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Evaluation of the bactericidal effect of some disinfectants against *E. coli* and salmonella isolated from poultry hatcheries

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ABSTRACT

Poultry production considers one of the most important activities for economy and income in many countries. Proper management and hygiene in poultry hatchery mostly depend on efficient cleaning and sanitation of the hatchery environment and hatching eggs for production high quality newly hatched chicks. The aim of our study was to evaluate the effectiveness of some disinfectants (Aquavinol® 5%, Presept® 2.5%, Poviment® and MM8 ®) at various concentrations 1%, 1.5% and 2% at different contact times 30, 60, 90 and 120 minutes against *E.coli a*nd salmonella which were isolated from the poultry hatcheries at a titer of 3×10^6/cm^2. Our results showed that Aquavinol® 5% was the highest powerful disinfectant against these pathogens, followed by Presept® 2.5% and then Poviment®, while MM8® was the weakest disinfectant at the same conditions. Our study concluded that the prevention and control of infections depend on the disinfectant of choice that must be applied with sufficient concentration at proper exposure time to achieve the best powerful effect on microorganisms with low costs

1. INTRODUCTION

The proper sanitation of poultry hatcheries and usage of highly efficient disinfectants are important for the success in commercial avian hatcheries (Moustafa *et al.,* 2009). High efficiency of sanitation following good cleaning and hygienic maintenance are highly essential for avian production and for minimizing the dissemination of infectious diseases (Lazarov *et al*., 2018). Evaluation the efficiency of some disinfectants on bacterial population indicated that different constituents of disinfectants give negative influence on the total bacterial loads (Aygun *et al.,* 2011; Darmus, 2012).

A sanitation and bio-security program should be improved in all areas, personnel, equipment, vehicles at each working point and applying disinfectants in bactericidal concentrations such as quaternary ammonium compounds, peroxides, glutaraldehyde, phenolics, chlorine, or formaldehyde (Deeba *et al.,* 2003; Haynes and Smith 2003; Ledoux and Lines 2003; OIE 2008). A proper disinfectant must be highly effective against microbes, more resistant to environmental conditions. It must also be highly biodegradable, not toxic to live tissues, odourless and relatively cheap (Olesiak and Stepniak 2012).

There are different factors that may influence the disinfectants efficiency such as number of microorganisms, temperature, exposure time, pH, kind of surfaces material, concentration of disinfectants (Desoky, 2008) as well as organic matters *(*Stringfellow *et al., 2009).* It was found that *Salmonella typhimurium* was highly affected by aldekol (QAC and Glutaraldehyde) (Youseif *et al*., 2001).

Salmonella species may come to avian flocks from several sources, e.g., from the vectors, feed, environment, and due to the absence of effective biosecurity and hygienic measures (Soria *et al.,* 2017). Salmonella species are the most important cause of many dangerous diseases in avian and other poultry species involved in high economic losses in the poultry industry led to reduction in the poultry production through chicken mortality and illness (Hameed *et al.,* 2014). Salmonellosis does not just lead to chronic and acute diseases in avian flocks, but the diseased birds are considered one of the major important reservoirs for a different serovars of Salmonella continuously transferred to humans (Wang *et al.,* 2020).

Colibacillosis in poultry acts as one of the primary causes of chicks' mortality and morbidity accompanied with high economic losses in the avian industry (Gholami-ahangaran *et al.,* 2016). Before that *E. coli* had been considered as kind of the major continuously identified microorganism included the risk of omphalitis (infection of the yolk sac) (Amer *et al.,* 2017). The objective of this study was to evaluate the effectiveness of some disinfectants (Aquavinol®5%, Presept®2.5%, Poviment® and MM8®) at various concentrations 1% ,1.5% and 2% at different contact times 30, 60, 90 and 120 minutes against *E. coli* and salmonella which were isolated from the poultry hatcheries at a titer of 3×10^6 / cm^2 to identify the most powerful disinfectant.

2. MATERIAL AND METHODS

2.1. Preparation of isolated bacterial strains according to Sutton *et al. (*2002)

Escherichia coli and salmonella were isolated from poultry hatcheries and then were identified serologically after that were prepared by Seed-Lots systems (seed-lot culture

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maintenance techniques) to obtain two working suspensions of concentration $3x10^6$ CFU/0.1 mL suspension through*:*

2.1.1. The tested strains grown on Lauryl Sulphate broth at 35 °C for 24 hours.

2.1.2. Sterile Phosphate buffer with pH 7.2 was used for making heavy test suspensions via inoculating it in harvested microbial suspensions (with sterile swab or loop).

2.1.3. The suspensions were measured via making serial dilutions then plate counts were done using Eosin Methylene Blue agar plates and Xylose Lysine Deoxycholate agar plates (XLD) which are suitable for each microorganism and choose suspensions of concentration 3×10^6 CFU/ 0.1 mL as working suspensions.

2.2. Preparation of tested disinfectants according to Linton et al. (1987)

2.2.1. Tested disinfectants:

2.2.1.1. Aquavinol® 5%: Manufactured by Aqua chemicals Egypt. Composition: 6% phenolic crystals and 40% coal tar oils.

2.2.1.2. Presept® 2.5%: Marketed by Advanced sterilization products (a Johnson and Johnson company). Composition: Tablets each contain 2.5 gm sodium dichloroisocyanurate (NaDCC).

2.2.1.3. Poviment®: Manufactured by: SFT (Sabsabi for trading). Composition: each 1000 ml contain PVP iodine 30000 mg and Ment.

2.2.1.4. MM8®: Manufactured by: SFT (Sabsabi for trading). Composition: contain per ml 125 mg Quaternary ammonium compound, 50 mg glutaraldehyde, 130 mg isopropanol and 3 mg pine oil.

Tested Disinfectants (Aquavinol® 5%, Presept® 2.5%, Poviment® and MM8®) were prepared to obtain the final dilutions by using USP purified water at pH 5-7 from sterilized tap water.

2.2.2. Antibacterial effectiveness test:

The tested disinfectants were diluted to 90% of used concentrations with known volumes of previously settled bacterial suspensions during the test acts as a matter of challenge for accounting the dilution error and difference during the actual situation of bactericidal agents preparation. Finally, Four commercially available disinfectants were applied in vitro at various concentrations used were 1%, 1.5% and 2% within various contact times of 30, 60, 90 and 120 minutes on contaminated area by *E. coli* **(**O91: H21 strain) and S. *typhimurium* at a titer of $3\times10^6/cm^2$ to evaluate the effectiveness of used disinfectants against these *pathogens.*

2.3. The Surface challenge test was performed in vitro:

This test was applied according to Clontz (2008).

Accurately, large squares (20 cm × 20 cm) of the surfaces area were used for application of these disinfectants at various dilutions at temperature of 30°C. 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. after application of each prepared disinfectant at intervals of 30, 60, 90 and 120 minutes, 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 37 °C for 24 hours. 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.

3. RESULTS

Table 1 showed that Aquavinol® 5% succeeded in completely reducing the number of tested *E.coli* when used at 1% ,1.5% and 2% concentrations by 100% at all concentrations within 120 minutes followed by Presept® 2.5% reduced the bacterial count of tested *E.coli* when used at 1% , 1.5% and 2% concentrations by 99.9% ,100% and 100% respectively within 120 minutes and then Poviment® reduced the number of tested *E.coli* when used at 1% ,1.5% and 2% concentrations by 97.2% ,99.4% and 100% respectively within 120 minutes finally MM8® reduced the bacterial count of tested *E.coli* at 1% ,1.5% and 2% concentrations by 90.3% , 98% and 99.7%, respectively within 120 minutes .

Table 2 showed that Aquavinol® 5% succeeded in completely reducing the number of tested salmonella when used at 1%, 1.5% and 2% concentrations by 99.9%,100% and100%, respectively within 120 minutes. This is followed by Presept® 2.5% which reduced the bacterial count of tested salmonella when used at 1% ,1.5% and 2% concentrations by 96.8%, 98.5% and 100%, respectively within 120 minutes and then Poviment® reduced the number of tested salmonella when used at 1% ,1.5% and 2% concentrations by 82% ,96.7% and 99.7% respectively within 120 minutes. Finally, MM8® reduced the number of tested salmonella when used at 1% ,1.5% and 2% concentrations by 71%, 82.3% and 98.9%, respectively within 120 minutes

Table 3 showed that Aquavinol® 5%, Presept® 2.5%, Poviment® and MM8® were more effective against *E. coli* than salmonella at 1% ,1.5% and 2% concentrations within 120 minutes. on the other hand, Aquavinol® 5% was the highest powerful disinfectant against these pathogens followed by Presept® 2.5% and then Poviment®, while MM8® was the weakest disinfectant against the same microorganisms at the same conditions.

4. DISCUSSION

Sanitation process is an important point in hatchery management, it is necessary for controlling of diseases and improvement quality of newly hatched chicks.

Our results indicated that Aquavinol® 5% succeeded in completely eliminating *E. coli* by 100% when used at all concentrations within 120 minutes and maximally eliminated salmonella organisms when used at 1% ,1.5% and 2% concentrations by 99.9%,100% and 100%, respectively within 120 minutes. On the other hand, MM8® couldn't completely eliminate the tested microorganisms at 1% ,1.5% and 2% concentrations within 120 minutes but only minimized the bacterial count. Aquavinol® 5% was the most effective disinfectant against *E. coli* and the salmonella organisms followed by Presept® 2.5% and then Poviment , while MM8® was the weakest one. This result agreed with that of Metawea, (2000) who reported that commercial phenolic compounds disinfectants have the

highest effect against tested microorganism. It also agreed with those of Lyutskanov *et al.* (2010) who found that the most efficiency was achieved with application of Sanifort with 0.025% solution and sodium hydroxide with 2% solution (99% sodium dichloroisocyanurate dihydrate) as well as the treatment by Dezinfect-B with 3% solution that including 1.6% iodine with contact times of one hour. The results were are consistent with Mclaren *et al.* (2011) who stated that phenolic compounds disinfectants are more consistently effective and with Ramesh *et al.* (2002) who showed that the salmonella organisms could be influenced and completely eliminated by 0.05% concentration of sodium hypochlorite. On the other hand, this result disagrees with those of Kamel, (2016) who indicated that Quaternary ammonium compounds combined with glutaraldehyde (Virudox-G) have powerful effect against *S. typhimurium* but phenolic crystals (phenodex) have lowest effect against the same microorganism, The result also disagrees with those of Nehal Alm Eldin, (2019) who stated that potassium peroxy monosulphate (BioVX) is highly effective compound against *Salmonella typhimurium*, while (iodine) Biodine is low effective against the same pathogen at the same conditions. As well as the results disagreed with that of Moustafa *et al.* (2009) who reported that (Quaternary ammonium compound and glutaraldehyde) and Per-acetic acid proved their effectiveness in controlling the contamination of poultry hatchery and may be applied as good alternatives for formaldehyde and with Youseif *et al.* (2001) who found that *Salmonella typhimurium* was highly affected by aldekol (QAC and Glutaradehyde).

Table 1 Efficacy of some Disinfectants against E. *coli at a titer* of 3×10^6 / cm^2 at different concentrations.

		90 min. (R %)		120 min. (R %)	
Disinfectants	Conc. %	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*
Aquavinol® 5%	1%	98.7	97.2	100	99.9
	1.5%	99.9	99.5	100	100
	2%	100	97.3	100	100
Presept® 2.5%	1%	95.3	79.3	99.9	96.8
	1.5%	99.8	91.0	100	98.5
	2%	99.5	96.9	100	100
Poviment®	1%	75.7	67.7	97.2	82.0
	1.5%	88.3	72.7	99.4	96.7
	2%	97.3	92.7	100	99.7
MM8®	1%	63.2	56.7	90.3	71.0
	1.5%	73.7	67.7	98.0	82.3
	2%	91.7	84.7	99.7	98.9

Table 2 Efficacy of some Disinfectants against salmonella *at a titer* 3×10^6/cm^2 at different concentrations.

Disinfectants	Conc. %	30 min.	R %	60 min.	R %	90 min.	R %	120 min.	R %
Aquavinol® 5%	1%	1.3×10^6	56.7	5.1×10^5	83.0	3.8×10^4	98.7	-	100
	1.5%	9.4×10^5	68.7	2.3×10^5	92.3	8.7×10^3	99.9	-	100
	2%	3.7×10^5	87.7	5.6×10^4	98.1	-	100	-	100
Presept® 2.5%	1%	1.8×10^6	40.0	8.9×10^5	70.3	1.4×10^5	95.3	3.3×10^3	99.9
	1.5%	1.2×10^6	60.0	5.8×10^5	80.7	9.2×10^4	99.8	-	100
	2%	8.4×10^5	72.0	2.0×10^5	93.3	1.4×10^4	99.5	-	100
Poviment®	1%	2.2×10^6	26.7	1.4×10^6	53.3	7.3×10^5	75.7	8.2×10^4	97.2
	1.5%	1.5×10^6	50.0	9.1×10^5	69.7	3.5×10^5	88.3	1.9×10^4	99.4
	2%	1.0×10^6	66.7	6.3×10^5	79.0	8.0×10^4	97.3	-	100
MM8®	1%	2.6×10^6	13.3	1.9×10^6	36.7	1.1×10^6	63.2	2.9×10^5	90.3
	1.5%	2.3×10^6	23.3	1.1×10^6	63.3	7.9×10^5	73.7	6.0×10^4	98.0
	2%	1.8×10^6	40.0	9.4×10^5	68.7	2.5×10^5	91.7	9.7×10^3	99.7

Table 3 Efficacy of some Disinfectants against E. *coli* and salmonella isolated from poultry hatcheries *at a titer* 3×10^6 / cm^2 at contact times 90 , 120 minutes.

Disinfectants	Conc. %	30 min.	R %	60 min.	R %	90 min.	R %	120 min.	R %
Aquavinol® 5%	1%	1.6×10^6	46.7	9.3×10^5	69.0	8.5×10^4	97.2	3.1×10^3	99.9
	1.5%	1.0×10^6	67.7	6.7×10^5	77.7	1.4×10^4	99.5	-	100
	2%	6.4×10^5	78.7	1.8×10^5	94.0	7.9×10^4	97.3	-	100
Presept® 2.5%	1%	1.9×10^6	36.7	1.1×10^6	63.3	6.2×10^5	79.3	9.6×10^4	96.8
	1.5%	1.4×10^6	53.3	9.2×10^5	69.3	2.7×10^5	91.0	4.5×10^4	98.5
	2%	9.9×10^5	67.0	5.0×10^5	83.3	9.1×10^4	96.9	-	100
Poviment®	1%	2.4×10^6	20.0	1.7×10^6	43.3	1.0×10^6	67.7	5.4×10^5	82.0
	1.5%	1.9×10^6	36.7	1.3×10^6	56.7	8.2×10^5	72.7	9.7×10^4	96.7
	2%	1.2×10^6	60.0	9.8×10^5	67.3	2.2×10^5	92.7	8.0×10^3	99.7
MM8®	1%	2.8×10^6	6.7	2.3×10^6	23.3	1.3×10^6	56.7	8.7×10^5	71.0
	1.5%	2.5×10^6	16.7	1.8×10^6	40.0	1.0×10^6	67.7	5.3×10^5	82.3
	2%	2.0×10^6	33.3	1.1×10^6	63.3	4.6×10^5	84.7	3.1×10^4	98.9

R% (Reduction rate)

Moreover, the results disagreed with Metawea, (2003) who demonstrated that Quaternary ammonium compounds combined with glutaraldehyde (virocid, TH4+) were more efficient available commercial disinfectant.

This disagreement might be due to antimicrobial activity of glutaraldehyde that is highly pH dependent so there is variation in their efficiency in the field (Mclaren *et al.*, 2011) and disinfectants which derived from halogens have high efficacy in minimizing of salmonella at every conditions and with (Ramesh *et al.*, 2002) and Organic matter couldn't affect microbicidal activities of phenolic disinfectants and Quat's (Dosoky *et al.*, 2000) Moreover, the bactericidal activity of whole disinfectants was affected by increasing their concentrations and longer contact times according to Desoky (2008).

5. CONCULOSIONS

In conclusion, highly efficient cleaning and sanitation is considered as an essential plan for success of working process inside poultry hatcheries, so that effective disinfectants should be perfectly selected and applied with proper concentrations and suitable contact times to control environmental contamination and achieve high production.

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