



In vitro Efficacy Testing of Some Commercial Disinfectants against Pathogenic Bacteria Isolated from Different Poultry Farms

HASSAN A. AIDAROS, EMAN M. HAFEZ, HALLA E.K. EL BAHGY*

Veterinary Hygiene and management Department, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt.

Abstract | Proper management and hygiene are the keys for the poultry industry, profit mainly depends on efficient cleaning and disinfection. Disinfectants play an essential role in controlling pathogens in health care, animal production, and food-related industries. The effectiveness of a disinfectant is mainly dependent on the active compound chosen, its concentration, and the cleanliness of the surfaces to which it is applied. The purpose of this study was to evaluate the effectiveness of some disinfectants (Prophyl 2000®, G7®, Pron-Tech®, Alkadox®, and Biodine®) at different concentrations and contact times at 20, 40, 60, and 90 minutes against field isolated serotypes of *E. coli*, *Pasteurella multocida*, *Campylobacter jejuni*, and *Staphylococcus aureus* from chicken and duck farms production at a titer of 3×10^6 CFU (Colony Forming Unit)/cm² in the absence and presence of organic matter (O.M). The results showed that the efficiency of disinfectants was significantly increase with high concentration and long contact time. The Prophyl 2000® was the most powerful disinfectant against field pathogens, followed by G7®, Pron-Tech®, and Alkadox®, while Biodine® was the weakest disinfectant at the same conditions. Moreover, the organic matter hindered bactericidal power of many commercial disinfectants such as quaternary ammonium compounds (QUATS), halogen releasing agents including iodine, chlorine and its compounds, but some were not affected, such as glutaraldehyde. Finally, the success of the disinfection process in different poultry farms mainly depends on the selection of suitable disinfectants.

Keywords | Disinfectant, Poultry, *E.coli*, *Pasteurella multocida*, *Campylobacter jejuni*, *Staphylococcus aureus*.

Received | June 23, 2022; Accepted | August 05, 2022; Published | September 15, 2022

*Correspondence | Halla EK El Bahgy, Hygiene and Veterinary Care Department, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt; Email: Hala.mohamed@fvtm.bu.edu.eg

Citation | Aidaros HA, Hafez EM, El Bahgy HEK (2022). *In vitro* efficacy testing of some commercial disinfectants against pathogenic bacteria isolated from different poultry farms. Adv. Anim. Vet. Sci. 10(10): 2116-2123.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2022/10.10.2116.2123>

ISSN (Online) | 2307-8316



Copyright: 2022 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Commercial poultry production around the world is one of the most successful sectors, mainly due to its high growth rate, short generation interval and low investment per unit. Unfortunately, its progress is not enough to meet the increased demand (Shoib et al., 2018). Intensive poultry production leads to the transmission and spread of infectious diseases due to the presence of thousands of birds in an enclosed warm and dusty environment (Collins,

2007; Li et al., 2022).

Cleaning and disinfection are very important parts of farm hygiene management for prevention, control of contagious diseases and can impact on productivity, feed conversion and welfare (Luyckx et al., 2015; Gosling, 2018). An effective sanitation plan is based on the appropriate selection of ideal disinfectant, the type of contaminants present, and their sensitivity to the available disinfectants (Jiang et al., 2018).

High microorganism concentration, disinfectant concentration, presence of organic matter, pH (pressure of hydrogen ion concentration), temperature, contact time, and surface material type all affect disinfectant efficiency (Stringfellow et al., 2009; White et al., 2018). The commonly used chemical disinfectants for poultry farms are aldehydes, halogens (chlorines and iodophors), phenols, oxidizing agents, and quaternary ammonium compounds or combinations of more than one (Chidambaranathan and Balasubramaniam, 2019).

Glutaraldehyde is a very strong disinfectant that directly acts on bacterial proteins, enzymes, and metabolism leading to bacterial death. Also, it prevents the release of dihydrochloride from the outer layer of the bacterial spores to prevent sporulation (Castro Burbarelli et al., 2017; Rhee et al., 2021). It has a broad bactericidal spectrum with a highly efficient killing capacity for bacteria and virus. In addition to that, it exhibits a strong effect on the spores generated by *Clostridium*, which can cause necrotic enteritis, and thus is commonly used for the disinfection of bacterial spores during epidemics. So, it is more frequently applied for disinfection process in poultry farms (Brantner et al., 2014).

Quaternary ammonium compounds are effective antimicrobial agents due to their significant biocide activity, compatibility with the environment, and long-term durability (Ramzi et al., 2020). It is a cationic surfactant whose bactericidal effect depends on lipophilicity, altering cell permeability and resulting in extravasation of the bacterial content. The gram-positive bacteria are more sensitive to quaternary ammonium; this is due to the presence of more lipids on the cell wall (Battersby et al., 2017; Belter et al., 2022). Dimethyl benzyl ammonium chloride compound is among the earliest disinfectants within the QUATS family that have been widely used in various fields, such as food, medicine, oil field and industrial water treatment, owing to their broad antibacterial spectrum, good water solubility and environmental stability (Wang et al., 2022).

Halogen-liberating disinfectants exert their bactericidal effect by releasing active forms (iodine or chlorine) that have lethal effect on a wide range of microorganisms. The great problem of halogen releasing chemicals is related to their relatively high suppression by organic matter. The presence of chlorine as hypochlorite compounds acts as an oxidizing agent that destroys both the bacterial DNA and cell membrane (Qiao and Shao, 2010; Aksoy et al., 2020). Chlorine containing disinfectants can effectively kill different microbes like *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Enterococcus* and *Enterobacter*, even at a low concentration (Suwa et al., 2013). Currently, chlorine-releasing agents (sodium hypochlorite, bleaching powder,

sodium dichloroisocyanurate, chlorine dioxide) are widely used as disinfectants on different poultry farms (Boxall et al., 2003; Byun et al., 2021).

The aim of the present *in vitro* study is to improve the performance of the cleaning and disinfection process by selection of the most powerful commercial disinfectant; decrease the cost of the disinfection process through determination of the minimum concentration that has a high bactericidal effect; and avoid exposure of microorganisms to sub-inhibitory concentrations that may facilitate decreased susceptibility and the evolution of bacterial resistance.

MATERIALS AND METHODS

PREPARATION OF TESTED STRAIN

The standardized stable suspensions of tested isolated field strains from different chicken and duck farms production were *E. coli* (O114:K90), *Pasteurella multocida* (A:1), *Campylobacter jejuni* and *Staphylococcus aureus*, that were prepared by seed-lot culture maintenance techniques (seed-lot systems) according to (Kamal et al., 2019) to obtain 3×10^6 CFU/ 0.1 mL suspension concentration by growing the tested strains on buffered peptone water broth at 37°C for 24 hours expect *Campylobacter jejuni* at 42°C for 24 hours in presence of 10% CO₂ and 5% O₂ then cultured them on EMB (eosine methylene blue), blood agar media, *Campylobacter* selective media and paired parker media, respectively. The separated colony picked up and inoculated in peptone water broth. The suspensions were measured via making serial dilutions, then the plate counts were done using plate count agar media at 37°C for 24 hours expect *Campylobacter jejuni* at 42°C for 24 hours which are suitable media for all microorganism and choose suspensions of concentration 3×10^6 CFU/ 0.1 mL as working suspensions.

PREPARATION OF DISINFECTANT AGENTS

Commercial disinfectants were prepared according to manufacturer procedure or supplier guideline. The different concentrations of commercial disinfectant were prepared by using distilled water according to (Aksoy et al., 2020; Drauch et al., 2020).

TESTED DISINFECTANTS

Prophyl 2000®

It consists of glutaraldehyde (0.13%), alkyl dimethyl benzyl ammonium chloride (0.1%) and chloro 4 methyl 3 phenol (0.05%). It manufactures by Laboratoire Meriel – France company. The recommended dose is at concentration of 0.4% - 2%.

G7®

It contains of glutaraldehyde, 7 quaternary ammonium chloride 12%. It manufactures by Alpha trade company for chemical. The recommended dose is 0.5% concentration.

Prontech®

It consists of N-Alkyl (60% C14, 30% C16, 5% C18), dimethyl benzyl ammonium chloride 40%, urea (inert carrier) 60%. It manufactures by United promotion ink company. The recommended dose is at 0.2% (high dose), 0.1% (low dose).

Alkadox®

It contains a sodium hypochlorite and sodium carbonate. It manufactures by Chemi-care, A.R.E company. The recommended dose is at concentration of 1%.

Biodine 2.8®

It consists of iodine (2.8%), phosphoric acid, alcohol ethoxylate and dodecyl benzene sulfonic acid. It manufactures by Biolink – Egypt company. The recommended dose is at concentration of 0.5% - 1%.

THE SURFACE CHALLENGE TEST WAS PERFORMED IN VITRO

Accurately, large squares (20cm×20cm) of the surfaces area were used for application of these disinfectants at various dilutions at room temperature in the absence of organic matter and in the presence of such matter by using calf serum. Each large square was divided into small squares of 4 cm x 4 cm and were artificially contaminated with the cultured broth for 24 hours of the tested microorganisms and acts as the initial bacterial counts of the tested pathogens and were counted before disinfectants application. Further, 1ml of calf serum was separately applied. Application of each disinfectant preparation at certain concentrations that differ from type to other at intervals of 20, 40, 60 and 90 minutes was performed, using sterile swabs for picking up the viable microorganisms from previously contaminated small squares. Whole swabs were directly transferred into sterile cotton plugged test tubes that contain 10 ml nutrient broth and 1 ml of the neutralizer of the applied preparation was added and then incubated at 37C for 24 hours after every contact time. The used neutralizer was prepared according to the protocol recommended by (Douglas and Kampf, 2011). Accurately, the used neutralizer is composed of combination of 3% Tween 80, 0.3% Lethcin, 1% Histidine, 0.5% Sodium thiosulphate and 3% Saponine in phosphate buffered saline (PBS). Any detectable bacterial growth was confirmed by culturing on specific agar plates. The bacterial count for each dilution should be read then multiplied its average by the reciprocal of the same dilution level according to (Drauch et al., 2020). All previous steps were repeated in absence of organic matter.

STATISTICAL ANALYSIS

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 25 (IBM Corp. Released 2013). Data were treated as a complete randomization design. Multiple comparisons were carried out applying Duncun test. The significance level was set at < 0.05.

RESULTS

The Prophyl 2000® succeed to complete the reduction of tested *E.coli* at 2% conc. within 60 minutes in the absence of O.M and within 90 minutes contact time in the presence of O.M. Furthermore, the G7® could completely reduce the tested *E.coli* at conc. 1% within 60 minutes without O.M and within 90 minutes with the presence of O.M, followed by the Pron-Tech® at conc. 0.2% within 90 minutes and at conc. 0.5% within 90 minutes with the presence of O.M. Finally, the Alkadox® at conc. 1.5% within 90 minutes achieved 100% reduction of tested *E.coli* and 99.3% with the presence of O.M., whereas Biodine® only reduced bacterial count by 95% and 83.6% at conc. 1.5% within 90 minutes in absence and presence of O.M, respectively (Table 1).

Table 2 showed that the tested disinfectants that reduced tested *Pasteurella multocida* by 100 % were Prophyl 2000® at (2% conc. within 40 minutes without O.M and at 60 minutes contact time with the presence of O.M., G7® at (0.5% conc. within 60 minutes without O.M and at 90 minutes contact time with the presence of O.M.), Pron-Tech® at (0.5% within 40 minutes without O.M. and at 60 minutes contact time with the presence of O.M), Alkadox® at (1.5% at 60 minutes without O.M and at 90 minutes with the presence of O.M.) and finally Biodine® achieved 100% reduction of the tested *Pasteurella multocida* only at 1.5% conc. within 90 minutes contact time without presence of O.M.

The Prophyl 2000® disinfectant completely reduced the number of tested *Campylobacter jejuni* at (4% conc. within 20 minutes, within 40 minutes at the same conc.), G7® at (.5% conc. within 40 minutes, 1% at the same time), Pron-Tech® at (0.2% conc. within 40 minutes, 0.5% within 60 minutes), Alkadox® at (1% conc. within 60 minutes, 1.5% within 90 minutes) and Biodine® at (1% conc. within 60 minutes, 1.5 % within 90 minutes) in the absence and presence of O.M, respectively (Table 3).

The tested disinfectants that reduced the tested *Staphylococcus aureus* by 100 % were Prophyl 2000® at (4% conc. within 40 minutes, within 90 minutes at the same conc.), G7® at (.5 % conc. within 60 minutes, 1% at the same time), Pron-Tech® at (.5% conc. within 60 minutes, within 90 minutes at the same conc. and Alkadox® at (1% conc.

Table 1: Commercial disinfectants efficacy against *E. coli* (3.0×10⁶ CFU/ cm²) in relation to the different concentrations and contact times.

Disinfectants	Concentration (%)	Reduction % in absence of organic matter				Reduction % in presence of organic matter			
		20 min	40 min	60 min	90 min	20 min	40 min	60 min	90 min
Prophyl 2000®	0.4	55.60 ^{fgD}	73.70 ^{cdC}	85.90 ^{bcB}	98.10 ^{abA}	39.80 ^{deD}	61.00 ^{eC}	70.80 ^{dB}	82.10 ^{cA}
	2.0	71.20 ^{bc}	88.40 ^{bb}	100.00 ^{aa}	100.00 ^{aa}	53.30 ^{bc}	79.70 ^{bb}	98.50 ^{aa}	100.00 ^{aa}
	4.0	79.80 ^{ac}	94.30 ^{ab}	100.00 ^{aa}	100.00 ^{aa}	62.90 ^{ac}	84.00 ^{ab}	100.00 ^{aa}	100.00 ^{aa}
G7®	0.25	43.50 ^{hd}	60.20 ^{gc}	72.60 ^{eb}	84.70 ^{da}	32.10 ^{fgD}	46.70 ^{hc}	56.30 ^{fb}	69.90 ^{ea}
	0.50	62.90 ^{deC}	75.40 ^{eb}	99.10 ^{aa}	100.00 ^{aa}	46.70 ^{cd}	67.60 ^{dc}	93.00 ^{bb}	99.50 ^{abA}
	1.00	68.70 ^{bcC}	85.90 ^{bb}	100.00 ^{aa}	100.00 ^{aa}	57.10 ^{bc}	74.40 ^{cb}	98.90 ^{aa}	100.00 ^{aa}
Pron-Tech®	0.1	40.10 ^{hiD}	54.90 ^{hc}	67.50 ^{fb}	78.00 ^{ca}	29.60 ^{ghD}	41.30 ^{ic}	49.80 ^{gb}	60.20 ^{fa}
	0.2	58.50 ^{efD}	69.30 ^{deC}	90.60 ^{bb}	100.00 ^{aa}	43.30 ^{cdD}	60.00 ^{efC}	82.70 ^{cb}	97.40 ^{aA}
	0.5	65.00 ^{cdD}	76.90 ^{ec}	96.00 ^{ab}	100.00 ^{aa}	55.30 ^{bd}	68.20 ^{dc}	91.50 ^{bb}	100.00 ^{aa}
Alkadox®	0.5	37.90 ^{jiD}	53.00 ^{hc}	65.20 ^{fb}	74.50 ^{ca}	28.90 ^{ghD}	37.00 ^{jiC}	42.60 ^{hb}	55.10 ^{gA}
	1.0	56.80 ^{fgD}	68.00 ^{ec}	83.40 ^{cdB}	99.70 ^{abA}	43.30 ^{cd}	56.70 ^{fc}	70.00 ^{db}	95.00 ^{ba}
	1.5	62.30 ^{deD}	74.10 ^{ec}	88.70 ^{bb}	100.00 ^{aa}	54.00 ^{bd}	64.30 ^{deC}	78.50 ^{cb}	99.30 ^{abA}
Biodine®	0.5	35.10 ^{jd}	48.80 ^{ic}	58.40 ^{gb}	69.00 ^{fa}	26.10 ^{hd}	33.60 ^{jc}	39.50 ^{ib}	48.90 ^{ha}
	1.0	53.00 ^{gd}	62.70 ^{fgC}	74.90 ^{eb}	91.20 ^{ca}	36.70 ^{efD}	50.00 ^{gc}	63.30 ^{eb}	77.00 ^{da}
	1.5	58.60 ^{efD}	66.90 ^{efC}	80.30 ^{db}	95.00 ^{bcA}	42.40 ^{cdD}	56.00 ^{fc}	69.10 ^{db}	83.60 ^{ca}

a, b & c: There is significant difference (P=0.00) between any two means, within the same column have the different superscript letters.

A, B & C: There is significant difference (P=0.00) between any two means for the same attribute, within the same row have the different superscript letters.

Table 2: Commercial disinfectants efficacy against *Pasteurella multocida* (3.0×10⁶ CFU/ cm²) in relation to the different concentrations and contact times.

Disinfectants	Concentration (%)	Reduction % in absence of organic matter				Reduction % in presence of organic matter			
		20 min	40 min	60 min	90 min	20 min	40 min	60 min	90 min
Prophyl 2000®	0.4	67.20 ^{gd}	85.40 ^{dc}	96.00 ^{ab}	100.00 ^{aa}	55.10 ^{fd}	71.50 ^{efC}	80.30 ^{eb}	89.80 ^{ba}
	2.0	89.60 ^{bb}	100.00 ^{aa}	100.00 ^{aa}	100.00 ^{aa}	71.30 ^{cdB}	97.80 ^{abA}	100.00 ^{aa}	100.00 ^{aa}
	4.0	96.90 ^{ab}	100.00 ^{aa}	100.00 ^{aa}	100.00 ^{aa}	82.70 ^{ab}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}
G7®	0.25	56.20 ^{hd}	73.80 ^{fc}	82.90 ^{cb}	95.60 ^{aa}	47.10 ^{gd}	63.00 ^{gc}	68.20 ^{fb}	78.50 ^{ca}
	0.50	80.50 ^{cdB}	99.30 ^{aA}	100.00 ^{aa}	100.00 ^{aa}	67.00 ^{deC}	94.00 ^{bcB}	98.10 ^{abA}	100.00 ^{aa}
	1.00	91.30 ^{bb}	100.00 ^{aa}	100.00 ^{aa}	100.00 ^{aa}	79.20 ^{abB}	99.20 ^{abA}	100.00 ^{aa}	100.00 ^{aa}
Pron-Tech®	0.1	51.60 ^{hd}	65.00 ^{gc}	74.90 ^{db}	86.00 ^{ba}	38.90 ^{hd}	50.30 ^{hc}	57.60 ^{gb}	66.90 ^{da}
	0.2	74.90 ^{efC}	92.70 ^{bcB}	100.00 ^{aa}	100.00 ^{aa}	63.30 ^{ed}	83.00 ^{dc}	96.90 ^{abcB}	99.80 ^{aA}
	0.5	86.70 ^{bcB}	100.00 ^{aa}	100.00 ^{aa}	100.00 ^{aa}	74.60 ^{bcC}	91.30 ^{cb}	100.00 ^{aA}	100.00 ^{aA}
Alkadox®	0.5	45.60 ^{jd}	58.80 ^{hc}	69.10 ^{eb}	78.90 ^{ca}	33.10 ^{id}	41.90 ^{ic}	48.70 ^{hb}	60.40 ^{ea}
	1.0	69.80 ^{fgC}	88.10 ^{cdB}	100.00 ^{aa}	100.00 ^{aa}	56.70 ^{fd}	72.00 ^{efC}	94.10 ^{bcB}	99.00 ^{aA}
	1.5	78.40 ^{deC}	94.70 ^{bb}	100.00 ^{aa}	100.00 ^{aa}	68.00 ^{deC}	85.90 ^{db}	97.30 ^{abA}	100.00 ^{aA}
Biodine®	0.5	40.30 ^{jd}	52.70 ^{ic}	65.20 ^{eb}	74.00 ^{ca}	31.50 ^{id}	37.30 ^{ic}	45.00 ^{hb}	53.90 ^{fa}
	1.0	65.00 ^{gd}	79.60 ^{ec}	93.20 ^{bb}	99.00 ^{aa}	53.30 ^{fd}	66.70 ^{fc}	86.70 ^{db}	92.30 ^{ba}
	1.5	76.10 ^{ec}	87.90 ^{cdB}	99.60 ^{aA}	100.00 ^{aa}	64.70 ^{ed}	76.20 ^{ec}	92.00 ^{cb}	98.80 ^{aA}

a, b & c: There is significant difference (P=0.00) between any two means, within the same column have the different superscript letters.

A, B & C: There is significant difference (P=0.00) between any two means for the same attribute, within the same row have the different superscript letters.

Table 3: Commercial disinfectants efficacy against *Campylobacter jejuni* (3.0×10^6 CFU/ cm²) in relation to the different concentrations and contact times.

Disinfectants	Concentration (%)	Reduction % in absence of organic matter				Reduction % in presence of organic matter			
		20 min	40 min	60 min	90 min	20 min	40 min	60 min	90 min
Prophyl 2000®	0.4	75.90 ^{efD}	92.30 ^{cC}	98.90 ^{aB}	100.00 ^{aA}	62.50 ^{fgD}	76.30 ^{gC}	83.60 ^{cB}	91.90 ^{bA}
	2.0	99.30 ^{aB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	87.00 ^{aB}	99.20 ^{abA}	100.00 ^{aA}	100.00 ^{aA}
	4.0	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	90.30 ^{aB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}
G7®	0.25	69.00 ^{gD}	77.90 ^{cC}	86.50 ^{bB}	96.90 ^{aA}	56.00 ^{hD}	69.10 ^{gC}	77.20 ^{dB}	84.80 ^{cA}
	0.50	91.90 ^{bB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	82.70 ^{bB}	97.60 ^{abA}	100.00 ^{aA}	100.00 ^{aA}
	1.00	99.80 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	88.60 ^{aB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}
Pron-Tech®	0.1	62.50 ^{hD}	71.80 ^{fC}	80.40 ^{eB}	88.70 ^{bA}	47.90 ^{iD}	58.50 ^{hC}	68.10 ^{eB}	75.30 ^{dA}
	0.2	86.10 ^{cB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	71.60 ^{de}	94.70 ^{bcB}	98.60 ^{abA}	100.00 ^{aA}
	0.5	93.90 ^{bB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}	79.10 ^{bc}	95.00 ^{abcB}	100.00 ^{aA}	100.00 ^{aA}
Alkadox®	0.5	53.70 ^{iD}	60.90 ^{gC}	72.30 ^{dB}	81.80 ^{cA}	40.00 ^{iD}	46.90 ^{iC}	59.20 ^{fB}	67.00 ^{eA}
	1.0	77.20 ^{efC}	95.80 ^{bcB}	100.00 ^{aA}	100.00 ^{aA}	66.70 ^{efD}	87.70 ^{deC}	95.40 ^{abB}	99.70 ^{aA}
	1.5	84.60 ^{cdB}	99.50 ^{abA}	100.00 ^{aA}	100.00 ^{aA}	75.20 ^{cdC}	91.40 ^{cdB}	99.60 ^{aA}	100.00 ^{aA}
Biodine®	0.5	45.10 ^{jD}	54.90 ^{hC}	68.70 ^{dB}	76.90 ^{cA}	36.20 ^{iD}	44.10 ^{iC}	53.80 ^{gB}	61.40 ^{fA}
	1.0	73.00 ^{fgC}	84.90 ^{dB}	100.00 ^{aA}	100.00 ^{aA}	60.00 ^{ghD}	81.60 ^{fC}	93.70 ^{bbB}	97.00 ^{aA}
	1.5	79.90 ^{deC}	92.00 ^{cB}	100.00 ^{aA}	100.00 ^{aA}	71.50 ^{deD}	83.90 ^{efC}	96.10 ^{abB}	100.00 ^{aA}

a, b & c: There is significant difference (P=0.00) between any two means, within the same column have the different superscript letters.

A, B & C: There is significant difference (P=0.00) between any two means for the same attribute, within the same row have the different superscript letters.

Table 4: Commercial disinfectants efficacy against *Staphylococcus aureus* (3.0×10^6 CFU/ cm²) in relation to the different concentrations and contact times.

Disinfectants	Concentration (%)	Reduction % in absence of organic matter				Reduction % in presence of organic matter			
		20 min	40 min	60 min	90 min	20 min	40 min	60 min	90 min
Prophyl 2000®	0.4	59.20 ^{fgD}	78.10 ^{efC}	89.50 ^{eB}	100.00 ^A	44.30 ^{eD}	67.80 ^{deC}	75.20 ^{eB}	86.40 ^{bA}
	2.0	75.80 ^{bcB}	97.80 ^{abA}	100.00 ^{aA}	100.00 ^A	56.70 ^{bcC}	93.30 ^{abB}	99.70 ^{aA}	100.00 ^{aA}
	4.0	87.60 ^{aB}	100.00 ^{aA}	100.00 ^{aA}	100.00 ^A	69.10 ^{aB}	98.00 ^{aA}	100.00 ^{aA}	100.00 ^{aA}
G7®	0.25	49.50 ^{hD}	64.70 ^{iC}	77.10 ^B	89.10 ^A	34.90 ^{fD}	57.70 ^{fC}	64.20 ^{fB}	75.40 ^{cA}
	0.50	67.10 ^{deC}	79.00 ^{efB}	100.00 ^{aA}	100.00 ^A	53.30 ^{cdD}	82.30 ^{cC}	96.70 ^{abB}	99.90 ^{aA}
	1.00	80.40 ^{bC}	92.60 ^{bcB}	100.00 ^{aA}	100.00 ^A	70.00 ^{aC}	89.50 ^{bB}	100.00 ^{aA}	100.00 ^{aA}
Pron-Tech®	0.1	46.20 ^{hiD}	58.50 ^{jC}	70.90 ^{eB}	82.00 ^A	31.60 ^{fgD}	45.30 ^{gC}	52.90 ^{gB}	63.10 ^{dA}
	0.2	61.90 ^{efD}	74.20 ^{fgC}	93.80 ^{bB}	100.00 ^A	53.30 ^{cdD}	70.30 ^{dC}	91.30 ^{bcB}	98.60 ^{aA}
	0.5	78.10 ^{bC}	92.30 ^{cB}	100.00 ^{aA}	100.00 ^A	67.10 ^{aB}	81.20 ^{cC}	96.00 ^{abB}	100.00 ^{aA}
Alkadox®	0.5	41.40 ^{ij}	55.70 ^{jk}	66.90 ^{eB}	77.10 ^A	30.20 ^{fgD}	39.50 ^{hC}	45.20 ^{hB}	57.80 ^{eA}
	1.0	58.50 ^{fgD}	71.30 ^{ghC}	89.90 ^{eB}	100.00 ^A	50.00 ^{dD}	63.30 ^{eC}	87.30 ^{cB}	97.50 ^{aA}
	1.5	69.70 ^{deD}	85.20 ^{dC}	97.50 ^{abB}	100.00 ^A	59.30 ^{bD}	72.00 ^{dC}	91.90 ^{bcB}	100.00 ^{aA}
Biodine®	0.5	37.60 ^{iD}	51.00 ^{kC}	60.90 ^{fB}	70.80 ^A	28.50 ^{gD}	34.90 ^{hC}	42.20 ^{hB}	50.40 ^{fA}
	1.0	56.00 ^{gD}	66.80 ^{hC}	83.70 ^{dB}	95.00 ^A	43.30 ^{eD}	56.70 ^{fC}	76.00 ^{eB}	88.70 ^{bA}
	1.5	70.80 ^{cdD}	81.40 ^{deC}	92.30 ^{bcB}	100.00 ^A	54.70 ^{bcdD}	67.40 ^{deC}	81.50 ^{dB}	94.10 ^{aA}

a, b & c: There is significant difference (P=0.00) between any two means, within the same column have the different superscript letters.

A, B & C: There is significant difference (P=0.00) between any two means for the same attribute, within the same row have the different superscript letters.

within 90 minutes ,1.5% at the same time) in the absence and presence of O.M , respectively. The Biodine® disinfectant completely reduced the tested *Staphylococcus aureus* only at conc. 1.5% within 90 minutes without presence of O.M (Table 4).

DISCUSSION

Preventive medicine is essential to combat poultry infectious diseases as biosecurity. As part of biosecurity strategies, effective cleaning and disinfection protocols are required to prevent disease transmission among farms (Gómez-García et al., 2022) The disinfection process is the most critical point in biosecurity plan of poultry farms and plays a key role in control of diseases and achievement of profit. The implementation of effective disinfection process at poultry production sites is of great importance for consumers and for public health, so it is important to evaluate the effectiveness of commonly used disinfectants on some pathogenic bacteria and determine the suitable state for their application to achieve the maximum benefit by determining the most powerful disinfectants and the lowest effective concentration.

Our results summarized that Prophyl 2000® is a very effective disinfectant against tested strains of *E. coli*, *Pasteurella multocida*, *Campylobacter jejuni*, and *Staphylococcus aureus* within 60 minutes' contact time in the absence and presence of organic matter. This is due to its aldehyde-based disinfectants that are considered one of the most powerful disinfectants and affect a wide range of bacteria and their spore through alkylation of hydroxyl, carbonyl and amino groups, which affect DNA, RNA and protein synthesis and also less affected by organic matter (Gosling, 2018; Drauch et al., 2020; Abdel-Latef and Mohammed, 2021). In addition, the G7® disinfectant is the combination of glutaraldehyde and quaternary ammonium compounds that is strong and very effective complex but less powerful than Prophyl 2000® due to the quaternary ammonium compound, which is one of the disinfectants affected by the presence of organic matter (Battersby et al., 2017; Figueroa et al., 2017; Drauch et al., 2020). Additionally, the Pron-Tech® that contains dimethyl benzyl ammonium chloride compound within the QUATS family is probably one of the best chemicals to inhibit microbial germination by penetrating the bacterial cell membrane by electrostatic gravity to cause the internal substances to leak out, which eventually results in cell lysis and death (Abdel-Latef and Mohammed, 2021; Wang et al., 2022).

Alkadox® disinfectant contains sodium hypochlorite (chlorine releasing agent) that has destructive action on bacterial cell membranes and oxidative action on irreversible bacterial enzymes, but are considered less stable disin-

fectant and less effective in the presence of organic matter (Guastalli et al., 2016). Moreover, the Biodine® is an iodine compound disinfectant that exhibits a broad range of microbicidal activity against bacteria by iodination of lipids and oxidation of cytoplasmic membrane compounds but couldn't completely eliminate the tested microorganisms within 90 minutes expect at high concentration and only minimized the bacterial count at low concentration, that is the least effective tested disinfectants that highly affected by the presence of organic matter (Aksoy et al., 2020).

There is a highly significant difference between bacterial efficacy of tested disinfectants at same contact time and concentration of tested disinfectant in presence and absence of O.M as remaining organic material in poultry farms is known to decrease the efficacy of disinfectants. This negative effect was also reported from the field once to focus on the importance of proper cleaning procedures before applying disinfectants (Drauch et al., 2020). Furthermore, the efficiency of disinfectants was significantly increase with high concentration and long contact time that clearly affect the reduction percentage of disinfection to pathogens (Wales et al., 2021).

Finally, our results summarized that Prophyl 2000® was the most effective disinfectant against *E. coli*, *Pasteurella multocida*, *Campylobacter jejuni* and *Staphylococcus aureus*, while the Biodine® was the weakest one. In addition, the organic matter is one of the most serious factors affecting the bactericidal power of some commercial disinfectants (Sato et al., 2019; Saadatpour and Mohammadipanah, 2022).

CONCLUSION

However, there are various types of disinfectants furthermore; the selection of the most effective one is not easy decision. So, the evaluation process of commercial disinfectants helps the poultry producer to put their hand on the most powerful one that can achieve the most successful sanitation plan.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Faculty of Veterinary Medicine / Benha University for supporting this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

This study is the first in Egypt to evaluate G7® and Pron-Tech® commercial disinfectants against field pathogenic serotypes of *E. coli*, *Pasteurella multocida*, *Campylobacter jejuni*, and *Staphylococcus aureus* isolated from chicken and duck.

AUTHORS CONTRIBUTION

HAA and HEKE designed the concept for this research and scientific paper. EMH had sampled and HEKE and EMH had analyzed the data and interpreted the results. All authors have read and approved the final manuscript.

REFERENCES

- Abdel-Latef GK, Mohammed AN (2021). Efficiency evaluation of some novel disinfectants and anti-bacterial nanocomposite on zoonotic bacterial pathogens in commercial Mallard duck pens for efficient control. *J. Adv. Vet. Anim. Res.* 8(1): 105. <https://doi.org/10.5455/javar.2021.h492>
- Aksoy A, Kahlout KEM El, Yardimci H (2020). Comparative evaluation of the effects of binzalkonium chloride, iodine, gluteraldehyde and hydrogen peroxide disinfectants against avian *Salmonellae* focusing on genotypic resistance pattern of the *Salmonellae* serotypes toward benzalkonium chloride. *Brazilian J. Poult. Sci.* 22. <https://doi.org/10.1590/1806-9061-2019-1055>
- Battersby T, Walsh D, Whyte P, Bolton D (2017). Evaluating and improving terminal hygiene practices on broiler farms to prevent *Campylobacter* cross-contamination between flocks. *Food Microbiol.* 64: 1–6. <https://doi.org/10.1016/j.fm.2016.11.018>
- Belter B, McCarlie SJ, Boucher-van Jaarsveld CE, Bragg RR. (2022). Investigation into the Metabolism of Quaternary Ammonium Compound Disinfectants by Bacteria. *Microbial Drug Resist.* <https://doi.org/10.1089/mdr.2022.0039>
- Boxall NS, Perkins, NR, Marks D, Jones B, Fenwick SG, Davies PR (2003). Free available chlorine in commercial broiler chicken drinking water in New Zealand. *J. Food Protect.* 66(11): 2164–2167. <https://doi.org/10.4315/0362-028X-66.11.2164>
- Brantner CA, Hannah RM, Burans JP, Pope RK (2014). Inactivation and ultrastructure analysis of *Bacillus* spp. and *Clostridium perfringens* spores. *Microscop. Microanaly.* 20(1): 238–244. <https://doi.org/10.1017/S1431927613013949>
- Byun KH, Han SH, Yoon JW, Park SH, Ha SD (2021). Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on *Salmonella* Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. *Food Cont.* 123: 107838. <https://doi.org/10.1016/j.foodcont.2020.107838>
- Castro Burbarelli MF, Valle Polycarpo G, Lelis KD, Granghelli CA, Pinho ACC De, Queiroz SRA, Fernandes AM, Souza RLM De, Moro MEG, Andrade Bordin R de (2017). Cleaning and disinfection programs against *Campylobacter jejuni* for broiler chickens: productive performance, microbiological assessment and characterization. *Poult. Sci.* 96(9): 3188–3198. <https://doi.org/10.3382/ps/pex153>
- Chidambaranathan AS, Balasubramaniam M (2019). Comprehensive review and comparison of the disinfection techniques currently available in the literature. *J. Prosthodont.* 28(2): e849–e856. <https://doi.org/10.1111/jopr.12597>
- Collins ML (2007). The role of intensive poultry production industry in the spread of avian influenza. A report by Compassion in World Farming February.
- Douglas H, Kampf G (2011). Efficacy of three surface disinfectants against spores of *Clostridium difficile* biotype O27. *Elsevier Int. J. Hyg. Environ. Health.* 214: 172–174. <https://doi.org/10.1016/j.ijheh.2010.10.004>
- Drauch V, Ibesich C, Vogl C, Hess M, Hess C (2020). In-vitro testing of bacteriostatic and bactericidal efficacy of commercial disinfectants against *Salmonella* *Infantis* reveals substantial differences between products and bacterial strains. *Int. J. Food Microbiol.* 328: 108660. <https://doi.org/10.1016/j.ijfoodmicro.2020.108660>
- Figueroa A, Hauck R, Saldias-Rodríguez J, Gallardo RA (2017). Combination of quaternary ammonia and glutaraldehyde as a disinfectant against enveloped and non-enveloped viruses. *J. Appl. Poult. Res.* 26(4): 491–497. <https://doi.org/10.3382/japr/pfx021>
- Gómez-García M, Argüello H, Pérez-Pérez L, Vega C, Puente H, Mencía-Ares Ó, Carvajal A (2022). Combined in-vitro and on-farm evaluation of commercial disinfectants used against *Brachyspira hyodysenteriae*. *Porcine Health Manag.* 8(1): 1–8. <https://doi.org/10.1186/s40813-021-00244-9>
- Gosling RJ (2018). A review of cleaning and disinfection studies in farming environments. *Livestock.* 23(5) : 232–237. <https://doi.org/10.12968/live.2018.23.5.232>
- Guastalli BHL, Batista DFA, Souza AIS, Guastalli EAL, Lopes PD, Almeida AM, Prette N, Barbosa FO, Stipp DT, Freitas Neto OC (2016). Evaluation of disinfectants used in pre-chilling water tanks of poultry processing plants. *Brazilian J. Poult. Sci.* : 217–224. <https://doi.org/10.1590/1806-9061-2015-0110>
- Jiang L, Li M, Tang J, Zhao X, Zhang J, Zhu H, Yu X, Li Y, Feng T, Zhang X (2018). Effect of different disinfectants on bacterial aerosol diversity in poultry houses. *Front. Microbiol. Frontiers.* : 2113. <https://doi.org/10.3389/fmicb.2018.02113>
- Kamal MA, Khalaf MA, Ahmed ZAM, Jakee J El (2019). Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. *Vet. World.* 12(12): 20–25. <https://doi.org/10.14202/vetworld.2019.2025-2035>
- Li Y, Arulnathan V, Heidari MD, Pelletier N (2022). Design considerations for net zero energy buildings for intensive, confined poultry production: A review of current insights, knowledge gaps, and future directions. *Renewable Sustain. Energy Rev.* 154: 111874. <https://doi.org/10.1016/j.rser.2021.111874>
- Luyckx K, Dewulf J, Weyenberg S Van, Herman L, Zoons J, Vervaeke E, Heyndrickx M, Reu K De (2015). Comparison of sampling procedures and microbiological and non-microbiological parameters to evaluate cleaning and disinfection in broiler houses. *Poult. Sci.* 94(4): 740–749. <https://doi.org/10.3382/ps/pev019>
- Qiao N, Shao Z (2010). Isolation and characterization of a novel biosurfactant produced by hydrocarbon-degrading bacterium *Alcanivorax dieselolei* B-5. *J. Appl. Microbiol.* 108(4): 1207–1216. <https://doi.org/10.1111/j.1365->

- Ramzi A, Oumokhtar B, Filali Mouatasssem T, Benboubker M, Ouali Lalami A El (2020). Evaluation of antibacterial activity of three quaternary ammonium disinfectants on different germs isolated from the hospital environment. *BioMed research international* 2020Hindawi, <https://doi.org/10.1155/2020/6509740>
- Rhee CH, Kang YE, Han B, Kim YW, Her M, Jeong W, Kim S (2021). Virucidal efficacy of seven active substances in commercial disinfectants used against H9N2 low pathogenic avian influenza virus. *J. Appl. Poult. Res.* 30(4): 100198. <https://doi.org/10.1016/j.japr.2021.100198>
- Saadatpour F, Mohammadipanah F (2022). Enhancement of bactericidal effect of Chlorhexidine using choline augmentation as a natural additive. *American J. Infect. Control.* 50(1): 39–48. <https://doi.org/10.1016/j.ajic.2021.05.012>
- Sato Y, Ishihara M, Nakamura S, Fukuda K, Kuwabara M, Takayama T, Hiruma S, Murakami K, Fujita M, Yokoe H (2019). Comparison of various disinfectants on bactericidal activity under organic matter contaminated environments. *Biocont. Sci.* 24(2): 103–108. <https://doi.org/10.4265/bio.24.103>
- Shoab M, Ashraf I, Chaudhary KM, Talib U, Usman M, Khan AA (2018). Farm and Disease Management by Commercial Poultry Farmers. *Curr. Res. Agric. Sci.* 5(2): 42–47. <https://doi.org/10.18488/journal.68.2018.52.42.47>
- Stringfellow K, Anderson P, Caldwell D, Lee J, Byrd J, McReynolds J, Farnell M (2009). Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions. *Poult. Sci.* 88(6): 1151–1155. <https://doi.org/10.3382/ps.2008-00455>
- Suwa M, Oie S, Furukawa H (2013). Efficacy of disinfectants against naturally occurring and artificially cultivated bacteria. *Biolog. Pharmaceut. Bulletin.* 36(3): 360–363. <https://doi.org/10.1248/bpb.b12-00721>
- Wales AD, Gosling RJ., Bare HL, Davies RH (2021). Disinfectant testing for veterinary and agricultural applications: A review. *Zoon. Pub. Health.*, 68(5): 361–375. <https://doi.org/10.1111/zph.12830>
- Wang S, Sun J, Shan B, Fan W, Ding R, Yang J, Zhao X (2022). Performance of dodecyl dimethyl benzyl ammonium chloride as bactericide and corrosion inhibitor for 7B04 aluminum alloy in an aircraft fuel system. *Arabian J. Chem.* 15(7): 103926. <https://doi.org/10.1016/j.arabjc.2022.103926>
- White D, Gurung S, Zhao D, Farnell Y, Byrd J, McKenzie S, Styles D, Farnell M (2018). Evaluation of layer cage cleaning and disinfection regimens. *J. Appl. Poult. Res.* 27(2): 180–187. <https://doi.org/10.3382/japr/pfx056>