Improvement of broiler meat microbial quality by using different treatments inside chilling tank

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ABSTRACT

The decontamination of poultry meat can help to reduce human foodborne infections. A study was conducted to improve the bacteriological quality of broiler carcases using different decontaminants (peracetic acid 100 ppm, chlorine 30 ppm and peracetic acid 100 ppm plus chlorine 30 ppm) in chilling tank of local poultry plant at Belbies city city. Broiler carcases were analyzed for total aerobic bacteria, *S. aureus*, enterobacteriaceae, and pseudomonas count. After chilling with per acetic acid 100 ppm, chlorine 30 ppm and peracetic acid 100 ppm plus chlorine 30 ppm, counts were 4.58 ± 0.51, 2.48 ± 0.34, 2.82 ± 0.28, 2.75 ± 0.28 & 4.91 ± 0.56, 2.69 ± 0.35, 3.1 ± 0.31, 3.14 ± 0.32 & 3.12 ± 0.29, 2.18 ± 0.24, 2.30 ± 0.26, 2.66±0.25 $\log_{10}$ CFU/mL of chill for total aerobic bacteria, *S. aureus* count, enterobacteriaceae count, and pseudomonas count, respectively. There was a little difference in microbial populations per milliliter of chill water among treatments. Effect of decontaminants artificially inoculated broiler breast with *Campylobacter jejuni* were also studied. Public health importance, the beneficial economic and applicable values of the different decontaminants were discussed.

Key words: Broiler carcass, chilling, tanks, peracetic acid, chlorine, decontaminants.

1. INTRODUCTION

Broiler carcass contamination may occur during different processing steps (evisceration, washing of carcass, chilling and cooling) where microorganisms are disseminated **(USDA, 2002)**. To minimize microbial contamination of broiler carcasses during processing, several disinfectant compounds can be added to the chilling tanks. Chlorine compounds 5 ppm (as a maximum level), can be added to the chilling tanks in poultry slaughterhouses. Five disinfectants (chlorine dioxide, alkyl dimethyl benzyl ammonium chloride, acidified sodium chlorite, per acetic acid, and sodium hypochlorite) were evaluated by **(Guastalli *et al.*, 2016)** to examine their effects on the indicator microorganism's populations and on *Salmonella enteritides* in the absence and presence of organic matter. During carcass chilling, cold water play a main role as

cross contamination vehicle between broiler carcasses. In addition, treated cold water with disinfectant as chlorine help in reduction of microbial population **(Demirok *et al.*, 2013).** By immersion of poultry carcasses in cold water; level of microbial contamination are decreased, and may increase again during refrigeration storage **(Hinton *et al.*, 2004).** In United States of America, Campylobacteriosis (from poultry meat) constituted about 6.5% of all foodborne diseases cases (**Crim *et al.*, 2014**).

The present study aimed to evaluate the effect of alternative four disinfectant compounds (per acetic acid 100 ppm, chlorine 30 ppm and per acetic acid 100 ppm plus chlorine 30 ppm) on the populations of total bacterial, *S. aureus*, enterobacteriaceae, and pseudomonas count. In addition, our study evaluated the effect of mentioned disinfectants on artificially inoculated broiler breast with *Campylobacter jejuni.*

2. MATERIALS AND METHODS

To evaluate the effect of selected disinfectants on broiler carcasses; two separate experiments were conducted: one experiment regarding the effect on microbial population, and the second on artificially inoculated broiler breast with *Campylobacter jejuni*.

2.1. Experiment I

2.1.1. Sampling

Five replicates were performed for each treatment. Experiments were performed at local poultry processing plant at Belbies city.

2.1.2. Broiler carcasses treatment

Four buckets were filled with 25 L of cold water (16-18°C). Peracetic acid 100 ppm, Chlorine 30 ppm and peracetic acid 100 ppm plus chlorine 30 ppm were added to one bucket each. The fourth bucket contained only water (negative control). Three carcasses were immersed in each bucket for 20 minutes in order to simulate the pre-chilling processing step.

2.1.3. Detection and enumeration of bacteria:

2.1.3.1. Total Aerobic mesophilic count (**Health Protection Agency, 2004**): Total viable counts (TVC) were determined on Plate Count Agar (Oxoid), after incubation for 2 days at 30 °C.

2.1.3.2. Coagulase positive *S.aureus*: an inoculum of 0.1 ml of serial dilution 10^{-1}&10^{-2} was evenly surface distributed on Baird Parker agar base supported with egg yolk tellurite emulsion Agar (Oxoid CM0275) according to **(APHA,2001)**. After incubation at 37°C for 48 hrs, counting of all typical colonies. For confirmation, five typical colonies were selected and transferred to

brain heart infusion broth tubes (BHIB) (Oxoid CM1135) for subsequent culture and preservation. Gram stain, catalase, mannitol fermentation, DNAs and coagulase tests were applied on suspected colonies to identify coagulase *S. aureus* (**Quinn et al., 2011**).

2.1.3.3. Enterobacteriaceae count [ISO 21528-2 (ISO, 2004): Enumeration was carried out on Violet Red Bile Glucose (VRBG) agar. Only red or pink colonies with or without the precipitate were considered.

2.1.3.4. Determination of Pseudomonas: (Kielwein, 1969): 0.1 ml dilutions were transferred to the surface of GSP (Glutamate Starch Phenol-red agar), then spread as quickly as possible over the surface of the agar plate. The plates were inverted after approximately 15 min and incubated at 25°C for 72 hours. The numbers of Pseudomonas were estimated by counting colonies >1 mm in diameter, with violet to blue.

2.1.3.5. Isolation of *Campylobacter jejuni*: (Rasschaert *et al.*, 2007): 10 g ± 0.1 g of the surface of the meat samples were transferred to 90 ml Preston enrichment broth (Nutrient Broth No.2, Oxoid, Basingstoke, UK + Supplement) and macerated for 30 sec. in a Stomacher 400 bag mixer.

2. Experiment II

2.1. *In-vitro* inoculation of *Campylobacter jejuni*

1- Preparation of chicken fillets:

Chicken carcasses about 2000 ± 45 g. were obtained from a local abattoir and kept in the refrigerator at 4±1 °C until use. The breast muscle was removed and sliced into parts (4 x 6 x 1) cm. Twenty five grams of breast muscle was used for microbiological detection to establish if the fillets were harboring indigenous *Campylobacter jejuni* prior to inoculation. *Campylobacter jejuni* free chicken fillets were introduced for artificial inoculation and decontamination trials.

2- Preparation of *Campylobacter jejuni*:

PCR identified *Campylobacter jejuni* were enriched in a sterile nutrient broth for 24 hours, then centrifuged at 4000 round per minutes (rpm) for 5 minutes. The supernatant was removed followed by addition of a sterile normal saline then centrifuged at 4000 (rpm) for 5 minutes. The supernatant was removed then addition of a sterile normal saline with few quantities and shacked with vortex in comparing with ½ McFarland tube equal (10^8 cfu/ ml).

3- Inoculation of chicken fillets with *Campylobacter jejuni*:

Each part of chicken fillets was placed into a sterile Ziploc plastic bag then 0. 1 ml of the diluted *Campylobacter jejuni* culture (10^8 cfu/ml) was distributed over the surface of the each chicken

fillets by a sterile insulin syringe. The Ziploc locked and massaged from outside for a distribution of *Campylobacter jejuni*. The inoculated fillets were left for 25 to 30 min at a room temperature to allow attachment and adsorption of the inoculated bacteria **(Morrison and Fleet, 1985)**.

STATISTICAL ANALYSES

Microbial population results were transformed in log10 and means compared by Tukey's test at 5% ($p<0.05$) significance level. Statistical Analysis System (SAS) software version 9.1 was used for analysis.

3. RESULTS AND DISCUSSION

Hygienic conditions occur during meat manufacturing process can be assessed by indicator microorganisms **(Huffman, 2002).** Chicken meat is frequently contaminated with different microorganisms causing foodborne diseases in human beings specially campylobacter and *S. aureus.* So, trials should be adopted to reduce these microorganisms in poultry carcasses (**Kottwitz *et al.* 2010; Silva *et al.* 2010 and Jacobsen *et al.* 2012**), disinfectants addition in pre-chilling tanks play a role in minimizing microbial populations in broiler carcasses during processing. Pre-chilling without addition of any antimicrobials to chill tanks has low effect on bacterial reduction in poultry carcasses **(Voidarou *et al.*, 2007)**.

Table (1) Effect of decontaminants on bacterial counts log_{10} CFU/ml of chilling water.

	Control group	Peracetic acid 100	Chlorine 30	Peracetic acid100 plus chlorine30
APC	5.8 ± 0.56^{a}	4.58 ± 0.51^{b}	4.91 ± 0.56^{ab}	3.12 ± 0.29^{c}
Reduction count		1.22	0.89	2.68
Staphylococcus count	3.95 ± 0.41^{a}	2.48 ± 0.34^{b}	2.69 ± 0.35^{b}	2.18 ± 0.24^{c}
Reduction count		1.47	1.26	1.77
Enterobacteriaceae count	3.9 ± 0.32^{a}	2.82 ± 0.28^{b}	3.1 ± 0.31^{b}	2.30 ± 0.26^{c}
Reduction count		1.08	0.8	1.6
Pseudomonas count	3.8 ± 0.38^{a}	2.75 ± 0.28^{b}	3.14 ± 0.32^{ab}	2.66 ± 0.25^{c}
Reduction count		1.05	0.66	1.14

Means of the same rows carrying different superscript letters are significantly different.

Table (1) showed the effect of decontaminants on bacterial counts log10 CFU/ml of chilling water. It is cleared that the higher reduction count of total aerobic count (2.68 CFU/ml) in Peracetic acid100 plus chlorine 30 group and the lowest reduction count for pseudomonas count

in Chlorine 30 group (0.66 log10 CFU/ml). All of the examined disinfectants reduced total aerobic counts (p>0.05) by different reduction count ranged from 0.89 to 2.68 log10 CFU/ml. Peracetic acid100 plus chlorine30 declined the total enterobacteriaceae count (2.30±0.26 CFU/mL) compared with the control one (3.9±0.32 CFU/mL) (p<0.05). Peracetic acid100 plus chlorine30 group reduced all tested bacterial populations Significantly (Table, 1).

Table (2) Effect of decontaminants on bacterial counts log_{10} CFU/g of broiler meat

	Control group	Peracetic acid 100	Chlorine 30	Peracetic acid100 plus chlorine30
APC	4.38 ± 0.49^{a}	3.18 ± 0.27^{b}	3.65 ± 0.41^{ab}	2.95 ± 0.39^{c}
Reduction count		1.2	0.73	1.43
Staphylococcus count	3.56 ± 0.27^{a}	2.58 ± 0.36^{b}	2.92 ± 0.28^{ab}	2.41 ± 0.34^{b}
Reduction count		0.98	0.64	1.15
Enterobacteriaceae count	3.45 ± 0.28^{a}	2.35 ± 0.24^{b}	2.72 ± 0.27^{b}	2.11 ± 0.21^{c}
Reduction count		1.1	0.73	1.34
Pseudomonas count	3.14 ± 0.31^{a}	2.21 ± 0.22^{b}	2.46 ± 0.31^{ab}	2.13 ± 0.26^{b}
Reduction count		0.93	0.68	1.01

Means of the same rows carrying different superscript letters are significantly different.

Peracetic acid100 plus chlorine 30 showed the best results in reducing total microbial count, Staphylococcus count, Enterobacteriaceae count and Pseudomonas count whereas Peracetic acid 100 and Chlorine 30 exhibited little reduction effect on these populations either in chill tanks (Table 1) or broiler carcasses (Table 2).

The results of the present study showed that Peracetic acid at 100 ppm, Chlorine at 30 ppm and Peracetic acid 100 ppm plus chlorine 30 ppm decreased tested microbial populations in different ratios. However, **Mead *et al.* (2000)** mentioned that using of chlorine at concentration of 50 ppm in chilling tanks (during pre-chilling stage) did not eliminate contamination of broiler carcasses by *Escherichia coli*. The addition of chlorine at 20 ppm concentration in pre-chilling tanks declined the coliforms, *Escherichia coli* and *Campylobacter* spp. and total aerobes count, in poultry carcasses, without effect on *Salmonella* spp (**Buhr *et al.*, 2005)**.

Figure (1) showed the prevalence of Campylobacter species in broiler meat and giblets. It is declared that *C. jejuni* has a higher prevalence than other species. Also, gizzard appeared as a most contaminated part by the campylobacter spp. and that can be explained as the gizzard has many sources of contamination during broiler processing especially during evisceration process and its cleaning. *Campylobacter jejuni* was isolated and identified using PCR (Figure 2).

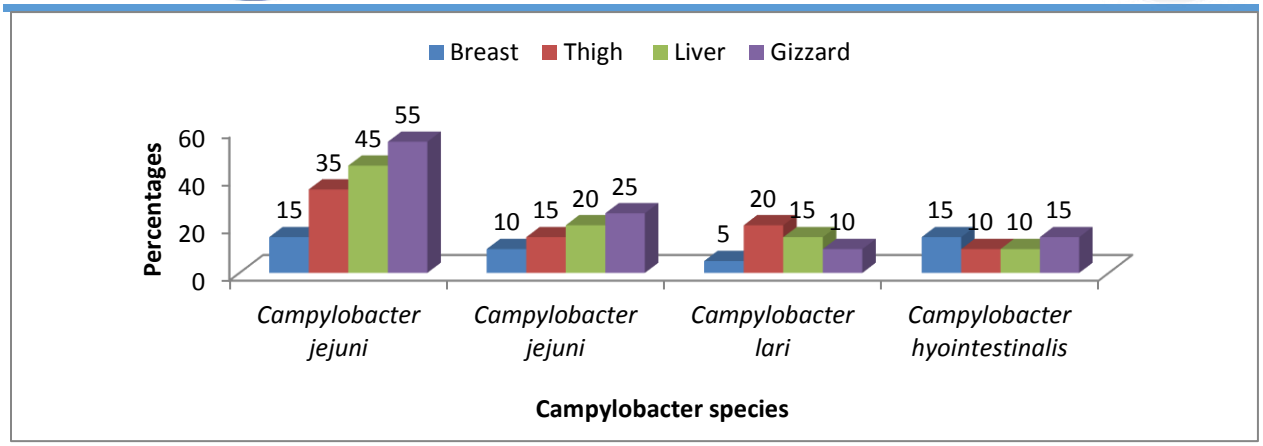

Figure (1) Prevalence of Campylobacter species in broiler meat and giblets

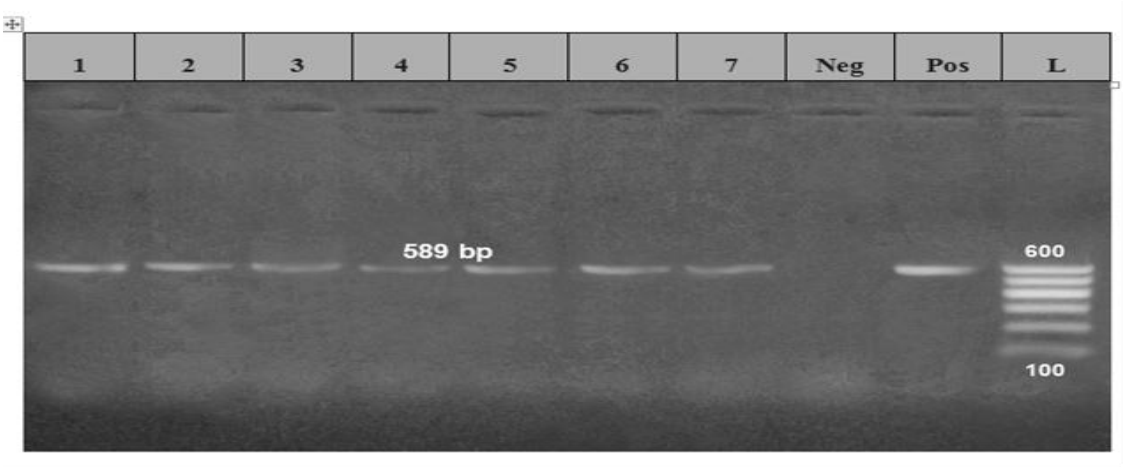

Figure (2) *Campylobacter jejuni map*A PCR (L: 100 bp Ladder, 1, 2, 3,4,5,6 and 7 *C. jejuni* positive isolates). Pos: positive control. Neg: Negative control

Table (3) Effect of decontaminants on *Campylobacter jejuni* log_{10} CFU/g of artificially inoculated broiler breast (N= 5)

	Control group	Peracetic acid 100	Chlorine 30	Peracetic acid100 plus chlorine30
Minumum	6.8	5.42	5.94	5.11
Maximum	7.23	5.69	6.23	5.36
Mean± SE	7.03 ± 0.62^{a}	5.55 ± 0.56^{b}	6.12 ± 0.52^{ab}	5.18 ± 0.54^{c}
Reduction count		1.48	0.91	1.85

Means of the same rows carrying different superscript letters are significantly different.

Results tabulated in Table (3) mentioned the effect of decontaminants on *Campylobacter jejuni* $\log_{10}$ CFU/g of artificially inoculated broiler breast. In control group, the mean±SE of *Campylobacter jejuni* count was 7.03 ± 0.62 $\log_{10}$ CFU/g. Reduction count for both Peracetic acid at 100 ppm and Peracetic acid100 ppm plus chlorine at 30 ppm are very similar; 1.48 $\log_{10}$ CFU/g and 1.85 $\log_{10}$ CFU/g, respectively while chlorine at 30 ppm showed more little reduction count (0.91 $\log_{10}$ CFU/g). **Bashor *et al.* (2004)** mentioned that chlorine addition at 25 to 35 ppm affect the *Campylobacter* spp. count in poultry carcasses and reduce it to 0.5 log CFU/mL of washing tank solution.

In the present study, peracetic acid was more effective in reducing microbial populations than chlorine-based compounds and this result agreed with that obtained by **Bauermeister *et al.* (2008)**, the chlorine is easily neutralized by organic compounds so that weakness in antimicrobial effect (**Northcutt & Lacy, 2000**). **Demirok et al. (2013)** declared that the immersion chill has an important role in reduction of pathogenic microorganisms like *Campylobacter* (43%) and *Salmonella* (39.7%) and that may be due to the effect of washing and occurance of chlorine in the chilled water.

4. REFERENCES

American Public Health Association (APHA)(2001). Compendium of methods for the microbiological examination of food (4th ed.). Washington, DC: Author.

Bashor MP, Curtis PA, Keener KM, Sheldon BW, Kathariou S, Osborne JA. (2004). Effects of carcass washers o*n Campylobacter* contamination in large broiler processing plants**.** Poultry Science;83(7):1232-1239.

Bauermeister LJ, Bowers JWJ, Townsend JC, McKee SR (2008). Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. Journal of Food Protection; 71 (6):1119-1122.

Buhr RJ, Bourassa DV, Northcutt JK, Hinton Junior, A, Ingram KD, Cason JA (2005). Bacteria recovery from genetically feathered and featherless broiler carcasses after immersion chilling. Poultry Science;84(9):1499- 1504.

Crim SM, Iwamoto M, Huang JY, Griffin PM, Gilliss D, Cronquist AB (2014). Incidence and trends of infection with pathogens transmitted commonly through food-foodborne diseases active surveillance network, 10 U.S. Sites, 2006–2013. Morbidity and Mortality Weekly Report; 63 (15):328-332.

Demirok, E.; Veluz, G.; Stuyvenberg,W.V.; Castaneda, M.P.; Byrd, A.; Alvarado, C.Z. (2013). Quality and safety of broiler meat in various chilling systems. Poultry Science, 92, 1117–1126.

Guastalli BHLI, Batista DFAI, Souza AISI, Guastalli EALII, Lopes PDI, Almeida AMI, Prette NIII, Barbosa FOI, Stipp DTIII,IV and Freitas Neto OCI (2016).

Evaluation of Disinfectants Used in Pre-Chilling water Tanks of Poultry Processing Plants. Brazilian Journal of Poultry Science ISSN 1516-635X May - Jun 2016 / v.18 / n.2 / 217-224.

Jakobsen L, Garneau P, Bruant G, Harel J, Olsen SS, Porsbo LJ (2012). Is *Escherichia coli* urinary tract infection a zoonosis? Proof of direct link with production animals and meat. European Journal of Clinical Microbiology & Infectious Diseases; 31(6):1121-1129.

Health Protection Agency (2004). Aerobic plate count at 30° C: surface plate method. National Standard Method F 10 Issue 1. http://www.hpastandardmethods.org.uk/pdf_sops.asp.

Hinton, A., Jr.; Cason, J.A.; Ingram, K.D. (2004). Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. Int. J. Food Microbiol. 91, 155–165.

Huffman, R. (2002). Current and future technologies for the decontamination of carcasses and fresh meat. *Meat Science*, 62, 285-294.

International Organization for Standardization (2004). Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of *Enterobacteriaceae*. Part 2: Colony-count method. enumeration. EN ISO 21528-2. ISO, Geneva.

Kielwein, G. (1969). Ein Nährboden zur selektiven Züchtung von Pseudomonaden und Aeromonaden. Arch. Lebensmittelhygiene, 20: 131-138.

Kottwitz LBM, Oliveira TCRM, Alcocer I, Farah SMSS, Abrahمo WSM, Rodrigues DP. (2010). Avaliaço epidemiolٍgica de salmonelose ocorridos no perيodo de 1999 a 2008 no estado do Paranل, Brasil. Acta Scientiarum Health Sciences; 32(1):9-15.

Mead GC, Allen VM, Burton CH, Corry JE. (2000). Microbial cross-contamination during air chilling of poultry. British Poultry Science 2000;41(2):158- 162.

Morrison G. J. and Fleet G. H. (1985). Reduction of Salmonella on Chicken Carcasses by Immersion Treatments. Journal of Food Protection, Vol. 48, No. 11, pp. 939-943.

Northcutt JK, Lacy MP. (2000). Odor problems associated with chlorineus age in poultry processing plants [abstr.]. Poultry Science; 78 (Suppl.1):47.

USDA (2002). Food safety and Inspetion Service .Prevalence of *Salmonella* on young chicken carcasses in the U.S.: 1999-2000. 2002 [cited 2014 Dec 9]. Available from: http://www.fsis.usda.gov/OA/speeches/2002/ IAFP_poster/iafp.pdf.

Voidarou C, Vassos D, Kegos T, Koutsotoli A, Tsiotsias A, Skoufos J (2007). Aerobic and anaerobic microbiology of the immersion chilling procedure during poultry processing. Poultry Science; 86 (6):1218- 1222.

Quinn PJ, Markey BK, Leonard FC, Fitzpatrick ES, Fanning S, Hartigan P. Veterinary microbiology and microbial disease (2nd ed.) (2011). Oxford: Wiley-Blackwell.

Rasschaert G., Houf K., Van Hende J., De Zutter L. (2006). *Campylobacter* contamination during poultry slaughter in Belgium. J. Food Prot. 69 27–33. 10.4315/0362-028X-69.1.27

Silva EP, Bergamini AMM, Oliveira MA. (2010). Alimentose agentes etiolgicos envolvidos em toxinfecçes na regiмo de Ribeiro Preto, SP, Brasil – 2005 a 2008. Boletim Epidemiolgico Paulista; 7 (77):4-10.

تحسين الجودة الميكروبية للحوم بداري التسمين باستخدام معاملات مختلفة داخل خزان التبريد

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إن وسائل تحسين سلامة لحوم بداري التسمين تكون بالحد من تلوثها خلال خط الإنتاج. إزالة التلوث من لحوم الدواجن يمكن أن يساعد في الحد من العدوى التي تنتقل عن طريق الأغذية. في هذه الدراسة؛ تمت مناقشة بعض أساليب تقليل التلوث في لحم بداري التسمين. أجريت الدراسة لتحسين التأثير الجرثومي لاستخدام معاملات مختلفة في خزانات التبريد في مسلخ دواجن محلي بمدينة الزقازيق (Peracetic acid 100، Chlorine 30 and Peracetic acid 100 plus chlorine 30) أثناء الغمر بتبريد ذبائح بداري التسمين كخمسة مكررات. تم تحليل لحوم اللاحم للبكتيريا الهوائية (APC) ، البكتيريا العنقودية الذهبية ، الأمعائيات ، وعدد البكتيريا الزائفة. وبعد عملية التبريد مع حمض Peracetic 100، Chlorine 30 و Peracetic acid 100 plus chlorine 30 ، كان العد الكلي الميكروبي: 4.58 ± 0.51 و 2.48 ± 0.34 و 2.82 ± 0.28 و 2.75 ± 0.28b و 4.91 ± 0.56 و 2.69 ± 0.35 و 3.1 ± 0.31 و 3.14 ± 0.32 و 3.12 ± 0.29 ، 2.18 ± 0.24 ، 2.30 ± 0.26 ، 2.66 ± 0.25 مستعمرة بكتيرية لوغاريتمية/مل من مياه التبريد للبكتيريا الهوائية ، البكتيريا العنقودية الذهبية ، الأمعائيات ، وعدد البكتيريا الزائفة ، على التوالي. كان هناك اختلاف بسيط في التعداد البكتيري لكل ملليلتر من ماء التبريد بين المعاملات المختلفة. وقد تم أيضا دراسة تأثير إزالة التلوث على ميكروب الكامبيلوباكتر من صدور ذبائح بداري التسمين والمحقونة صناعيا بالميكروب. تم التركيز على أهمية المعزولات للصحة العامة وكذلك القيم التحسينية والاقتصادية المفيدة لمزيلات التلوث المستخدمة ومدي تطبيقها في مسالخ الدواجن.

الكلمات المفتاحية: ذبائح بداري التسمين، peracetic، الكلور، مزيلات التلوث.