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RESEARCH ARTICLE

Antibiofilm Effect of Commonly Used Chemical Disinfectants on Certain Bacterial Species Isolated from Poultry Abattoirs

Amr M.M. Abd-Elall, Ayman Megahed, Mariam H. El-Bana and Nagwan Gamal

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, 44511, Zagazig, Egypt
Corresponding author: nagwangamal364@gmail.com

Abstract

This study was to assess biofilm formation capacity of Salmonella, E. coli and Staph. aureus isolated from poultry abattoirs. Efficacy of disinfectants against biofilms produced by these bacterial species were also evaluated. Therefore, 100 samples were collected from two poultry slaughterhouses (slaughterhouse 1 located at Belbeis city and slaughterhouse 2 located at Mashtool city) at different localities of Sharkia governorate, Egypt. After that biofilm formation ability of the tested bacterial species was assessed by microtiter plate (MTP) method. The effectiveness of five commercial disinfectants widely used in poultry abattoirs, including hydrogen peroxide, sodium hypochlorite, virkon S, glutaraldehyde, and copper sulphate with different concentrations (1, 2 and 5%) on the removal of biofilms produced by S. Typhimurium, E. coli O2H6, and Staph. aureus after different contact times of 10, 60 and 120 m was also evaluated. Out of 100 collected samples, 10 (10%) of Salmonella: 40 (40%) E. coli and Staph. aureus as 35 (35%) were identified. About 90, 92.5 and 91.4% of the Salmonella, E. coli and Staph. aureus isolates had the ability to produce biofilm. Virkon S (5%) was the most powerful disinfectant which removed 98.6 and 95.7% of S. Typhimurium and E. coli O2H6 biofilms after 120 min contact time, followed by 89.7% of S. aureus biofilm at the same concentration and contact time. Additionally, sodium hypochlorite (5%) for 120 min had a great efficacy and achieved 92.3% reduction of Staph. aureus biofilm. Meanwhile, removed 94.6% and 91% of S. Typhimurium and E. coli O2H6 biofilms. Copper sulphate wasn't a powerful enough disinfectant to eliminate biofilms. It can be inferred that the inhibitory effect of the used disinfectants against biofilms in this study increased with increasing concentration and contact time with biofilms**.**

Key words:

Poultry slaughterhouse, Biofilm, Salmonella, E. coli, Staph aureus, Disinfectant, Hypochlorite.

Introduction

In Egypt, Poultry slaughterhouses face several issues and challenges. One of them is bacterial infection, specifically with *Salmonella*, *Staphylococcus*, and *E. coli*. Moreover, those microbes represent a serious threat to public health and cause substantial economic losses for the poultry production [1].

It had been significantly revealed that Salmonella, Staphylococcus, and E. coli formed a slimy matrix composed of extracellular polymeric substances (EPS) establishing a biofilm [2]. Biofilms are defined as communities of microorganisms that adhere to biotic or abiotic surfaces. Microorganisms have a natural capacity to adhere to moist surfaces, multiply, and incorporate themselves in a slimy matrix formed from extracellular polymeric substances (EPS), establishing a biofilm [3].

Moreover, EPS was responsible for the stability of biofilm and developed resistance to unpleasant environmental conditions such as the host immune system, disinfectants, antibiotics, dehydration, salinity and UV exposure

[4]. The biofilm formed in poultry slaughterhouses had a great significance as it was a permanent source of microbial contamination causing food contamination and endangering consumers' health [5]. There are many reports indicating that the biofilm had a negative impact in poultry slaughterhouses causing food spoilage, outbreak of food borne diseases and enhancing resistance against cleaning and sanitation [5]. Bacterial biofilm promoted chemical and biological reactions causing rusting of metal in pipelines, tanks and concrete & reducing the shelf- life of equipment and buildings [6].

One of the most effective controlling programs against biofilm is using powerful disinfectants. The most common disinfectants used in chicken slaughterhouses are sodium hypochlorite (NaOCl), hydrogen peroxide (H2O2), Verkon S), glutaraldehyde and copper sulphate [7-10]. These disinfectants must be safe, effective, easily handled, leaving no poisonous residues that could badly affect the final products [11]. Furthermore, the broiler slaughter industry produced wastes rich in lipids and protein and accumulated on surfaces promoting the biofilm formation that was frequently a source of public health problems [12]. Thus, effective cleaning and sanitation should be applied before disinfection to prevent the accumulation of microbial cells and particulates on the surface of equipment [13].

This study was to identify the occurrence of biofilm producing bacteria in chicken slaughterhouses in Egypt. Evaluation of their capacity of biofilm formation in vitro. The effectiveness of five disinfectants at various concentrations and exposure times in decreasing the biofilm produced by the tested bacterial strains were also assessed.

Material and methods

Bacteriological isolation of the tested microorganisms.

Investigated poultry slaughterhouses and collected samples

This study was carried out to isolate some bacterial strains from two poultry slaughterhouses located at Sharkia governorate, Egypt.

A total of 100 samples were aseptically obtained from the investigated chicken slaughterhouses including washing water as well as swabs from tables, knives, eviscerated carcasses and worker' s hand. With a minimum of delay, samples were transported in an aseptic manner in an icebox to the laboratory for further investigations.

Sample processing, cultivation and identification

Cotton swabs were incubated in five ml TSB [14]. Five ml of washing water samples are mixed with 225 ml of pre-enrichment broth [15]. The TSB tubes were aerobically incubated at 37 °C for 24 hrs.

Briefly, a loopful of the 24-hour-incubated TSB tubes was inoculated into the surfaces of XLD (Himedia, India), EMB (Himedia, India), and Baird Parker agar (Himedia, India) for the selective isolation of Salmonella, E. coli, and S. aureus, respectively. Incubation conditions, colony features, and biochemical identification were performed [16]. At the Food Analysis Centre, Faculty of Veterinary Medicine, Benha University, Egypt, serotyping of the biochemically identified Salmonellae and E. coli bacteria was carried out.

Invitro production of biofilm by isolated microorganisms.

The microtiter plate method was used to evaluate each bacterial strain's capacity of biofilm production in pure culture. The biofilm formation of Salmonella (n=10),

E. coli (n=40) and Staph. aureus (n=35) isolates were detected by the microtiter plate assay [17] with some modifications.

The bacterial suspension was prepared from freshly grown agar plates of each strain and adjusted to 0.5 McFarland (1.5 x 108 CFU/mL) in Müller-Hinton broth (MHB). About 100 L of bacterial suspension were added to each well of 96 microtiter tissue culture plates, and the plates were then incubated at 37°C for 24 hours. For removal of planktonic cells, each well's liquid media was removed, and the wells were then rinsed with phosphate-buffer saline (PBS) three times. After that and before staining, the created biofilms were fixed by soaking them in 150 µL of ethanol for 15 min, then were stained for 15 min with 150 µL of 0.1% crystal violet.

After rinsing the stained microplate wells three times with PBS to remove the excess stain, the plates were allowed to dry for 30 minutes. Finally, 150 µL of 95% ethanol were added to each well and kept for 15 minutes in order to resolubilize the dyes of biofilms that lined the walls of the microplate. Negative controls were inoculated with 100 µL of sterile MHB, which served as negative controls, whereas positive controls were inoculated with both MHB and bacterial isolates. The experiment was carried out in triplicate. Using a microplate reader, the microplates were spectrophotometrically measured at 570 nm.

The categorization of results was done as no biofilm production (0), weak (+), moderate (+++), and strong (+++ or more) biofilm production using the calculation of cut of value (ODc) shown below [5]:

No biofilm production: OD ≤ ODc; Weak biofilm production: ODc< OD ≤ 2 × ODc; Moderate biofilm production; 2 × ODc< OD ≤ 4 × ODc; Strong biofilm production: 4 × ODc< OD.

The ODc = Average OD of negative control + (3 × standard deviation of negative control).

The OD for each isolate = Average OD of the isolate – ODc.

In vitro antibiofilm assay using chemical disinfectants.

Disinfectants

Five chemical disinfectants with different modes of action, compressing hydrogen peroxide, sodium hypochlorite and Virkon S act by oxidation, glutaraldehyde act by protein cross-linking and copper sulphate acts by protein denaturation, were chosen among those frequently used in Egypt to decontaminate poultry slaughterhouses. At 1, 2, and 5% concentrations and 10, 60, and 120 m contact times, different disinfectants were tested.

Microorganisms

Salmonella typhimurium, E. coli O2:H6, and Staph. aureus were chosen for this study.

Antibiofilm assay

The antibiofilm assay of disinfectants was performed with some modifications [18] and summarized as follows:

The bacterial suspension was prepared from freshly grown agar plates of each strain and adjusted to 0.5 McFarland (1.5 x 108 CFU/mL) in Müller-Hinton broth (MHB). Formation of the biofilm by the tested bacteria was performed as previously described in experiment II. All wells, excluding the blank and positive control wells, were inoculated with 200 µL of each concentration of the tested disinfectants. For each concentration, the plates incubated for different contact times of 10, 60, and 120 m. After incubation period, 200 µL of tween 80 was added to cease the antimicrobial effect of disinfectants. After that, phosphate-buffered saline (PBS) was used to wash the plates multiple times. The

wells were then stained for 15 min with 150 µl of 0.1% crystal violet. After rinsing the stained microplate wells three times with PBS to remove the excess stain, the plates were left to dry for 30 minutes. To resolubilize the dyes of the biofilms that lined the wells of the microplate, 150 µL of 95% ethanol were added to each well and left for 15 min. For each strain, three wells inoculated with MHB only (negative control) and another three wells were treated with bacterial inoculums without treatments (positive control). Lastly, the experiment was carried out in triplicate. Using a microplate reader, the microplates were spectrophotometrically measured at 570 nm. The following equation was used to calculate the biofilm reduction percentages [18]:

Reduction/Removal Percentage = [(C-B) - (T-B) / (C-B)] *100%

Where B: Absorbance of blank (no biofilm, no treatment); C: Absorbance of control (biofilm, no treatment); T: Absorbance of test (biofilm and treatment).

Statistical analysis

All the numerical data were collected and then subjected to arcsine transformation which is typically applied to stabilize the variance of data when dealing with proportions or percentages that are close to 0% or 100%, especially when the data exhibits a binomial mean distribution according to the formula [ASIN(SQRT(A1)] as A1 Represent to the [X/100)]. Then they are tested for normality by the Anderson-Darling test. Statistical analysis was done, using SPSS software (version 16.0; Chicago, USA). The data were expressed as mean ± standard error (SEM). The One-Way ANOVA followed by post hoc "Duncan's test" was done to reveal the significant differences in the reduction (%) of biofilm produced by certain bacterial species after different contact times with modern disinfectants [19].

Results

Regarding the frequency of Salmonella, E. coli, and Staph. aureus found in chicken slaughterhouses in various areas of the Sharkia governorate in Egypt, as well as their capacity to form biofilm (Table 1).

About 10% of the samples taken from poultry abattoirs was positive for Salmonella, 90% of the isolates had the capacity of biofilm production, including 60% of isolates produced strong biofilm and 30% produced weak biofilm after incubation period at 37°C for 24 hours.

E. coli was identified in 40% of samples collected from poultry abattoirs. It was evident that 92.5% of isolates had the capacity to produce biofilm, where 17.5% of isolated E. coli possessed a strong biofilm production ability, 45% possessed moderate ability and 30% possessed weak ability to produce biofilm after incubation at 37°C for 24 hours.

This study showed that 35% of the samples obtained from poultry slaughterhouses contained Staph. aureus. Moreover, 91.4% of isolated Staph. aureus was able to produce biofilm. However, 20% of isolates were strong biofilm producers, 22.9% of isolates were moderate biofilm producers and 48.6% of isolates were strong biofilm former after incubation at 37°C for 24 hours.

S. Typhimurium, E. coli O2:H6, and Staph. aureus were chosen to evaluate the effectiveness of certain common disinfectants used for disinfection of poultry abattoir to remove the biofilms produced in vitro by these strains (Tables 2-4 and Figures 1-3).

Concerning the biofilm produced by S. Typhimurium, virkon S was the most powerful disinfectant against S. Typhimurium when used at concentration of 5% for 120 min. This treatment

achieved 98.6% biofilm reduction. Otherwise, sodium hypochlorite 5% and glutaraldehyde 5% showed a high significant reduction of the biofilm produced by S. Typhimurium with percentages of 94.6 and 90.7% after 120 min contact time. However, a moderate efficacy against the biofilm achieved by the highest concentration and contact time of hydrogen peroxide and copper sulfate with 70% reduction of the biofilm.

On this basis, Virkon S 5% was the most effective disinfectant against E. coli O2:H6 and eliminated 95.7% of the biofilm after 120 min contact time. Furthermore, it was found that sodium hypochlorite (5%), hydrogen peroxide (5%) and glutaraldehyde (5%) showed a great potency against E. coli O2:H6 biofilm and achieved 91, 85.8 and 72.2% biofilm reduction after 120 min contact time. However, copper sulphate with the same concentration and contact time was the less efficient disinfectant against E. coli O2:H6 biofilm, where only 40.3% of biofilm were removed.

Concerning the biofilm produced by Staph. aureus, sodium hypochlorite and Virkon S were the most effective disinfectants against Staph. aureus, where 92.3 and 89.7% of the biofilm used at 5% concentration for 120 min were eliminated. However, hydrogen peroxide (5%), glutaraldehyde (5%) and copper sulphate (5%) for 120 min. had a moderate efficacy against Staph. aureus biofilm, where 69.6, 67.5 and 65.6% of the biofilms, respectively were removed.

Table 1: The degree of biofilm production by the isolated microorganisms from the investigated poultry slaughterhouses.

Microorganism	No of tested isolates	Degree of biofilm production							
		Strong producer		Moderate producer		Weak producer		Total	
		No.	%	No.	%	No.	%	No.	%
Salmonella	10	6	60	3	30	0	0	9	90
E. coli	40	7	17.5	18	45	12	30	37	92.5
Staph. aureus	35	7	20	8	22.9	17	48.6	32	91.4

Table 2: The mean+-Se of the reduction of *S. Typhimurium* biofilm after different contact times with different modern disinfectants.

Disinfectant	Conc. %	Reduction % of biofilm produced by *S. Typhimurium* after different contact times with modern disinfectants.			P- value
		S. Typhimurium			
		10 m	60 m	120 m	
		Mean± SEM	Mean± SEM	Mean± SEM	
Hydrogen Peroxide (H_2O_2) (H_2O_2)	1	12.5±3.81	32.2±1.0969	36.4±2.078*	0.07
	2	23 ±2.36	37.3±1.84	53.7±3.810*	
	5	28.3±1.443	57.4±1.32*	69.7 ±1.385*	
Sodium hypochlorite (NaOCl)	1	49±1.381	64.5±1.90	89.4±1.501*	0.6
	2	71.9±3.0022	72.6±2.0784	90±0.69282*	
	5	75.2±2.07	93.8±1.385*	94.6±1.327*	
Virkon S	1	49.4±1.3279	69±3.233162*	73±1.501111*	0.6
	2	81.5±3.5795*	89.2±3.5795*	94±0.92376*	
	5	83±2.367136	95±2.367136*	98.6±0.6928*	
Glutaraldehyde	1	30±1.501111	39.4±1.6161	52.3±0.9237	0.01*
	2	49.1±1.558846	54.3±1.0969	74.3 ±1.039*	
	5	55.2±1.096	70±2.078*	90.7±2.424*	
Copper Sulphate	1	0±0	10±2.829	22±2.078	0.07
	2	0±0	13.4±1.501	40.6±2.193*	
	5	16.6±2.0784	32.4±3.2331	70.3±0.9237	

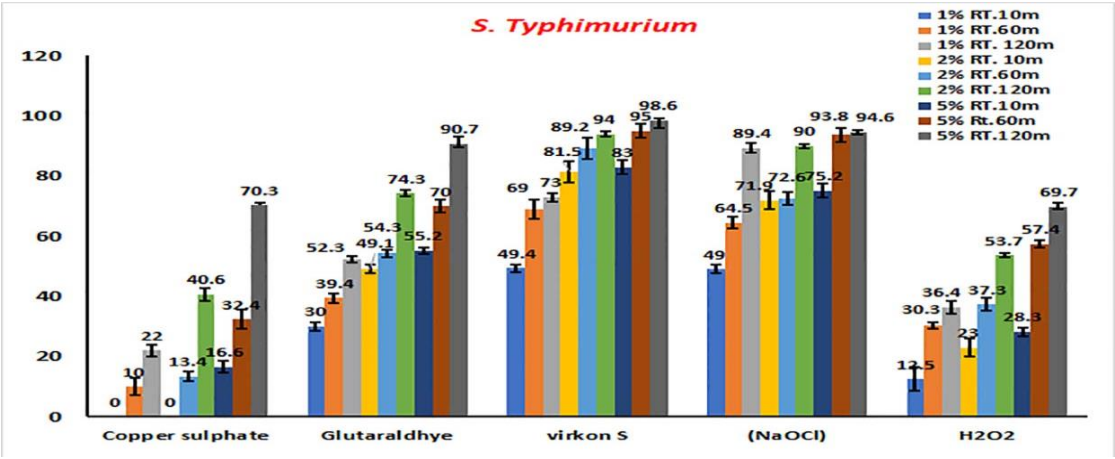

Figure 1: Bar chart showing the reduction % of biofilm produced by *S. Typhimurium* species after different contact times with the different commercial disinfectants. The data are expressed as the mean ±Se; differences are considered significant at p≤ 0.05 (one way a nova test followed by the post Hoc Duncan test).

Table 3: The mean+-Se of the reduction of *E. coli O2:H6* biofilm after different contact times with different modern disinfectants.

Disinfectant	Conc. %	Reduction % of biofilm produced by *E. coli O2:H6* after different contact times with modern disinfectants.			P- value
		E. coli O2:H6			
		10 m	60 m	120 m	
		Mean± SEM	Mean± SEM	Mean± SEM	
Hydrogen Peroxide (H_2O_2) (H_2O_2)	1	42±2.136	50±2.25	66.9±3.11	0.2
	2	63.3±1.38	71.9±1.38	74.3±1.61*	
	5	79.7±0.865*	80.7±2.65*	85.8 ±2.078	
Sodium hypochlorite (NaOCl)	1	55.2±1.09	56.1±3.81	57±3.695	0.2
	2	68.5±4.1569*	81.8±3.6950*	86.7±1.3279*	
	5	77±1.385641*	83.7±3.9837*	91±3.059*	
Virkon S	1	61.5±2.0784*	76.8±1.5011*	85±2.655811*	0.2
	2	65.9±1.6165	79.3±1.0969*	91.8±2.7712*	
	5	76±1.674316*	79.5±1.0969*	95.7±1.3856*	
Glutaraldehyde	1	7±3.233162	15±2.078461	44.3±3.2331	0.001*
	2	25.6±1.9052	40±1.5011	67.2±1.6743	

	5	54.3±1.61	66.7±3.69	72.2±2.77*	
Copper Sulphate	1	0±0	0±0	2±0.40	0.001*
	2	0±0	6.6±1.443	22.4±2.611*	
	5	0±0	7.5±1.3276	40.3±3.7536*	

The data are expressed as the mean ±SEM; differences are considered significant at p≤ 0.05 (one way a nova test followed by the post Hoc Duncan test).

* The subscribed symbols refer to the significant differences between the variable disinfectant related to the concentration of each and the bacterial biofilm reduction percentage.

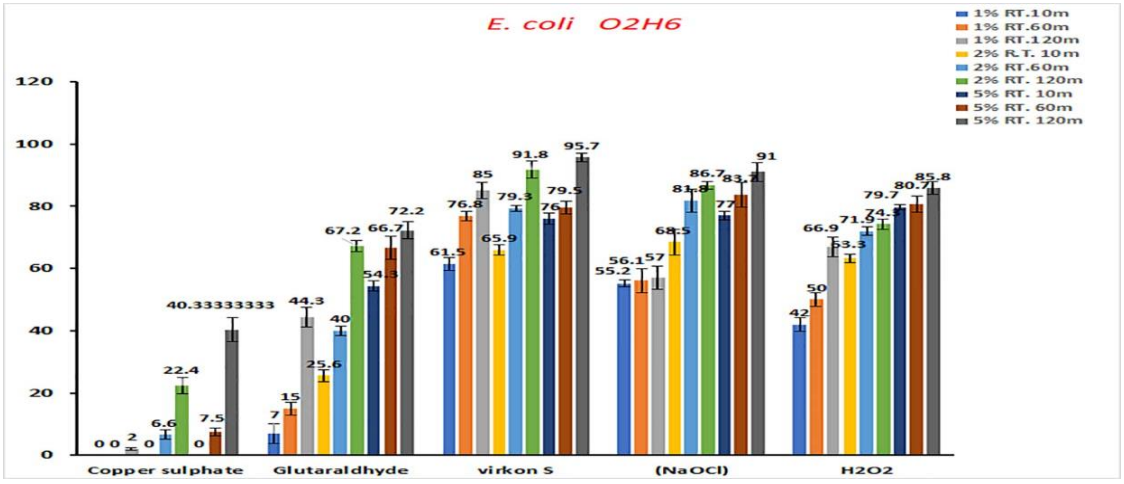

Figure 2: Bar chart showing the reduction % of biofilm produced by *E. coli O2:H6* species after different contact times with the different commercial disinfectants. The data are expressed as the mean ±Se; differences are considered significant at p≤ 0.05 (one way a nova test followed by the post Hoc Duncan test).

Table 4: The mean+-Se of the reduction of *Staph. aureus* biofilm after different contact times with different modern disinfectants.

Disinfectant	Conc. %	Reduction % of biofilm produced by *Staph. aureus* after different contact times with modern disinfectants.			P- value
		Staph. aureus			
		10 m	60 m	120 m	
		Mean± SEM	Mean± SEM	Mean± SEM	
Hydrogen Peroxide (H_2O_2)	1	45.9±1.3	49.6±3.002	53±2.655	0. 5
	2	46±1.21	50±2.136	59.5± 2.13*	
	5	47.4±1.67	57±3.57	69.6± 1.616*	
Sodium hypochlorite (NaOCl)	1	47.7±2.48	53.7±0.750	64.5±2.82*	0.07
	2	48.5±2.4826	68±3.6373*	84.8±1.212*	
	5	69±2.3671*	84.8±1.3856*	92.3±2.3671*	
Virkon S	1	56±2.944486*	67±2.020726*	73.1±0.923*	0.3
	2	60.3±2.4826*	69.3±1.2124*	88±3.002221*	
	5	69.5±1.8475*	89.4±1.5011*	89.7±1.0969*	
Glutaraldehyde	1	37.2±0.923	58.3±3.1176	60±2.713546*	0.5
	2	53.6±1.5011	55.4±3.983	62.3±3.983	
	5	57±3.637	59.1±2.655*	67.5±2.07	
Copper Sulphate	1	15±1.38	28.2±3.290	29.5 ±4.1562	0.01*
	2	27.6 ±1.039	33.4±3.757	45±3.117	
	5	58.9±2.598*	59.3±1.96*	65.6 ±2.771*	

The data are expressed as the mean ±SEM; differences are considered significant at $p \leq 0.05$ (one way a nova test followed by the post Hoc Duncan test).

* The subscribed symbols refer to the significant differences between the variable disinfectant related to the concentration of each and the bacterial biofilm reduction percentage.

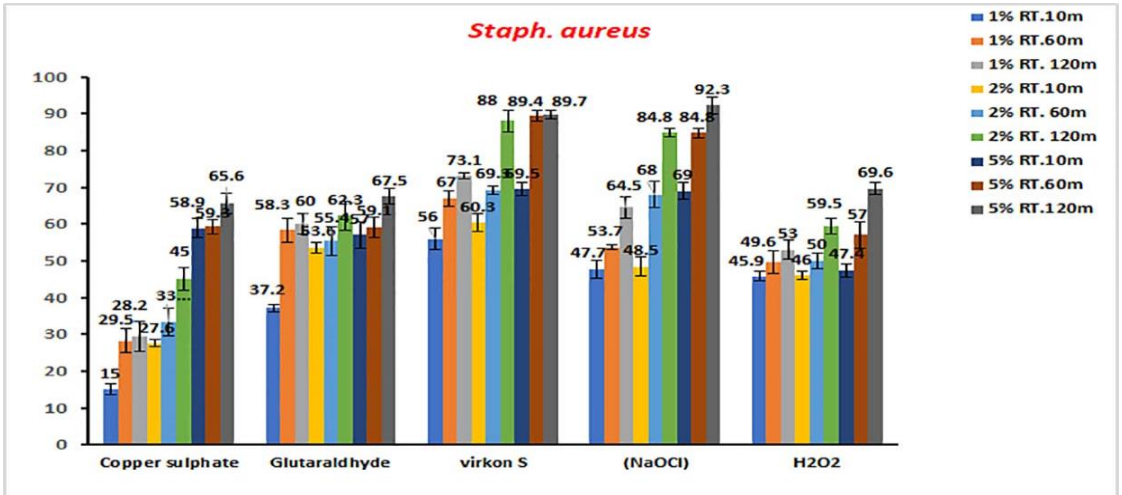

Figure 3: Bar chart showing the reduction (%) of biofilm produced by *Staph. aureus* species after different contact times with different commercial disinfectants. The data are expressed as the mean ±Se; differences are considered significant at $p \leq 0.05$ (one way a nova test followed by the post Hoc Duncan test).

Discussion

Virulence of *Salmonella*, *E. coli* and *Staph. aureus* is attributed to the adhesive properties of biofilms [20]. Biofilms represent a serious threat in poultry slaughterhouses causing food deterioration, biocorrosion damage to equipment and human illness from foodborne diseases [21].

Salmonella spp were detected in 10% of samples collected from the investigated poultry slaughterhouses. About 90% of the isolates had the ability of biofilm production by the microtiter plate method at 37° C for 24 h, where 60% were strong and 30% were moderate biofilm producers. our results were consistent with the other findings [22] who reported that out of 114 of Salmonella isolates recovered from poultry slaughterhouses in northern Malaysia, 69.3% were strong, whereas 30.7% had a moderate biofilm former after incubation at 28°C for 48 hrs. On contrast, our findings disagreed with those who found that 70 and 29% of Salmonella recovered from poultry slaughterhouses in Brazil produced weak and moderate biofilm producers. However, there was no evidence for strong biofilm producer Salmonella [23].

The prevalence of E. coli in the investigated poultry abattoirs was 40 and 92.5% of the isolates had the capacity to produce a biofilm with different extents. About 17.5% of isolates possessed a strong biofilm forming ability, 45% of isolates possessed a moderate ability, while 30% of isolates showed a weak ability. Our finding was nearly similar to who demonstrated that 30, 40 and 30% of E. coli isolates recovered from poultry slaughterhouse in southern Brazil, had strong, moderate and weak biofilm production capacity, respectively, after incubation at 36° C for 24 hrs [2]. In a previous study, 55.8% of E. coli isolates were able to produce biofilm by using TCP assay after incubation at 25° C for 24 hrs [24].

Staph. aureus was found in 35% of samples obtained from the examined poultry slaughterhouses. About 91.4% of isolates had a biofilm production capacity. A strong producer represented 20% of the biofilm-producing Staph. aureus, whereas 22.9% were moderate, whereas 48.6% showed weak producers. Our results were in accordance with a previous study [25] who revealed that 22.22% of Staph. aureus isolates had a strong biofilm former, while 11.11% showed moderate, and 44.44% were weak biofilm former. Our findings contradicted the other findings [26] who recorded that the most of Staph. aureus isolates recovered from poultry slaughterhouses in Nanjing, China hadn't the capacity to produce a biofilm.

In Egypt, the most widespread serovars identified in poultry slaughterhouses were E. coli O2:H6, S. Typhimurium, and Staph. aureus [2, 27]. Additionally, they were mostly implicated in the formation of biofilms [28-30]. A significant risk to human health can be attributed to a high incidence of Salmonella, E. coli and Staph. aureus in meat and on contact surfaces [31]. Therefore, it is crucial to keep coming up with new strategies to reduce the risk of foodborne illnesses in slaughterhouses.

The long-term persistence of bacterial biofilm is prompted by the use of sublethal disinfectant concentrations in poultry slaughterhouses [23]. This requires the appropriate choice of the disinfectant at the recommended concentration and for a proper contact time. so that five commercial disinfectants that are frequently used in poultry abattoirs at concentrations of 1, 2% and 5% were tested in vitro for their capacity to remove S. Typhimurium, E. coli O2:H6, and Staph. aureus biofilms after different contact times (10, 60 and 120 min.).

Efficacy of disinfectants against S. Typhimurium biofilm was showed in Table (2) and Figure (1). Virkon S (5%) was the most powerful disinfectant against S. Typhimurium biofilm after 120 min exposure time. This treatment achieved 98.6% biofilm reduction.

However, sodium hypochlorite (5%) and glutaraldehyde (5%) showed a great reduction of the biofilm produced by S. Typhimurium with percentages of 94.6 and 90.7% after 120 min exposure time. A moderate efficacy against a biofilm achieved by the highest concentration and contact time of hydrogen peroxide and copper sulfate with 70% reduction of biofilm.

Similar results were previously recorded by Balasubramanian et al. [32] who reported that Virkon S (1%) had a high efficacy and achieved complete reduction of 2-days-old Salmonella biofilms after 5 min contact time. Our results were contradicted with Vieira et al. [29] who showed that sodium hypochlorite 1% for 45 min and glutaraldehyde 2% for 60 min had low effectiveness against 2-day old Salmonella typhimurium biofilms. In another study, S. Typhimurium biofilms had a great resistance against copper sulphate due to presence of the Cu-resistance genes [33].

Regarding the efficacy of disinfectants on removing biofilm produced by E. coli O2:H6, Table (3) and Figure (2) showed that Virkon S (5%) was the most effective disinfectant against E. coli O2:H6, where 95.7% of E. coli O2:H6 biofilm were removed after 120 min exposure time. Furthermore, it was found that sodium hypochlorite (5%), hydrogen peroxide (5%) and glutaraldehyde (5%) after 120 min exposure time showed a great potency against E. coli O2:H6 biofilm, where 91, 85.8 and 72.2% biofilm reduction were eliminated, respectively. Copper sulphate with the same concentration and contact time was a lowest efficient disinfectant against E. coli O2H6 biofilm, where only 40.3% of biofilm were removed.

The obtained results were a nearly similar to previous study [32] who demonstrated that Virkon S with a concentration of 4% completely removed 7-day-old E. coli biofilms after 10 min contact time. Lower estimates (65 %) of biofilm reduction of E. coli using sodium hypochlorite at a concentration of 0.25% for 10 min. exposure time were recorded by Günther et al. [34]. The results of this study disagreed with those of another studies [29] who showed that glutaraldehyde (2%) for 60 min. eliminated 100% of 2-day old E. coli biofilm.

Sodium hypochlorite and Virkon S achieved a significant reduction against Staph. aureus biofilm with percentages of 92.3 and 89.7%, when used at a concentration of 5% for 120 min. Hydrogen peroxide (5%), glutaraldehyde (5%) and copper sulphate (5%) for 120 min. showed a moderate efficacy against Staph. aureus biofilm and reduced 69.6, 67.5 and 65.6% of the biofilm, respectively.

Our results were in accordance with previous results of Bayoumi et al. [35] who found that sodium hypochlorite at concentration 250 mg/L showed a significant reduction against 3- day- old Staph. aureus biofilm after 30 s contact time, while it was insufficient to eliminate all biofilm. In another study, the high efficacy of sodium hypochlorite may be attributed to its decomposition into sodium hydroxide and hypochlorite, which is a strong oxidizing agent [36]. Our findings contradicted the findings of Rushdy and Othman [37] who recorded that H2O2 with MIC 3.75% was a highly effective and completely removed 6-day old Staph. aureus biofilm after 20 min contact time.

Conclusion

The current work provided a more information on biofilm formation capacity of Salmonella, E. coli and Staph. aureus isolated from poultry abattoirs. The susceptibility of the biofilm to the various commercial disinfectants was also assessed. Most of the Salmonella, E. coli and Staph. aureus isolates possess the ability of biofilm production ranged from strong to weak. Particularly oxidizing disinfectant (Virkon S, hypochlorite and

peroxide) showed a great efficacy against biofilm especially with increasing concentration (5%) and contact time (120 min.). The study throws a light on the magnitude of spread of biofilm producing bacteria in our poultry slaughterhouses in Egypt. Thus, a continuous controlling program should be adopted to minimize the problem and its complications.

Conflict of Interest

The authors declare no conflict of interest.

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الملخص العربي

تأثير المطهرات الكيميائية الشائعة الاستخدام علي الاغشية الحيوية المنتجة بواسطة بعض انواع البكتريا المعزولة من مجازر الدواجن

عمرو محمد محمد عبد العال وأيمن مجاهد ومريم حسن البنا ونجوان جمال
قسم الصحة العامة البيطرية – كلية الطب البيطري – جامعة الزقازيق- الزقازيق-44511- مصر.

تم إجراء هذه الدراسة لتقييم مدى قدرة ميكروبات السالمونيلا والإيشريشياكولاي والبكتريا العنقودية المعزولة من مجازر الدواجن على إنتاج الغشاء الحيوي معمليا. بالإضافة لذلك فحص مدى قدرة المطهرات الكيميائية شائعة الإستخدام على إزالة الغشاء الحيوى المنتج معمليا تحت تركيزات وأوقات تلامس مختلفة. لهذا الهدف تم تجميع عدد 100 عينة من مجزرين للدواجن التي تم فحصهما بمحافظة الشرقية – مصر. بعد ذلك تم تقييم قدرة البكتريا المختبرة علي إنتاج الشريط الحيوي بطريقة الميكروتيتر. بالإضافة إلى ذلك ، فحص فاعلية خمسة مطهرات شائعة الإستخدام في مجازر الدواجن بما في ذلك بيراوكسيد الهيدروجين ، هيبوكلوريت الصوديوم ، فيركون إس ، جلوتارالدهيد ، وكبريتات النحاس بتركيزات مختلفة (1 ، 2 و 5٪) وأوقات تلامس مختلفة (10 ، 60 ، 120دقيقة) علي إزالة الأغشية الحيوية التي تنتجها S. Typhimurium والإيشريشياكولاي O2:H6 والبكتريا العنقودية. من بين 100 عينة تم جمعها ، تم تحديد 10 (10٪) عزلات سالمونيلا ، و 40 (40٪) عزلة من الايشريشياكولاي وكذلك 35 (35٪) عزلة من البكتريا العنقودية. بالإضافة الي ذلك, وجد أن 90٪ و 92.5٪ و 91.4٪ من عزلات والإيشريشياكولاي والبكتريا العنقودية منتجين للأغشية الحيوية. وقد أظهرت النتائج أن الفيركون إس هو اكثر المطهرات كفاءة حيث أزال 98.6٪ و 95.7٪ من الأغشية الحيوية ل S. Typhimurium ولإيشريشياكولاي O2:H6 عند إستخدامه بتركيز 5٪ لمدة 120 دقيقة بينما أزال 89.7٪ من الغشاء الحيوي للبكتريا العنقودية. بالإضافة إلى ذلك ، فإن هيبوكلوريت الصوديوم 5٪ لمدة 120 دقيقة أظهر فعالية كبيرة وازال92.3٪ من الغشاء الحيوي للبكتريا العنقودية بينما أزالت 94.6٪ و 91٪ من الأغشية الحيوية المنتجه بواسطة S. Typhimurium والإيشريشياكولاي O2:H6. بينما كبريتات النحاس كان أقل المطهرات فعالية للتخلص من الأغشية الحيوية. وقد إتضح أن فعالية المطهرات المستخدمة ضد الاغشية الحيوية تزداد بزيادة تركيزها وزمن ملامستها للأغشية الحيوية.