



## Assessment of Drinking Water Quality and New Disinfectants for Water Treatment in a Small Commercial Poultry Farm

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### ABSTRACT

This work designed to monitor the hygienic quality of drinking water supply in a small commercial poultry farm and to assess the efficiency of different new disinfectant compounds against some pathogenic bacterial isolates. A total of 60 water samples was collected from both the main source and drinkers for physico-chemical and bacteriological examination. The sensitivity pattern of 40 strains of bacterial isolates to commonly used disinfectants in poultry facilities for water supply treatment was evaluated using the broth macro-dilution method. Results, the mean values of both alkalinity and total hardness were found in the highest rate of  $183.0 \pm 17.6$  and  $345.6 \pm 7.6$  mg/l compared to their values in the main water source. Furthermore, the mean value of ammonia in drinkers besides nitrite, and phosphate discovered at the highest level compared to the main water supply ( $1.36 \pm 0.31$ ,  $3.4 \pm 0.46$ ,  $26.3 \pm 0.78$  mg/l, respectively). Both *E. coli* and *Shigella* spp. in drinkers were detected at the highest isolation rate (22.6%). *Salmonella kentucky* (*S. kentucky*: O20, 8 H I) isolates were found at the highest rate of 57.1%. Whilst the pathogenic *E. coli* serotyping Poly3 (O157) recorded at 66.67%, followed by *E. coli* O114 33.33%. Biocidal efficiency of Klorsept 25 disinfectant against investigated pathogenic bacterial isolates was 100% at a concentration of 2.0 mg/l after 180 min of exposure. Whilst the efficiency of calcium hypochlorite Ca (Ocl)<sub>2</sub> against *E. coli* and *S. kentucky* was 100% at a concentration of 0.5 mg/l and exposure time 120 min. The susceptibility of all bacterial isolates to H<sub>2</sub>O<sub>2</sub> disinfectant at a concentration of 5.0 % was 100% within 60 min contact time. In conclusion, the investigation of hygienic quality of water supply should be occurred periodically to ensure the safety of water source for poultry chick's health. The sensitivity of the studied pathogenic bacterial isolates is 100% to Klorsept 25 disinfectant at a concentration of 2.0 mg/l, calcium hypochlorite (Ca (Ocl)<sub>2</sub>) at 0.5 mg/l, and H<sub>2</sub>O<sub>2</sub> at 5.0 % at exposure time does not exceed 180 min.

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### Introduction

Water is an important and vital nutrient for all birds. Improving drinking water quality can help in maintaining the poultry health. Factors such as physio-chemicals, heavy metals, and microbial load should be investigated to evaluate water sources and ensure its level within an acceptable range (Maharjan *et al.*, 2017).

Drinking water should be of adequate physio-chemicals properties and microbiological load. There are different factors affecting drinking water quality in poultry farms that include pH, total hardness, mineral content, and microbial load (Singh, 2019). High values of certain water physio-chemicals such as pH, total dissolved solids, nitrite, and salinity affected feed intake and resulted in decreased body weight of broiler chicks (Reutor, 2010). Moreover, bacterial load in water supplies has a serious impact on broiler health, Grizzle *et al.* (1997) recorded

that exposure of broiler chicks to water contaminated with *E. coli* (500 CFU/ml) led to a lowering of body weight at 4-6 weeks of age. In addition, Van der Sluis (2002) clarified that poor water quality led to low vaccines and medication efficiency through the water line system.

Interestingly, drinking water has a role as a contributing factor in the occurrence of foodborne diseases when using contaminated water throughout the processing and production of food (FAO/WHO, 2008; EFSA, 2013). Cleaning and disinfection process are essential for commercial poultry farms and the efficiency of these operations affect greatly the quality of the final poultry product (Ahmed, 2017). Reportedly, the disinfection of drinking water supplies has an efficient role in mitigating the microbial contaminants in water sources (Sapers, 2001; Parish *et al.*, 2003; Gil *et al.*, 2009; Goodburn and Wallace, 2013).

The disinfection process of water supply is considered the major step to ensure hygienic water quality and to protect both bird health and welfare. Nowadays, there are several methods should be applied to save clear water through the disinfection of water source (Yang, 2016). In addition, disinfect-

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tion is the main part of an effective biosecurity program in poultry operations that prevents the entry of disease agents to facilities (Newell et al., 2011).

In poultry flocks, several sanitizers and disinfectants are available to use and applied at the farm level, but they had drawbacks in its use (Watkins, 2007). Recently, the most common disinfectants have no effect on antimicrobial-resistant bacteria so, there is a need to seek alternative water disinfectants and/or sanitizers. Although chlorine is widely used in water treatment, hydrogen peroxide has a strong bactericidal effect on different micro-organisms in water supplies (Shuval et al., 1995; Pedahzur et al., 2000; Liberti et al., 2003).

This study aimed to evaluate the hygienic quality of drinking water source at the poultry farm level and to assess the efficiency of different new disinfectant compounds against some pathogenic bacterial isolates and also to seek about antimicrobial alternatives that can be used in the safety disinfection process of water supplies in the investigated farm.

## Materials and methods

### Study area and period

This study was carried out in a private poultry farm located in Beni-Suef (coordinates 29° 04' N-31° 05' E) province, Egypt, during the period from February to August 2019. The farm contained 4000 chicks in two building units and kept in deep litter system at the stocking rate 3-4m<sup>2</sup>/bird. Food and water were provided ad libitum from manual feeders and drinkers (n=40 each). The water supply is tap water in the investigated farm. The hygienic measures inside the farm are fair.

### Water sampling

A total of 60 water samples were collected from both the main source (n= 20) and drinkers (n= 40). Samples collected aseptically from the main water supply in sterilized glass bottles (2 liters capacity) after flaming the tap outlet and allowing the water to run for 5 min then all samples were stored in a cool box and transported to the laboratory in the Faculty of Veterinary Medicine, Beni-Suef University, for physico-chemical and bacteriological examination as described by APHA (2005).

### Physico-chemical analysis and heavy metals estimation

Water physico-chemical parameters were measured using Multiparameter Photometer (bench, HI83200, HANNA, Romania). The examined parameters included pH (HI93710-01, Hanna® kits), ammonia (using Nessler method with reagent kits HI93700-01), alkalinity (colorimetric method, using kits, HI93755-01). Measurement of nitrite high range (NO<sub>2</sub>-N) and nitrate (NO<sub>3</sub>-N) using the ferrous sulfate method (kits: HI93708-01), and the cadmium reduction method (HI93728-01 reagent kit), respectively. Meanwhile, the EDTA method was used for measuring calcium hardness (HI93720-01), and magnesium hardness (HI93719-01). Sulphate was determined by Turbidity spectrophotometric method according to APHA (1998). The Phosphate level was measured using Stannous

chloride method (APHA, 1992). On the other hand, the estimation of heavy metals involved iron (HI93721-01), copper (HI93702-01), and zinc [(HI93731A-0) + (HI93731B-0)], while arsenic (AS) was estimated by detection Kit (Hach Company, USA).

### Isolation and Identification of pathogenic bacteria from drinking water

Water samples were examined for some bacterial pathogens, which included *E. coli*, *klebsiella* spp., *shigella* spp. and *Salmonella* spp. For isolation of *E. coli* and *klebsiella* spp., samples were enriched on Tryptic soya broth (Oxoid, Basingstoke, UK) at 37°C for 18–24 h, then loopful from each tube showing turbidity was streaked onto MacConkey Lactose Agar (Oxoid, Basingstoke, UK) plates. Colonies of lactose fermenting pink, and smooth were further streaked onto Eosin Methylene Blue (EMB: Oxoid, Basingstoke, UK) agar plates as the method described by Brown (2005). In addition, for isolation of *shigella* spp. and *Salmonella* spp., after pre- enrichment stage using buffered peptone water, samples were enriched on Rappaport and vassiliadis broth (Oxoid, Basingstoke, UK) and incubated at 42°C for 24 h, then a loopful was streaked on xylose lysine deoxycholate (XLD: Oxoid, Basingstoke, UK) then incubated at 37°C for 24-48 h, based on the morphological characters, the suspected colonies were picked up for further identification. Biochemical tests (HiMedia Rapid Biochemical Identification Kit), were used for identification of bacteria, which included indole production, methyl red, Voges-Proskauer and citrate utilization, and urease test (Ewing, 1986; Ahmad et al., 2009).

### Serological identification of pathogenic bacteria

All *E. coli* isolates were identified using commercial latex kits for diagnosis of Enteropathogenic types *E. coli* O157 and O114 besides slide agglutination tests were performed to identify the O-antigen. Furthermore, latex agglutination test was performed for *Salmonella* spp. using a Hi-latex identification kit (Hi-Media) for genus confirmation, in the presence of control positive and negative organisms and control latex (Kok et al., 1996). Identification of bacterial isolates was performed in the Animal Health Research Institute, Dokki, Egypt.

### Sensitivity test of pathogenic bacteria to different disinfectants

The sensitivity pattern of 40 strains of bacterial isolates to commonly used disinfectants in poultry facilities was evaluated using the broth macro dilution method. Disinfectants included in the study were Klorsept 25 (sodium dichloroisocyanurate, 2.5g (Medentech, Ireland), bleaching powder (Calcium hypochlorite, 65% active chlorine), Terminator (active aldehyde, glutaraldehyde and a cationic surfactant, quaternary ammonium (QAC) (Bomac laboratories, Ltd, New Zealand), Viricosanity (peracetic acid 50%, and alkyl dimethyl ammonium chloride, oregano and eucalyptus extract, Taba for chemical industry, Egypt), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 6%, Puremisr, Egypt). Disinfectants were evaluated at the recommended concentrations according to the manufacturer's direction as shown in Table 1.

Table 1. Different testing disinfectants compounds used for drinking water purification in poultry farms

Testing disinfectants	Recommended Concentration	Active ingredients
Klorsept 25	4.3 g/L	Sodium dichloro-isocyanurate
Bleaching powder Ca (OCl) <sub>2</sub>	0.5 mg/L	Calcium hypochlorite
Terminator	0.33 ml/L	Active aldehyde, Glutaraldehyde, a cationic surfactant, and quaternary ammonium
Viricosanity	0.5 g/L	Per acetic acid 50% and alkyl dimethyl ammonium chloride
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	3% and 5%	H <sub>2</sub> O <sub>2</sub>

*In-vitro sensitivity testing method*

The tested dilutions of different disinfectants [Klorsept 25 (4.3g/L), bleaching powder (0.5mg/L), Terminator (0.33ml/L), Viricosanity (0.5g/L), and hydrogen peroxide (3.0, and 5.0%)] were prepared using distilled water. One hundred microliter of bacterial strains ( $1 \times 10^6$  CFU/mL) was added to different sterilized test tubes contained 2 mL of separate disinfectant dilutions, then incubated for different contact times (1 h., 2 h., and 3 h). Thereafter, one mL of the inoculate was transferred to tubes contained nutrient broth and incubated for 24 h. at 37°C. All tubes that exhibited turbidity with a thin layer on the surface and/or precipitate in the bottom of tubes were considered positive compared to the control negative tube (nutrient broth contained 1 mL of the tested bacteria) and control positive (nutrient broth contained 1 mL of the tested disinfectant). Finally, 1 $\mu$ L of bacterial suspension from positive tubes was inoculated in a nutrient agar plate and incubated at 37°C for 24 h. The efficiency of the tested disinfectants was confirmed throughout the absence of microbial growth (Pilotto et al., 2007).

*Statistical analysis*

The obtained data were collected and analyzed using the Chi-square test as a non-parametric test and One-way ANOVA (parametric test). The statistical program was SPSS version 22.0, Statistical Package for Social Sciences. The data were expressed as [mean ( $\pm$ ) SE]. The accepted significance level was at  $P < 0.05$ .

**Results***Physico-chemical analysis and heavy metals estimation*

The mean values of temperature in the main water supply (25.6 $\pm$ 1.3) were lower than its record in drinkers' samples (27.6 $\pm$ 2.3°C). Moreover, the pH value in both the main source and drinkers was recorded within the permissible limit according to WHO (6.8-8.5) as shown in Table 2.

The level of water turbidity in drinkers was significantly higher than 36.7 $\pm$ 3.8 NTU compared to its level in the main water supply (0.46 $\pm$ 0.13 NTU), whereas the permissible limit

of turbidity should not be increased than 0-4 NTU.

The mean values of both alkalinity and total hardness were found in the highest rate of 183.0 $\pm$ 17.6 and 345.6 $\pm$ 7.6 mg/l respectively, compared to their values in the main water source. On the other hand, ammonia value in drinkers besides nitrite, and phosphate discovered at the highest level (1.36 $\pm$ 0.31, 3.4 $\pm$ 0.46, 26.3 $\pm$ 0.78 mg/l, respectively) compared to the main water supply. Whilst the nitrate and sulfate levels in both the main water and drinkers (45, and 500 mg/l, respectively). were within the permissible limit

Estimation of heavy metals (arsenic (As), copper (Cu), iron (Fe), zinc (Zn)) in both the main water supply and drinkers were recorded at the safe level according to WHO guideline where their levels in drinkers were 0.05 $\pm$ 0.02, 0.21 $\pm$ 0.04, 0.19 $\pm$ 0.01, and 0.27 $\pm$ 0.08 mg/l, respectively. Meanwhile, the heavy metal level in the main water supply was 0.01 $\pm$ 0.007, 0.13 $\pm$ 0.02, 0.02 $\pm$ 0.01, 0.17 $\pm$ 0.01 mg/l, respectively.

*Isolation and Identification of pathogenic bacteria from drinking water*

The frequency of pathogenic bacterial isolates from drinking water supply in the investigated poultry farm (Table 3) showed that the distribution of positive bacterial isolates was 41/60; 68.3% in all investigated water samples throughout the study period. Moreover, the most predominant bacterial isolates were *Shigella* spp. (10/41; 24.4%) followed by *E. coli* (9/41; 21.9%), *K. pneumonia* (8/41; 19.5%), *S. kentucky* (8/41; 19.5%), *S. garoli* (6/41; 14.6%). In addition, the percentage of pathogenic bacteria in tap water supply revealed that *Shigella* spp. was isolated in the highest percentage (3/10; 30.0%, whilst each of *E. coli*, *K. pneumonia* and *S. kentucky* found at the same percentage (2/10; 20.0% each) followed by *S. garoli* (1/10; 10.0%). On the other hand, the pathogenic bacteria in drinkers exhibited that both *E. coli* and *Shigella* spp. were discovered at the highest isolation rate (7/31; 22.6%) followed by *K. pneumonia*, *S. kentucky* (6/31; 19.3% each) and *S. garoli* (5/31; 16.1%).

The total viable count (TVC) in main water supply was recorded in the least count compared to its percentage in drinkers' water samples (10.8 $\times$ 10<sup>3</sup>, and 170.0 $\times$ 10<sup>3</sup> CFU/ml, respectively). Furthermore, the total coliform counts in main water source and drinkers were significantly high. Both are exc

Table 2. Mean values of physico-chemical parameters and heavy metals (mean $\pm$  SE) in drinking water supply in the investigated farm

Tested parameter	Water sampling		Permissible limit (P.L) according to WHO, (2011)
	Main water supply (Tap water)	Drinkers	
<b>Physico-chemical parameters</b>			
Temperature	25.6 $\pm$ 1.3	27.6 $\pm$ 2.3	-
pH	7.2 $\pm$ 0.5	8.4 $\pm$ 0.8	6.8-8.5
Turbidity (NTU)	0.46 $\pm$ 0.13 <sup>b</sup>	36.7 $\pm$ 3.8 <sup>a</sup>	0-4 NTU
Total alkalinity (CaCO <sub>3</sub> ) (mg/L)	86.6 $\pm$ 5.4 <sup>b</sup>	183.0 $\pm$ 17.6 <sup>a</sup>	180
Total hardness (mg/L)	101.2 $\pm$ 3.8 <sup>b</sup>	345.6 $\pm$ 7.6 <sup>a</sup>	180.0-200.0
Ammonia (NH <sub>3</sub> -N) (mg/L)	0.15 $\pm$ 0.08 <sup>b</sup>	1.36 $\pm$ 0.31 <sup>a</sup>	0.5
Nitrite (NO <sub>2</sub> -N) (mg/L)	0.04 $\pm$ 0.01 <sup>b</sup>	3.4 $\pm$ 0.46 <sup>a</sup>	3
Nitrate (NO <sub>3</sub> -N) (mg/L)	0.062 $\pm$ 0.01 <sup>b</sup>	7.48 $\pm$ 1.6 <sup>a</sup>	0.0-45.0
Sulfate (mg/L)	20.0 $\pm$ 3.1 <sup>b</sup>	61.6 $\pm$ 11.3 <sup>a</sup>	500
Phosphate (mg/L)	7.8 $\pm$ 1.6 <sup>b</sup>	26.3 $\pm$ 0.78 <sup>a</sup>	
<b>Heavy metals estimation (mg/L)</b>			
Arsenic (AS)	0.01 $\pm$ 0.007	0.05 $\pm$ 0.02	10
Copper (Cu)	0.13 $\pm$ 0.02	0.21 $\pm$ 0.04	0.6
Iron (Fe)	0.02 $\pm$ 0.01	0.19 $\pm$ 0.01	0.3
zinc (Zn)	0.17 $\pm$ 0.01	0.27 $\pm$ 0.08	5

-The mean values with different superscript letters a&b in the same row differ significantly at  $P \leq 0.05$

- WHO (2011) Guidelines for drinking water quality. 4th ed., World Health Organization (WHO), Geneva.

Table 3. Frequent distribution of pathogenic bacteria isolated from drinking water supply in the investigated farm.

Samples	Total		Distribution of bacterial isolates				Bacterial counting		
	Examined No.	Positive No. (%)	Salmonella spp.		K. pneumonia	Shigella spp.	TVC (CFU/mL)	TCC (CFU/100mL)	FCC (CFU/100mL)
			S. garoli	S. kentucky					
Tap water	20	10 (50.0)	1 (10.0)	2 (20.0)	2 (20.0)	3 (30.0)	10.8x10 <sup>3</sup> ± 1.1x10 <sup>3b</sup>	2.8x10 <sup>3</sup> ±2.1x10 <sup>3b</sup>	2x10 <sup>3</sup> ±1.3x10 <sup>3b</sup>
Drinkers	40	31 (77.5)	7 (22.0)	5 (16.1)	6 (19.3)	7 (22.6)	170.0x10 <sup>3</sup> ±130x10 <sup>3a</sup>	500.0x10 <sup>3</sup> ±350x10 <sup>2a</sup>	232.4x10 <sup>3</sup> ±156x10 <sup>2a</sup>
Total	60	41 (68.3)	9 (21.9)	6 (14.0)	8 (19.5)	10 (24.4)			

- The distribution of different bacterial isolates from drinking water supply was significantly differ at P ≤ 0.05

- TVC: Total viable count; TCC: Total coliform count; FCC: Fecal coliform count; CFU/ml: Colony forming unit per milliliter

Table 4. Serological identification of pathogenic *E. coli* and *Salmonella* spp.

Serological type	Identified pathogenic bacteria		Total No. (%)
	<i>E. coli</i>	<i>Salmonella</i> spp.	
Poly3 (O157)	6 (66.67)	<i>S. garoli</i> (O7, 6 H I)	6 (33.33)
O114	3 (33.33)	<i>S. kentucky</i> (O20, 8 H I)	3 (33.33)
Total No. (%)	9 (90)	14 (14)	23 (23)

eeded the standard requirement (zero total coliform count/100 ml) according to WHO guidelines. Moreover, the fecal coliform in both tap water and manual drinkers was 2x10<sup>3</sup>±1.3x10<sup>3</sup>, and 232.4x10<sup>3</sup>±156x10<sup>2</sup> CFU/ml, respectively.

### Serological identification of pathogenic bacteria

Serotyping of pathogenic bacterial isolates were identified for each of *E. coli* and *Salmonella* spp. as shown in Table 4. It has been found that out of 14 *Salmonella* spp. strains, there was *S. garoli* (O7, 6 H I) isolated at the percentage of 6/14; 42.8%. Meanwhile, *S. kentucky* (O20, 8 H I) isolates were found at the highest rate of 8/14; 57.1%. On the other hand, out of 9 *E. coli* strains, the pathogenic *E. coli* serotyping Poly3 (O157) recorded at 6/9;66.67% meanwhile, *E. coli* O114 was identified at the percentage of 3/9; 33.33%.

### Sensitivity test of pathogenic bacteria to different disinfectants

The biocidal efficiency of Klorsept 25 disinfectant against *E. coli*, *S. garoli*, *S. kentucky*, *K. pneumonia*, and *Shigella* spp. isolates were 100% at a concentration of 2.0 mg/l after 180 min of exposure. Whilst the efficiency of calcium hypochlorite Ca (Ocl)<sub>2</sub> against *E. coli* and *S. kentucky* was 100% at a concentration of 0.5 mg/l and exposure time 120 min. In contrast, its efficacy on *S. garoli*, *K. pneumonia*, and *Shigella* spp. was lower (83.3, 50.0 and 90 %, respectively) at the same concentration and the same exposure time. Meanwhile, terminator disinfectant against *S. garoli* and *S. kentucky* isolates was 100% at a concentration of 0.33 ml/l after 120 min of exposure. On the other hand, its effect on *Shigella* spp., *K. pneumonia*, and *E. coli* were declined (80, 75, and 66.7%, respectively) at the same concentration and exposure time. Oppositely, the biocidal efficiency of Virco-santy disinfectant was 0.0% against all bacterial isolates at any exposure time. In contrast, exposure of pathogenic bacteria to H<sub>2</sub>O<sub>2</sub> disinfectant at concentration of 5.0 % within 60 min contact time showed that the susceptibility of all bacterial isolates was 100% compared to H<sub>2</sub>O<sub>2</sub> (3.0 %) whereas the efficacy wasn't exceeded 87.5% at the same contact time as shown in Table 5.

### Discussion

Drinking water is a critical and vital nutrient for all living beings. Where poultry can survive with food shortage for a few weeks but will die within a few days without water. Besides, water constitutes more than 98% of all molecules in the body tissue and it is important for many biological processes, so that water quality affects poultry performance (Jamlianthang et al., 2018; El-sabrou and El-hanoun, 2019). In the current study, determination of physico-chemical parameters of drinking water supply and drinkers revealed that the pH value in both the main source and drinkers was recorded within the permissible limit. Oppositely, the level of water turbidity in drinkers was significantly higher, compared to its level in the main water supply. Both alkalinity and total hardness values were found in the highest rate compared to their values in the main water source. Whilst in drinkers, ammonia value besides nitrite, and phosphate values discovered at the highest level compared to the main water supply. Tesfamariam and Younis (2016) found that both value of pH and hardness of tap water supply were within the permissible limit guidelines that ranged from 6.98 - 7.09, and 108.46 -118.42 mg/L, respectively. Whilst the turbidity level was 7.50-836 NTU that exceeded the WHO permissible limit (5.00 NTU). Osei et al. (2019) recorded that the pH value of drinking water samples was ranged from 3.76 to 8.90, while turbidity level ranged from 0.20 to 617 NTU, and total hardness of 17.1 to 192.0.



Table 5. Sensitivity testing of bacterial isolates from drinking water supply to different types of disinfectant compounds in-vitro.

Tested disinfectant (Conc.)	Exposure time / h.	Sensitivity testing of bacterial strains																			
		<i>E. coli</i>						<i>Salmonella</i> spp.						<i>K. pneumonia</i>						<i>Shigella</i> spp.	
		(n=9)						(n=14)						(n=8)						(n=10)	
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R		
Klorsept 25 (1 Tablet/L)	1h.	88.9	11.1	0	66.7	0	33.3	62.5	25	12.5	50	12.5	37.5	70	10	20					
	2h.	100	0	0	83.3	16.7	0	87.5	12.5	0	75	25	0	80	0	20					
	3h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
Ca (Ocl) <sub>2</sub> (0.5 mg/L)	1h.	88.9	11.1	0	83.3	16.7	0	75	12.5	12.5	50	0	50	80	10	10					
	2h.	100	0	0	83.3	16.7	0	100	0	0	50	0	50	90	10	0					
	3h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
Terminator (0.33 mL/L)	1h.	44.4	33.3	22.2	66.7	16.7	16.7	87.5	12.5	0	75	0	25	60	20	20					
	2h.	66.7	22.2	11.1	100	0	0	100	0	0	75	12.5	12.5	80	20	0					
	3h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
Virco-santy (0.5 g/L)	1h.	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100					
	2h.	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100					
	3h.	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100					
H <sub>2</sub> O <sub>2</sub> -3.00%	1h.	55.5	0	44.5	83.3	0	16.7	75	25	0	87.5	12.5	0	50	0	50					
	2h.	88.9	0	11.1	100	0	0	75	25	0	87.5	12.5	0	100	0	0					
	3h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
H <sub>2</sub> O <sub>2</sub> 5.00%	1h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
	2h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					
	3h.	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0					

-S: sensitive; I: Intermediate; R: Resistant

- Data expressed as percentage

-The Sensitivity testing of bacterial strains against different disinfectant compounds was significantly differ at P≤0.05

Fridrich *et al.* (2014) reported that monitoring of water supplies should include the detection of physicochemical and microbiological parameters, which indicate organic pollution, especially contamination derived from animal waste, fertilizers, and others. Consequently, periodical investigation of water quality is an essential factor to improve hygienic quality of water sources (Behailu *et al.*, 2017).

Contamination of water supplies with a wide variety of microorganisms such as bacteria, viruses, and protozoa are fact cannot be ignored. These pathogens may originate from the fecal matter of birds (Leclerc *et al.*, 2002). Furthermore, about 90% of wastewater is discharged directly to surface water without any management in different developing countries, which negatively affect hygienic water quality besides more than 80% of diseases are caused by water contaminated by pathogenic microorganisms (UNICEF, 2004). In this context, the pathogenic bacterial isolates in drinkers exhibited that both *E. coli* and *Shigella* spp. were discovered at the highest isolation rate followed by *K. pneumonia*, *S. kentucky*, and *S. garoli*. Furthermore, the TVC in main water supply was recorded in the least count compared to its percentage in drinkers' water samples. In addition, the total coliform counts in main water source and drinkers were significantly high. Zaman *et al.* (2012) revealed that the prevalence rates of *E. coli* and *S. typhi* were high at 44% and 19%, respectively in drinkers, meanwhile their percentages in main water tanks were 28% and 9%, respectively. Moreover, they showed multidrug-resistant strains such as *E. coli*, and *S. typhi* at the percentages of 72% and 28%, respectively. Mohammed (2019) found that the pathogenic bacteria isolated from main tap water supply and trough were *K. pneumonia* at 100% and 24.3%, respectively and *E. coli*. At 0.0% and 56.8%, respectively. Talabi and Ogundana (2014) recorded that the total bacterial count in drinking water samples in the form of total coliform count and total viable bacteria count was 2.15 and 9.42 CFU/100 mL, respectively. Shahaby *et al.* (2015) detected both total coliform and fecal coliform at 9.5 CFU/100 mL in tap water supply.

The current study showed that the sensitivity pattern of pathogenic bacteria (*E. coli*, *S. garoli*, *S. kentucky*, *K. pneumonia*, and *Shigella* spp.) to Klorsept 25 disinfectant were 100% at a concentration of 2.0 mg/l after 180 min of exposure. Moreover, the sensitivity of *E. coli* and *S. kentucky* to calcium hypochlorite  $\text{Ca}(\text{OCl})_2$  was 100% at a concentration of 0.5 mg/l and exposure time 120 min. Li *et al.* (2013) found that both *Salmonella* spp. and *E. coli* are more resistant to chlorine than both enterococcal bacteria and total coliforms, where application of chlorine with a dose of 0.2–3.0 mg/L for 30 minutes led to reduction of coliform. Calcium hypochlorite is one of the disinfectant compounds that used in water treatment where it acts as oxidizing agent in microbial cell leading to damage bacterial cell walls (Lewis, 2010; Randtke, 2010). In addition, Bester (2015) recorded that sodium and calcium hypochlorite are the most commonly disinfectants that used for water treatment although chlorine is more effective against *E. coli* at higher dose (1.5 mg/L). Mohammed (2019) found that the efficiency of calcium hypochlorite against *E. coli* and *K. pneumonia* at a concentration of 2.0 mg/l was not exceeded 70% after 180 min, and 120 min, respectively. Moreover, in this study, the biocidal efficiency of Virco-santy disinfectant was 0.0% against all bacterial isolates. Whilst the efficiency of  $\text{H}_2\text{O}_2$  disinfectant against pathogenic bacteria was 100% at a concentration of 5.0 % within 60 min contact time. The mode of action of hydrogen peroxide depends on oxidizing the proteins and enzymes of pathogenic agents (Finnegan *et al.*, 2010). Hydrogen peroxide can destruct microorganisms by creating powerful oxidizing agents, hydroxyl radicals, from superoxide radicals (Linley *et al.*, 2012). Furthermore, hydrogen

peroxide is considered more powerful disinfectant for drinking water compared to chlorine due to it has been found that bacterial pathogens can survive in chlorine treated water (Bumanglag, 2016).

## Conclusion

The bactericidal efficiency of Virco-santy disinfectant is 0.0% against all bacterial isolates. The sensitivity pattern of pathogenic bacteria to  $\text{H}_2\text{O}_2$  disinfectant is 100% at a concentration of 5.0 % within 60 min contact time. In addition, the pathogenic bacteria (*E. coli*, *S. garoli*, *S. kentucky*, *K. pneumonia*, and *Shigella* spp.) are highly sensitive to Klorsept 25 disinfectant (100%) at a concentration of 2.0 mg/l after 180 min of exposure. Moreover, the sensitivity of *E. coli* and *S. kentucky* to  $\text{Ca}(\text{OCl})_2$  is 100% at a concentration of 0.5 mg/l and exposure time 120 min.

## Conflict of interest

All authors declare that there is no conflict of interest.

## References

- Ahmad, M.D., Hashmi, R.A., Anjum, A.A., Hanif, A., Ratyal, R.H., 2009. Drinking water quality by the use of congo red medium to differentiate between pathogenic and non-pathogenic *E. coli* at poultry farms. *J. Animal and Plant Sci.* 19, 108–110.
- Ahmed, M.F.A., 2017. The Efficiency of Disinfectants and Disinfection Programmes Commonly Adopted in Poultry Farms in Egypt. Ph.D. thesis in Veterinary medicine. Faculty of Veterinary Medicine, Cairo University, Egypt.
- APHA, 1992. Standard Methods for the Examination of Water and Wastewater. Published by American Public Health Association. Washington DC.
- APHA, 1998. Standard methods for the examination of water and wastewater, 20th Edition, Publisher, APHA American Public Health Association, Washington, DC.
- APHA., 2005. Standard Methods for Examination of Water and Wastewater. 21<sup>st</sup> ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.
- Behailu, T.W., Badessa, T.S., Tewodros, B.A., 2017. Analysis of Physical and Chemical Parameters in Ground Water Used for Drinking around Konso Area, Southwestern Ethiopia. *J. Anal. Bioanal. Tech. (JABT)* 8, 379
- Bester, C., 2015. Investigating the efficacy of sodium hypochlorite, calcium hypochlorite and peracetic acid on environmental *Escherichia coli* strains (Degree of Master of Science in Food Science). South Africa: University of Stellenbosch.
- Brown, A.E., 2005. Benson's microbiological applications: Laboratory manual in General Microbiology, Short Version 9<sup>th</sup> Edition. Publisher: McGraw-Hill Science/Engineering/Math
- Bumanglag, E.K.B., dela Cruz, J.F., Olarve, J.P., 2016. Effects of Hydrogen Peroxide and Silver Complex on the Potability of Drinking Water in Broiler Farms. *Austin J. Vet. Sci. and Anim. Husb.* 3, 1024.
- EFSA (Panel on Biological Hazards), 2013. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal.* 11, 3025.
- El-sabrou, K., El-hanoun, A., 2019. Does magnetised drinking water influence poultry's health and production?. *Worlds Poult. Sci. J.* 75, 411–415.
- Ewing, W.H., 1986. Edwards and Ewing's Identification of Enterobacteriaceae (4th Ed.) Elsevier, Netherlands. pp. 536
- Finnegan, M., Linley, E., Denyer, S.P., McDonnell, G., Simons, C., Mailard, J.-Y., 2010. Mode of Action of Hydrogen Peroxide and Other Oxidizing Agents: Differences between Liquid and Gas Forms. *J. Antimicrob. Chemother.* 65, 2108–2115.
- Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO), 2008. Microbiological hazards in fresh leafy vegetables and herbs. Meeting report. Mi-

- crobiol. Risk Assess. Ser. 14. 151. <http://www.fao.org/3/a-i0452e.pdf>;
- Fridrich, B., Krcmar, D., Dalmacija, B., Molnar, J., Pesic, V., Kragulj, M., Varga, N., 2014. Impact of wastewater from pig farm lagoons on the quality of local groundwater. *Agric. Water Manag.* 135, 40–53.
- Gil, M. I., Selma, M. V., López-Gálvez, F., Allende, A., 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *int. J. Food Microbiol.* 134, 37–45.
- Goodburn, C., Wallace, C.A., 2013. The microbiological efficacy of decontamination methodologies for fresh produce: a review. *Food Control.* 32, 418–427.
- Grizzle, J.M., Armbrust, T.A., Bryan, M.A., Saxton, A.M., 1997. The effect of water nitrate and bacteria on broiler growth performance. *J. Appl. Poult. Res.* 6, 48–55.
- Jamlianhang, Ranjana Goswami, L Hmar, Girin Kalita, R Buragohain, Hemen Das and K Khate, 2018. Effect of different sources of water on the performance of broilers in Mizoram. *IJCS.* 6, 1152–1155.
- Kok, T., Worswich, D., Gowans, E., 1996. Some Serological Techniques for Microbial and Viral Infections. *J. Med. Microbiol* (Collie, J.; Fraser, A.; Marmion, B. and Simmons, A., Eds.), 14<sup>th</sup> Ed., Edinburgh, Churchill Livingstone, UK.
- Leclerc, H., Schwartzbrod, L., Dei-Cas, E., 2002. Microbial agents associated with waterborne diseases. *Critical Reviews in Microbiology* 28, 371–409.
- Lewis, K.A., 2010. Hypo chlorination—sodium hypochlorite. In: Wallis-Lage C, editor. *White's handbook of chlorination and alternatives*. 5<sup>th</sup> ed. Hoboken, NJ: John Wiley and Sons Inc. pp. 452–526.
- Li, D., Zeng, S., Gu, A.Z., He, M., Shi, H., 2013. Inactivation, reactivation and regrowth of indigenous bacteria in reclaimed water after chlorine disinfection of a municipal wastewater treatment plant. *J. Environ. Sci. (China)* 25, 1319–1325.
- Liberti, L., Notarnicola, M., Petruzzelli, D., 2003. Advanced treatment for municipal wastewater reuse in agriculture. UV disinfection: parasite removal and by-product formation. *Desalination* 152, 315–324.
- Linley, E., Denyer, S.P., McDonnell, G., Simons, C., Maillard, J-Y., 2012. Use of Hydrogen Peroxide as a Biocide: New Consideration of its Mechanisms of Biocidal Action. *J. Antimicrob. Chemother.* 67, 1589–1596.
- Maharjan, P., Huff, G., Zhang, W., Watkins, S., 2017. Effects of chlorine and hydrogen peroxide sanitation in low bacterial content water on biofilm formation model of poultry brooding house waterlines. *Poult. Sci.* J. 96, 2145–2150.
- Mohammed, A.N., 2019. Resistance of bacterial pathogens to calcium hypochlorite disinfectant and evaluation of the usability of treated filter paper impregnated with nanosilver composite for drinking water purification. *J. Glob. Antimicrob. Resist.* 16, 28–35.
- Newell, D.G., Elvers, K., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N.J., Davies, R., Connerton, I., 2011. Biosecurity-Based Interventions and Strategies to Reduce *Campylobacter* Spp. on Poultry Farms. *Appl. Environ. Microbiol.* 77, 8605–8614.
- Osei, F.B., Boamah, V.E., Agyare, C., Abaidoo, R.C., 2019. Physicochemical Properties and Microbial Quality of Water Used in Selected Poultry Farms in the Ashanti Region of Ghana. *Open Microbiol. J.* 13, 121–127.
- Parish, M.E., Beuchat, L.R., Suslow, T.V., Haris, L.J., Garret, E.H., Farber, J.N., Busta, F.F., 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. In: *Analysis and evaluation of preventive control measure for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce*. *Compr. Rev. Food Sci. Food Saf.* 2, 161–173.
- Pedahzur, R., Katzenelson, D., Barnea, N., Lev, O., Shuval, H.I., Fattal, B., 2000. The efficacy of long-lasting residual drinking water disinfectants based on hydrogen peroxide and silver. *Water Sci. Technol.* 42, 293–298.
- Pilotto, F., Rodrigues, L.B., Santos, L.R., Klein, W.A., Colussi, F.M., Nascimento, V.P., 2007. Antibacterial efficacy of commercial disinfectants on dirt floor used in poultry breeder houses. *Braz. J. Poultry Sci.* 9, 127–131.
- Randtke, S.J., 2010. Chemistry of aqueous chlorine. In: Wallis-Lage C, editor. *White's handbook of chlorination and alternatives*. 5<sup>th</sup> ed. Hoboken, NJ: John Wiley & Sons Inc. pp. 68–173.
- Reutor, R., 2010. Water is the most important nutrients Nobel foundation. *Agricultural Division*. [http://www.nobel.org/Ag/Livestock/Water important nutrient.htm](http://www.nobel.org/Ag/Livestock/Water%20important%20nutrient.htm).
- Sapers, G.M., 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technol. Biotech.* 39, 305–311.
- Shahaby, A.F., Alharthi, A.A., Tarras, A.E. E.I., 2015. Bacteriological Evaluation of Tap Water and Bottled Mineral Water in Taif, Western Saudi Arabia. *Int. J. Curr. Microbiol. Appl. Sci.* 4, 600–615.
- Shuval, H., Fattal, B., Nassar, A., Lev, O., Pedahzur, R., 1995. Study of the synergism between oligodynamic silver and hydrogen peroxide as a long-acting water disinfectant. *Water Supply.* 13, 241–251.
- Singh, R.K., 2019. Role of poultry drinking water sanitation in poultry farming. <https://www.pashudhanpraharee.com/role-of-poultry-drinking-water-sanitation-in-poultry>.
- Talabi, A. O., Ogundana, A.K., 2014. Bacteriological Evaluation of Groundwater in Ekiti State, Southwestern Nigeria. *Int. J. Sci.* 3, 288–293.
- Tesfamariam, Z., Younis, Y.M.H., 2016. Assessment of physicochemical parameters and levels of heavy metals concentrations in drinking water of Asmara city, Eritrea III. *Am. J. Res. Commun.* 4, 30–44.
- UNICEF, 2004. United Nations Children's Fund and World Health Organization Joint Monitoring Programme for Water Supply and Sanitation. Meeting the MDG Drinking Water and Sanitation Target, A Mid-Term, Assessment of Progress. New York, NY: WHO and UNICEF.
- Van der Sluis, W., 2002. Water quality is important but often over estimated. *World Poultry Sci. J.* 18, 26–32.
- Watkins, S., 2007. Water Line Sanitation. Ross tech notes. University of Arkansas. [http://cn.staging.aviagen.com/tech-center/download/26/AviaTech\\_WaterLineSanitation](http://cn.staging.aviagen.com/tech-center/download/26/AviaTech_WaterLineSanitation).
- Yang, X.U., 2016. A study on antimicrobial effects of nanosilver for drinking water disinfection [PhD thesis]. Singapore: Department of Civil and Environmental Engineering, National University of Singapore.
- Zaman, A., Amin, A., Shah, A.H., Kalhor, D.H., Muhammad, Kh., Sha-keebullah, M. H., Wazir, H., 2012. Prevalence and vulnerability characterization of poultry pathogens isolated from water tanks and drinkers in commercial broiler farms. *Afr. J. Microbiol. Res.* 6, 691–695.