}	99.23%	7.67±0.045^{a}	68.70%	5.84±0.026^{b}	76.66
	3	8.07±0.077^{a}	77%	7.44±0.125^{a}	83.50%	7.65±0.098^{a}	62.50%
	P value	0		0		0	
After pre-chilling	1	6.04±0.093^{c}	97.80%	6.17+0.029^{b}	91.70%	0.00+0.00^{c}	100%
	2	6.31±0.064^{b}	99.48%	6.76+0.049^{a}	98%	5.30+0.042^{b}	71.40%
	3	7.64±0.023^{a}	91.50%	6.54+0.067^{a}	98%	7.06+0.066^{a}	73.30%
	P value	0		0		0	
After chilling	1	4.89±0.074^{b}	99.84%	5.94+0.032^{b}	95%	0.00+0.00^{c}	100%
	2	5.23±0.033^{b}	100%	4.84+0.029^{c}	99.95%	0.00+0.00^{c}	100%
	3	5.32±0.182^{a}	99.90%	6.26+0.065^{a}	99%	0.00+0.00^{c}	100%
	P value	0		0			

CFU=Colony Forming Unit; SE= Standard Error; TBC=Total Bacterial Count; TCC = Total Coliform Count; R%=reduction percent

^{a,b,c} different letters in columns refer to statistical difference between all slaughterhouses for same environmental sampling (c < b< a) (p value ≤0.05).

cessing meat must adhere to the same requirements as drinking water. A regular evaluation of the water's quality is suggested. The findings in Fig. 3 determined that the temperature is around 52–59°C while scalding, 4°C while chilling, and slowly rises to 16–19°C. The pH ranges from 6.6 to 8.1, and there are no obvious differences across slaughterhouses at different phases. According to Savell *et al*. (2005) the carcasses should be chilled below 4.4°C within four hours after slaughtering, which agrees with previous research that says poultry carcasses need to be chilled immediately after scalding for safety. These results were consistent with those made by James *et al*. (2006), who claimed that during industrial processing of poultry, the temperature of the poultry carcasses must be quickly reduced from 40°C to 4°C by chilling, which helps to ensure the safety of the final product. according to a microbiological analysis of samples of water from various processing phases shown in Table 4. In all three slaughterhouses, the MPN mean log rose when scalding water exceeded reach 1800 CFU/100 ml, Furthermore, in the second and third slaughterhouses, the water samples collected before the chiller still had significant levels of bacteria after chilling, but in the first slaughterhouse, the levels dropped to 2 CFU/100 ml. When *Campylobacter* was analyzed, only the second and third samples of water from the first abattoir had *Campylobacter* because those samples had been pre chiller (1.35 to 5.3 CFU/100 ml) and after scalding with the log between (2 and 3.3 CFU/100 ml). Overall, in the case of automatic processing facilities, examined systems employed pre-cooling operations before chilling, that exhibited greater reduction during the chilling stage. This made easier due to the use of sanitizer (chlorine) since it lessened the number of bacteria that were exposed to it and allowed chlorine to totally lower the *Campylobacter* count. This is in line with research by Demirok *et al*. (2013), who found that immersion chilling had the greatest effect on lowering the prevalence of Salmonella (39.7%) and *Campylobacter* (43%), because of the washing action and chlorine content of the chilled water. The microbiological status of water samples at several processing phases reveals statistical-

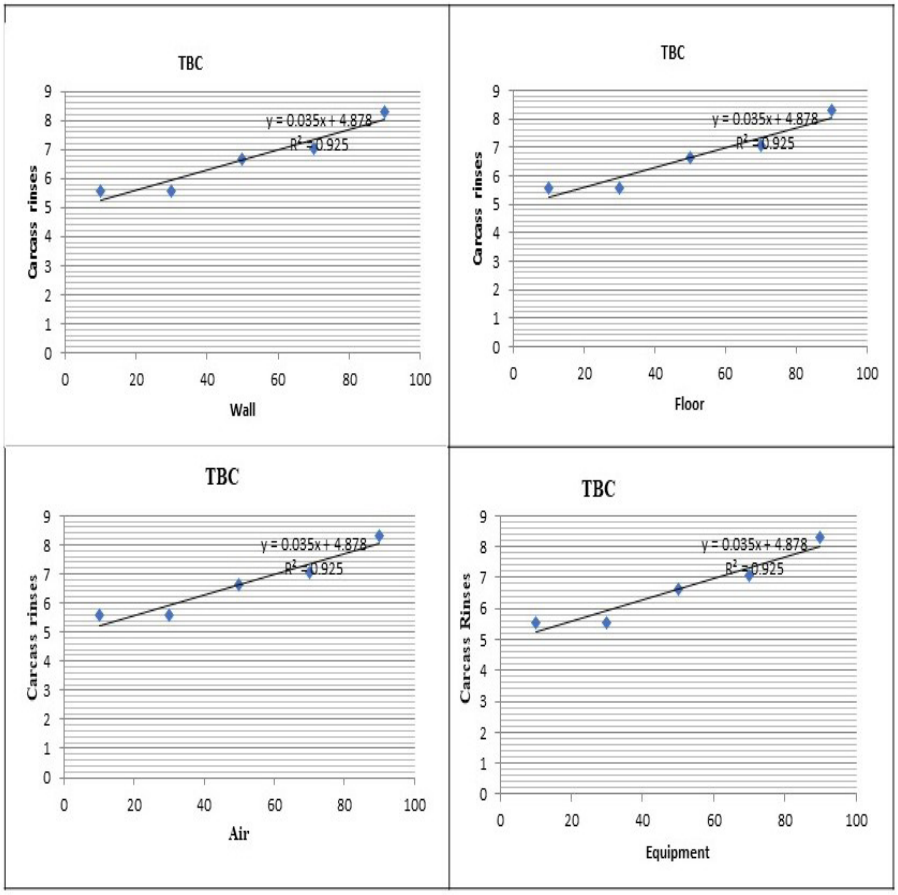

Fig 4: Regression plots showing correlations between microbial count of carcass rinses and different environmental samples (floor, air, wall, and equipment) as $\log_{10}$ CFU/ml.

Table 5. Pearson's Correlation (r) between carcass rinses and environmental samples.

		Wall	Floor	Air	Equipment	Carcass rinse
Wall	r	1	-0.05	-0.39	0.12	0.41
	sig. (1-tailed)		0.47	0.26	0.43	0.29
Floor	r		1	0.41	0.89	0.89
	sig. (1-tailed)			0.25	0.02	0.06
Air	r			1	0.1	0.14
	Sig.				0.44	0.43
Equipment	r				1	0.95
	sig. (1-tailed)					0.02
Carcass rinse						1

ly significant differences ($p < 0.05$) across the processes. all the samples at the various phases of processing were found to be positive for *Campylobacter* except before scalding and after chilling, Scaling is a critical stage in the production of broilers, and it has the potential to cross-contaminate carcasses with *Campylobacter* spp., according to Shane (1992). Due to the heat sensitivity of *Campylobacter* species, faeces and other organic materials may foster an environment that is conducive to their existence. Water recontamination is brought on by frequent bird entry into scald tanks Bryan & Doyle (1995). Like the findings of Cegar *et al*., (2022), who observed that all samples obtained from the worktable, washing machine, washing water, and scalding water at the beginning of the workday were *Campylobacter* negative. At the end of the workday (after slaughter), *Campylobacter* was found in the scalding water, the abattoir, and the worktable. Additionally, all samples obtained from the abattoir environment, according to Gruntar *et al*. (2015), *Campylobacter* were negative before the slaughtering, and it was positive after it. According to Kudirkienė *et al*. (2010), *Campylobacter* can remain in the atmosphere of slaughterhouses even after disinfection and is a possible cause of contamination in chicken meat. It is important to remember that broilers typically have high levels of *Campylobacter* colonization even when the animals don't show any symptoms. *Campylobacter* was detected during the whole slaughter procedure. Work surfaces, tools, water, and air can all get polluted as a result Seliwiorstow *et al*. (2016). The chlorination of chilling water in several poultry slaughterhouses using sodium hypochlorite at concentrations ranging from 20 to 100 ppm is shown in Table 4. Because of the chlorine, the final washers worked better. Chlorine was used to further minimize the number of microorganisms on the surface of the carcass skin. Following 100 ppm chlorination, the first slaughterhouse microbial counts were reduced. The percentage reduction was 100% for the *Campylobacter* count, 99.84% for the TBC, and 95% for the TCC. While chlorination at 50 ppm was successful in reducing counts (100% for TBC and *Campylobacter* count, while reaching 99.95% for TCC), Low amounts of chlorine at 20 ppm result in significant log decrease percentages for TBC, TCC, and *Campylobacter* count, respectively, of 99%, 99%, and 100%. There is no statistically significant difference between the three slaughterhouses. Because of its antibacterial properties, chlorine prevents bacteria from oxidising glucose. But when too much chlorine is used, it reacts with the meat to create tri-halo methane, which is poisonous and carcinogenic (Oğuz and Guler 2004). These outcomes are in line with those of Kameyama *et al*. (2012), who found a 1.5% and 70% reduction in the numbers of coliform and *Campylobacter* spp., respectively, using sodium hypochlorite and chlorine dioxide. Bashor *et al*. (2004) also reported that addition of chlorine at 25 to 35 ppm reduced *Campylobacter* spp. populations in broiler carcasses in 0.5 log CFU/mL of washing solution. However, another study by Lopes *et al*. (2007) found no evidence that adding those substances to pre-chilling tanks in slaughterhouses had any impact on the number of bacteria on broiler carcasses. In general, a decrease is shown right after washing steps, although results might vary depending on the study and washing settings (Berrang and Bailey, 2009) even a rise in bacterial numbers may be seen after washing (Stopforth *et al*., 2007). Our results demonstrates that there is an increase in the microbial load in the samples collected following the pre-cooling stage. Cleaning and disinfection are crucial processing processes to remove secondary contamination brought on by equipment (Ünlütürk and Turantaş, 1999).

There was a strong correlation between the microbial load of the carcass rinse and the count of the floor and equipment, as shown in Table 5 and Figure 4. This finding is like that of Peyrat *et al*. (2008), who found that bacteria could persist on the surface of abattoir equipment even after cleaning and sanitizing it and that it may contaminate carcasses while they are being processed. The quality and shelf life of carcass products are affected by the proliferation of microbes; hence, it is essential to prevent this growth, especially during commercial poultry processing (Babacan *et al*., 2020). According to Voidarou *et al*. (2007), the pre-chilling step alone, without any disinfectant, has little effect on reducing the presence of microorganisms in broiler carcasses during processing. Adding disinfectants to pre-chilling tanks is a crucial step to minimize the presence of microorganisms in chicken carcasses during processing.

CONCLUSION

The results of the study indicate that the environment of the slaughterhouse have been found to be contaminated to varying degrees that may affect carcass contamination. The findings of the environmental sampling point to a connection between pollution and carcasses. The effectiveness of the sanitation standard as well as the use of chlorine in chilled tanks should be checked to prevent carcass contamination. The proliferation of bacteria, particularly *Campylobacter*, and the contamination of broiler carcasses by the bacteria found in the intestinal material during processing could lead to hygienic issues. The primary strategies to reduce microbial load and *Campylobacter* contamination at the slaughtering stage are an enhanced cleaning process, including the use of disinfectants, and effective training of staff. Supervisors of slaughterhouses should be aware of the sanitary design's weak areas, including potential accumulation sites for organic residues and locations where microorganisms could survive.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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