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Biofilm Production Capacity Exerted by some Bacterial Pathogens Recovered from Poultry Farms in Egypt with a Trial of Control Using Chemical Disinfectants

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Abstract

Microbial biofilm is one of the most serious problems facing poultry farms all over the world and especially in Egypt. *Salmonella*, *E. coli* and *S. aureus* were the highest implicated bacteria in biofilm formation in poultry farms. Consequently, 440 samples were collected from 8- broiler and 8-layer farms at El- Sharkia Province, Egypt, during the period from (July 2021 till August 2022). The objective of the study was to evaluate biofilm development capacity of the tested bacterial species by the microtiter plate (MTP) assay. Also, the efficacy of five disinfectants commonly used in poultry farms (Sodium hypochlorite, hydrogen peroxide, Virkon S, glutaraldehyde and copper sulphate) with different concentrations (1, 2 and 5%) and different contact times (10, 60 and 120 m) on reducing the biofilms produced by *S.* Enteritidis, *E. coli* O78 and *S. aureus* was estimated. Results showed that out of 440 collected samples, 17 (3.8%), 200(45.5%) and 66 (15%) strains were identified as *Salmonella*, *E. coli* and *S. aureus*, respectively. 88.2%, 92% and 87.8% of the isolates of *Salmonella*, *E. coli* and *S. aureus* were biofilm producers. The most effective disinfectant was sodium hypochlorite which eliminated the biofilms of *S.* Enteritidis and *E. coli* O78 when used at concentration 5% for 120 m while 5% for 60 m against *S. aureus* biofilm. Additionally, hydrogen peroxide showed great efficiency and complete removal of biofilm of *S.* Enteritidis when used at concentration 2% for 120m and 5%f or 120 m against *S. aureus* biofilm, meanwhile removed 91% of *E. coli* O78 biofilm when used at concentration of 5% for 120 m. However, Copper Sulphate was insufficient disinfectant to be used against the biofilms. It can be concluded that the anti-biofilm efficiency of the disinfectants increases with the increase concentration and contact time with biofilms especially when using oxidizing disinfectants (hypochlorite and peroxides).

KEYWORDS
Biofilm, *Salmonella*, Disinfectant, Hypochlorite, Hydrogen peroxide

INTRODUCTION

In Egypt, the most important economic and animal protein sources is poultry production. Additionally, the most essential amino acids for humans, vitamins and minerals are found in chicken meat and eggs (Farrell, 2013). Poultry farms face numerous issues and difficulties. Infection with different bacteria, such as *Salmonella*, *Staphylococcus* and *E. coli*, is one of these issues. Furthermore, those microbes have a negative impact on public health and cause significant financial losses for the poultry production (Youssef *et al*., 2019).

Salmonella, *E. coli* and *S. aureus* were the most significant cause of food poisoning (WHO, 2018). Furthermore, there are many reports indicating that these microbes have serious problems in poultry farms, where *Salmonella* caused serious problems in poultry such as decreased egg production, profuse watery diarrhea (Tariq *et al*., 2022). *Salmonella* poses a public health concern due to its emergence/reemergence and high mutation rate (Jassim and Limoges, 2017). While the high prevalence of *E. coli* causing fatal diseases in poultry farms such as respiratory distress, loss of appetite, reduction of weight gain, closed eyes and cyanosis (Barnes, 1994). Moreover, the prevalence of *S. aureus* causing fatal diseases in poultry farms such as causing reduction of egg production, lameness.

There are many reports regarding the production of slimy matrix composed of extracellular polymeric substances (EPS) forming a biofilm by *Salmonella*, *Staphylococcus* and *E. coli* (Rodrigues *et al*., 2010). Bacterial biofilm is exopolymer-based matrices that include microbial aggregates and connected to either biotic or abiotic surfaces (Gutierrez and Bonnassie, 1995).

Furthermore, biofilm formation protects bacterial cells from unfavorable environmental conditions such as high temperatures, variations in pH, salinity, UV radiations, desiccation, disinfectants, antibiotics, shear forces, starvation and the host's immune defence mechanisms. Additionally, it is 10–1,000 times more resistant to disinfectants than its planktonic form (Sheffield and Crippen, 2012). Moreover, the biofilm is the main cause of persistence of pathogens in poultry farms causing increasing in the spreading of diseases (Barnes, 1994).

A greater understanding of the serious effect of bacterial biofilm is required for the development of effective control strategies such as using effective disinfectants. The most common disinfectants used in poultry farms are sodium hypochlorite (NaOCl) (Ismail *et al*., 2019), hydrogen peroxide (H_2O_2) (Marques

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et al., 2007), verkon s (Elsayed *et al*., 2020), glutaraldehyde (Günther *et al*., 2017) and copper sulphate (Sallami *et al*., 2022). These disinfectants must be safe, effective, easily used and not leaving any toxic residues (Arnold and Silvers, 2000). Additionally, the appropriate concentration of a disinfectant should be used. Each effective disinfectant should be tested before application against various bacterial strains and under conditions that are similar to those found in poultry farms.

The objective of the current study was to investigate the prevalence of biofilm producing bacteria in poultry farms in Egypt as well as their power to produce biofilms *In vitro*. Finally, evaluation of the efficacy of five disinfectants at different concentrations and contact times in reducing the biofilm produced by the tested bacterial strains.

MATERIALS AND METHODS

Investigated farms and collected samples

The current study was conducted to isolate some bacterial strains from different type poultry farms (8 broiler and 8 layer farms) located at different aspects of Sharkia governorate, Egypt. Samples were collected almost during the period from July 2021till August 2022.

A total of 440 samples were aseptically collected from the farms under investigation. These samples were collected from litter as well swabs from water troughs, feeders, eggshell, cloaca, and worker's hands. Samples were transported aseptically in an icebox to the laboratory for further investigations with minimum delay.

Sample processing, cultivation, and identification

One gram of each litter sample was thoroughly mixed with 9 ml of TSB in a sterile mortar, then the filtrate was aseptically collected in a sterile falcon tube (Thermo Fisher Scientific, UK). Cotton swab was directly incubated in 5 ml TSB (Weese *et al*., 2004). Finally, the TSB tubes were aerobically incubated at 37°C for 24 h.

Briefly, a loopful of the 24 h TSB tubes was streaked on the surface of XLD (Himedia, India), EMB (Himedia, India) and Baird parker agar (Himedia, India) for selective isolation of *Salmonella*, *E. coli* and *S. aureus*, respectively. Incubation conditions, colony characters as well as biochemical identification were carried according to Quinn *et al*. (1994). Serological identification of the biochemically identified *Salmonella* and *E. coli* strains was performed at the Food Analysis Center, Faculty of Veterinary medicine, Benha University, Egypt.

Invitro production of biofilm by the isolated microorganisms.

The tissue culture plate method was used to assess each bacterial strain's ability to produce biofilm in pure culture. The biofilm production of *Salmonella* (17 isolates), *E. coli* (50 isolates) and *S. aureus* (66 isolates) was determined by the TCP assay (Nair *et al*., 2015) with some modifications.

From fresh overnight cultured agar plates of each strain, the bacterial suspension was prepared in Müller- Hinton broth (MHB) and adjusted to 0.5 McFarland ($1.5x10^8$ CFU/mL). About 100 µL of bacterial suspensions was inoculated into each well of 96 microtiter tissue culture plates in triplicate then incubated at 37°C for 24 h. After that, the liquid media from each well was removed and the wells were washed three times by phosphate-buffer saline (PBS) to remove planktonic cells. After that, the produced biofilms were fixed before staining by submerging them in 150 µL of ethanol for 15 min, then were stained with 150 µl of 0.1% crystal violet for 15 min.

After that, the excess stain was removed by washing the stained microplate wells three times with PBS and then the plates were kept for 30 min till dryness. Finally,150 µL of 95 % ethanol were added to each well and kept for 15 min for resolubilizing the dyes of biofilms that lined the walls of the microplate. Blanks were only inoculated by 100 µL of sterile MHB, which were called negative controls while positive ones were inoculated by MHB and bacterial isolates. The experiment was performed in triplicate. The microplates were spectrophotometrically measured by a microplate reader at 570 nm.

To interpret results, categorization was done as no biofilm production (0), weak (+), moderate (++), and strong biofilm production (+++ or more) by the calculation of cut of value (ODc) shown below according to Stepanović *et al*. (2004):

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 disinfectants among that commonly used for disinfection of poultry farms in Egypt were selected to carry out this experiment: including hydrogen peroxide, sodium hypochlorite, Virkon S, glutaraldehyde and copper sulphate. The different disinfectants were challenged at concentrations of 1, 2 and 5% and contact time of 10, 60 and 120 m.

Microorganisms

S. Enteritidis, *E. coli* O78 and *S. aureus* were selected to be investigated in this study.

Antibiofilm assay

The antibiofilm assay of disinfectants was done with some modifications according to Abidi *et al*. (2014) and summarized as follow; from the fresh overnight cultured agar plates of each strain, the bacterial suspension was prepared in Müller-Hinton broth (MHB) and adjusted to 0.5 McFarland ($1.5x10^8$ CFU/mL). Production of the biofilm by the test microorganisms was carried out as previously explained in experiment II. 200µL of each concentration of tested disinfectants was transferred into each well except blank and positive control wells. The plates were incubated for different contact times (10 m, 60 m and 120 m) for each concentration. After incubation period, 200 µLof tween 80 was added at the end of each contact time to stop the antimicrobial action of disinfectants. Then, the plates were washed several times with phosphate-buffered saline (PBS). Subsequently, the wells were stained with 150 µl of 0.1% crystal violet for 15 min. The excess stain was removed by washing the stained microplate wells three times with PBS and then the plates were kept for 30 min till dryness. 150 µl of 95 % ethanol were added to each well and kept for 15 min for resolubilizing the dyes of biofilms that lined the walls of the microplate. For each strain, three wells were inoculated with bacterial inoculums without treatments (positive control) and another three wells treated with MHB only (negative control). Finally, the experiment was performed in triplicate. The

microplates were spectrophotometrically measured by a microplate reader at 570 nm. The reduction percentages of the biofilm were calculated by using the following equation according to Abidi *et al.* (2014):

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

Generalized linear mixed models were produced form reduced to full models to select the best model that fit the data to construct a series of statistical models describing how type of disinfectants with three different concentrations (1%, 2% and 5%) at three time points (10 m, 60 m, and 120 m) can affect biofilm reduction %. Restricted maximum likelihood (REML) was used for parameter estimation. Summaries for model objects fitted with lmer list standard errors and t-statistics for the fixed effects. Coefficient estimates for parameters and corresponding standard errors and p-values were reported. Adjusted tukey's test was run to test significance of differences between pairs of groups. Statistical analysis performed with R version 4.3.0 using lme4 package.

RESULTS

Regarding incidence rates of *Salmonella*, *E. coli* and *S. aureus* recovered from the poultry farms in different localities of Sharkia governorate, Egypt and their ability to produce biofilm (Table 1). *Salmonella* was identified in 3.8% (17/440) of samples collected from poultry farms and 88.2% (15/17) were able to produce biofilm, where 41.2% (7/17) were moderate and 47% (8/17) were weak biofilm producer.

E. coli was detected in 45.5% (200/440) of samples collected from poultry farms. Out of 50 isolates of *E. coli* tested for biofilm production, 92% (46/50) were able to produce biofilm, where 16% (8/50), 32% (16/50) and 44% (22/50) were strong, moderate and weak biofilm producers, respectively.

Results showed that *S. aureus* was detected in 15% (66/440) of samples collected from poultry farms. Out of 66 isolates of *S. aureus* tested for biofilm production, 87.8% (58/66) were able to produce biofilm, where 16.7% (11/66) and 71.2% (47/66) were moderate and weak biofilm producers, respectively.

S. Enteritidis, *E. coli* O78, and *S. aureus* were selected to test

Table 1. Degree of biofilm production by the isolated microorganisms from poultry farms.

Microorganisms	No of tested isolates	Degree of biofilm Production							
		Strong producer		Moderate producer		Weak producer		Total	
		No.	%	No.	%	No.	%	No.	%
Salmonellae	17	0	0	7	41.2	8	47	15	88.2
E. coli	50	8	16	16	32	22	44	46	92
S. aureus	66	0	0	11	16.7	47	71.2	58	87.8

Table 2. Estimated marginal means of disinfectants, concentration, and time for biofilm reduction produced by *S.* Enteritidis.

Time (Minutes)	Disinfectant Conc.	Hydrogen peroxide (H_2O_2)	Sodium hypochlorite (NaOCL)	Virkon S	Glutaraldehyde	Copper sulphate
10	1%	38.6	36.7	23.4	36.6	-11.8
60		55.9	54	40.7	53.9	5.5
120		71.2	69.3	56	69.2	20.8
10	2%	66.2	64.3	51	64.2	15.8
60		83.5	81.6	68.3	81.5	33.1
120		98.8	96.9	83.6	96.8	48.4
10	5%	78.8	76.9	63.5	76.8	28.3
60		96.1	94.2	80.8	94.1	45.6
120		111.3	109.5	96.1	109.3	60.9

Table 3. Estimated marginal means of disinfectants, concentration, and time for biofilm reduction produced by *E. coli* O78.

Time (Minutes)	Disinfectant Conc.	Hydrogen peroxide (H_2O_2)	Sodium hypochlorite (NaOCL)	Virkon S	Glutaraldehyde	Copper sulphate
10	1%	36.28	54.98	33.43	12.08	-11.78
60		48.96	67.66	46.12	25.49	0.91
120		61.88	80.85	59.04	38.42	13.83
10	2%	53.29	71.99	50.44	29.82	5.23
60		65.98	84.68	63.13	42.51	17.92
120		78.89	97.59	76.05	55.43	30.84
10	5%	66.38	85.08	63.53	42.91	18.32
60		79.06	97.76	76.22	55.59	31.01
120		91.98	110.68	89.14	68.52	43.93

the efficacy of some popular disinfectant used as farm disinfectants to eliminate the produced biofilms by these strains *In vitro* (Tables 2-4).

Concerning the biofilm produced by *S.* Enteritidis, by considering the copper sulphate as the reference disinfectant (unpublished data), hydrogen peroxide was the most effective disinfectant against *S.* Enteritidis and eliminated the biofilm when used at concentration of 2% and 5% for 120 min. This treatment achieved estimated margin 98.8 and 111.3 (100%) biofilm reduction, respectively. Moreover, sodium hypochlorite 5% for 120 min and glutaraldehyde 2% and 5% for 120 min eliminated one-day old biofilm of *S.* Enteritidis with estimated margin 109.5 (100%), 96.8 (100%) and 109.3 (100%) for biofilm production, respectively. Otherwise, virkon s showed a high efficiency against biofilm of *S.* Enteritidis but not eliminated it when used at concentration 5% for 120 min. this treatment achieved estimated margin 96.1 (90%) for biofilm reduction. However, copper sulphate 5% for 120 min showed estimated margin 60.9 (77.8%) for biofilm reduction.

On this basis, by considering the copper sulphate as the reference disinfectant (unpublished data), sodium hypochlorite was the most effective disinfectant against *E. coli* O78 and completely eliminated the biofilm when used at concentration of 5% for 120 min. this treatment achieved estimated margin 110.68 (100%) biofilm reduction. Furthermore, it was found that hydrogen peroxide 5% and virkon s 5% after 120 min contact time showed a high significant reduction of the biofilm produced by *E. coli* O78 with estimated margin 91.98 (91%) and 89.14 (88%), respectively. However, the highest concentration of copper sulphate and glutaraldehyde at concentration of 5% after 120 min contact time had the lowest biofilm reduction with estimated margin 68.52 and 43.93, respectively.

Concerning the biofilm produced by *S. aureus*, by considering the copper sulphate as the reference disinfectant, sodium hypochlorite was the most effective disinfectant against *S. aureus* when used at concentration of 5% for 60-120 min. this treatment achieved estimated margin 101.3-105.7 (100%) biofilm reduction. Otherwise, hydrogen peroxide 5% and virkon s 5% after 120 min contact time showed a high significant reduction of the biofilm produced by *S. aureus* with estimated margin 97.7 (100%) and 83.7 (91.4%), respectively. However, glutaraldehyde 5% and copper sulphate 5% showed lower efficiency in biofilm reduction of *S. aureus* after 120 min contact time with estimated margin 75.8 (70%) and58.5 (61%), respectively.

DISCUSSION

Biofilms produced by certain bacteria can adhere to surfaces in poultry farms and represent a serious issue and a high risk. Bacteria can release from biofilm matrix and colonize new surfaces as well as be transmitted to animals, poultry, and human (Semenyuk *et al*., 2014).

Salmonella organism was detected in 17 out of 440 examined samples from investigated poultry farms with an overall incidence (3.8%). Most of *Salmonella* isolates were able to produce biofilm with varying degrees (88.2%) ranged from moderate (41.2%) to weak (41.2%) producers. Díez-García *et al*. (2012) found that all 96 isolates of *Salmonella* were able to produce the biofilm by using the microtiter plate method and that nearly matches our findings. However, De Oliveira *et al*. (2014) recorded only 65.5% of the isolated *Salmonella* can produce biofilms. The obtained results can affirm that *Salmonella* spp. in poultry farms is among the popular biofilm produces.

An overall incidence of *E. coli* in the examined farms was 45.5% (200 out of 440 samples). A marked higher percentage (92%) of the tested *E. coli* were positive biofilm producers with varied degrees. 16% of the biofilm producing *E. coli* was strong, 32% were moderate and 44% were weak producers. Our finding is similar to those of Sharan *et al*. (2023) who demonstrated that out of 46 isolates of *E. coli* collected from layer farms in Ludhiana, Punjab, 17.4% (8/36) were strong, 8.7% (4/36) were moderate, and 43.8% (16/36) were weak biofilm former by using the microtiter plate method at 37°C for 48 h. Also, Wang *et al*. (2016) obtained similar records to that of ours. The present study as well as previous ones emphasize that *E. coli* is one of the very important biofilm formers in poultry industry.

S. aureus was detected in 15% (66/440) of samples collected from poultry farms with biofilm producing capacity in 87.8% of the examined samples. Moderate *S. aureus* biofilm production was shown in 16.7% (11/66), while weak capacity was shown in 71.2% (47/66) of the examined samples. The results of the study agreed with those of Rodrigues *et al*. (2010) who reported that most *S. aureus* isolates nearly 66.6% (8/12) were weak biofilm producer. However, in another study, 94.02% (63/67) of *S. aureus* isolates isolated from poultry farms in Egypt were biofilm producers, where 13.43% (9/67), 17.91% (12/67) and 62.68% (42/67) of the isolates were weak, moderate, and strong biofilm producers (Erfan and Marouf 2015). Instead of the difference in literatures regarding the biofilm producing capacity of *S. aureus* recovered from poultry farms but also throws light on its important role in complicating the problem in poultry farms.

The efficacy of disinfectants to control biofilm producing bacteria is surly dependent on concentration and contact time. Fraise (2008) reported that the effective disinfectant is that should eliminate 99.99% of the biofilm after its contact time with biofilm producing bacteria. This necessitates the proper selection of the disinfectant at the recommended concentration and for correct contact time. For the reason, five commercial disinfectants among that commonly used for decontamination process in poultry farms were put in challenge with *S.* Enteritidis, *E. coli* O78 and *S. aureus in vitro* to test their power on removal of the aforementioned microbes.

By considering the copper sulphate as the reference disin-

Table 4. Estimated marginal means of disinfectants, concentration, and time for biofilm reduction produced by *S. aureus*.

Time (Minutes)	Disinfectant Conc.	Hydrogen peroxide (H_2O_2)	Sodium hypochlorite (NaOCL)	Virkon S	Glutaraldehyde	Copper sulphate
10m		59.3	67.3	45.2	37.4	20.1
60m	1%	71.3	79.3	57.2	49.4	32
120m		75.7	83.7	61.6	53.8	36.4
10m		68.6	76.5	54.5	46.6	29.3
60m	2%	80.5	88.5	66.5	58.6	41.3
120m		84.9	92.9	70.9	63	45.7
10m		81.4	89.3	67.3	59.4	42.1
60m	5%	93.3	101.3	79.3	71.4	54.1
120m		97.7	105.7	83.7	75.8	58.5

fectant (unpublished statistical data), hydrogen peroxide was the most effective disinfectant against *S.* Enteritidis and eliminated the biofilm when used at concentration of 2% and 5% for 120 min (Table 2). This treatment achieved estimated margin 98.8 and 111.3 (100%) biofilm reduction, respectively. The great power of H2O2 on biofilm removal produced by *S.* Enteritidis in this study was collided to that of Marin *et al*. (2009) who showed that hydroxide peroxide with concentration 1% removed only 1.2% of *Salmonella* biofilm indicating that it had very low efficiency against *Salmonella* biofilm. De Carvalho (2007) attributed the high efficiency of hydrogen peroxide to the production of free radicals, which greatly affect the biofilms matrix.

Moreover, sodium hypochlorite 5% for 120 min and glutaraldehyde 2% and 5% for 120 min were eliminated one-day old biofilm of *S.* Enteritidis with estimated margin 109.5 (100%), 96.8 (100%) and 109.3 (100%) for biofilm production, respectively. Otherwise, virkon s showed a high efficiency against biofilm of *S.* Enteritidis but not eliminated it when used at concentration 5% for 120 min. Our finding is similar to those of Rodrigues *et al*. (2011) who recorded that sodium hypochlorite with low concentration as 3.125 mg /ml was highly efficient for eradication of 1- day old S. Enterica biofilm. Meanwhile, Marin *et al*. (2009) recorded that glutaraldehyde with concentration 1% removed only30% of *Salmonella* biofilm indicating that it was insufficient for elimination of *Salmonella* biofilm. In this study $CuSo_4$ was the least efficient disinfectant against *S.* Enteritidis and this may be due to most *Salmonella* isolates were resistant to the presence of the Cu-resistance genes which resulted from the excessive use of copper as a disinfectant and in poultry feed (Mustafa *et al*., 2021).

Regarding the efficacy of disinfectants on destroying the biofilm produced by *E. coli* O78 (Table 3), sodium hypochlorite was the most effective disinfectant against *E. coli* O78 and eliminated the biofilm when used at concentration of 5% for 120 min. this treatment achieved estimated margin 110.68 (100%) biofilm reduction. Furthermore, it was found that hydrogen peroxide 5% and virkon s 5% after 120 min contact time showed a high significant reduction of the biofilm produced by *E. coli* O78 with estimated margin 91.98 (91%) and 89.14 (88%), respectively. However, the highest concentration of copper sulphate and glutaraldehyde at concentration of 5% after 120 min contact time had the lowest biofilm reduction with estimated margin 68.52 and 43.93, respectively.

The obtained results are similar to those of Vieira *et al*. (2005) who recorded that sodium hypochlorite 1% completely eliminated biofilm of *E. coli* after 45 min contact time. However, our results are disagreed with those of Günther *et al*. (2017) who recorded that SHC at a concentration of 0.25% for 10 m contact time eliminated only 65% of the biofilm of *E. coli* indicating that sodium hypochlorite was effective but couldn't eliminate all pathogens in biofilms. Additionally, our finding is similar to those recorded by Balasubramanian *et al*. (2021) which showed that that virkon s with concentration 0.1% had low efficiency while virkon s with concentration 4% completely eliminated 7-day-old biofilm of *E. coli*. the biofilms indicating that the efficiency of virkon s increased when the concentration increased. In general, the variations between this study and other previous ones regarding the efficiency of disinfectants on removal of biofilm produced by *E. coli* arise due to the difference in used protocols, age of colony, concentration of disinfectant and contact time.

Efficacy of disinfectants on destroying biofilm produced by *S. aureus* (Table 4) showed that sodium hypochlorite was the most effective disinfectant against *S. aureus* when used at concentration of 5% for 60-120 min. with achieved estimated margin 101.3-105.7 (100%) biofilm reduction. Also, hydrogen peroxide 5% and virkon s 5% after 120 min contact time showed a high significant reduction of the biofilm produced by *S. aureus* with estimated margin 97.7 (100%) and 83.7 (91.4%), respectively. However, glutaraldehyde 5% and copper sulphate 5% showed lower efficiency in biofilm reduction of *S. aureus* after 120 min contact time with estimated margin 75.8 (70%) and58.5 (61%), respectively.

High efficiency (76.1%) of sodium hypochlorite at concentration 1% for 30 min. contact time on removal of one-day old biofilm of *S. aureus* was reported by Köse and Yapar (2017). Similarly, Rushdy and Othman (2011) found that H_2O_2 with MIC 3.75% was highly effective and eliminated *S. aureus* biofilm. Meanwhile, Köse and Yapar (2017) showed that hydrogen peroxide with concentration 5% eliminated 70% and 80.3% of one-day old *S. aureus* biofilm after 1 and 60 min., contact time. Reduction of *S. aureus* biofilm (96.9%) was recorded by treatment with virkon s 2% for 20 min was recorded by Elsayed *et al*. (2020). Lower estimates (44 %) of biofilm reduction of *S. aureus* using glutaraldehyde at a concentration of 1 % for 10 min. contact time were recorded by Günther *et al*. (2017).

CONCLUSION

Biofilm forming bacteria occur in poultry farms worldwide. Most of the bacterial isolates possess the ability for biofilm production which ranged from strong to weak producers. Chemical disinfectants from different categories (hypochlorite, peroxides, glutaraldehyde, and copper sulphate) show varied degrees of biofilm reduction at different concentrations and contact times. Particularly, oxidizing disinfectants are able to remove biofilms especially at higher concentration and contact time.

CONFLICT OF INTEREST

The authors declare that they have no conflct of interest.

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