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Effect of Aldehyde-Quaternary Ammonium Compound Based Disinfectant on Hatching Parameters of Embryonated Chicken Egg Contaminated with *E. Coli*

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ABSTRACT

E .coli is one of the most important pathogens that causes embryonic death and reduces hatchability that causing severe economic losses in poultry production. *E. coli* is resistant to some commonly used antibiotics in poultry, so we must have other approaches to control *E. coli*. The aim of this study is to investigate the effectiveness of glutaraldehyde (ALDEKOL GDA) disinfectant on *E. coli* O78 experimentally contaminating specific pathogen free embryonated chicken egg (SPF ECE) eggshell. In this study apply the antibiotic susceptibility of the identified isolate to know antibiotic *E coli* resistance. 150 fertile SPF embryonated chicken egg were divided into five equal groups (30 eggs per each group) Group number (1) be non-infected non-treated (control negative), while group number (2) considered non-infected treated (treatment control). Group (3) infected non-treated group, and those of groups 4 and 5 were direct wet spray disinfected using (Aldekol GDA®) one time for group (4), while group (5) was re-sprayed twice. All egg groups were candled for dead in shell embryos which were subjected to bacterial examination for reisolation of *E. coli* from egg shell, liver and yolk sac. Microbiological examination was carried out on live and dead in egg shell, liver and yolk sac. The obtained results, Resistance were detected to 17 of the 26 antibiotics commonly used in poultry. Results demonstrated that total bacterial counts were reduced as a result of treatment with Aldehyde – QAC based disinfectant. infected group 5 (infected and disinfected twice) showed highest hatchability (93.3%) followed by group 4 (infected and disinfected once) which showed 73.3% hatchability while group 3 (infected non treated group) showed the lowest hatchability (10%), group 2 hatchability was not affected. disinfection power increased against *E .coli* O78 when using disinfection more than one time. Reisolation of *E. coli* from group 3 egg shell, yolk sac and liver was the highest followed by group 4 that treated with disinfectant once while group 5 was the lowest. Conclusively ALDEKOL GDA disinfectant displays a high rate of hatchability and reduces embryonic mortalities and production of high quality chicks. Hatchability improved when ALDEKOL GDA disinfectant re-sprayed twice. ALDEKOL GDA was effective disinfectant, as reduce total bacterial counts and eliminate *E. coli* from fertile egg.

Keywords: ALDEKOL GDA, *E. coli*, Hatchability, disinfectant

1. Introduction

Contamination of commercial fertile egg is one of the most important problems that causing severe economic losses in poultry production. Fertile eggs may become contaminated by contaminated droppings either right away after they are laid or later during farm collection or by contaminated egg shell in the hatchery or from infected hatched check. Bacterial infections occurs worldwide causing enormous economic losses to the poultry production (Nhung *et al.,* 2017). *E. coli*

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is one of the most important poultry pathogens that causing embryonic death when contaminating fertile egg (Zohair, 2011 and Nolan *et al.,* 2020).

The bacteria which found in poultry dropping material may penetrate egg shell and the membrane because of the vacuum effect subsequent from the loss of heat after the lay, or as a result of unfavorable storage conditions and time, and may spread inside the egg's contents **(**Wang and Slavik, 1998, Barnes *et al.,* 2003). Microbial contamination of hatching eggs reduces hatchability and chick quality, so it is a important concern of poultry producers performing the greatest standards of hygiene in hatcheries to minimize the bacterial count (Cadirci, 2009).

Many researchers had tested alkaline or acidic disinfectant compounds for hatching eggs (Hafez *et al.,* 1991 and Scott, *et al.,* 1993). Glutaldehyde alone is less effective in the presence of organic matter, so the most disinfectants have a combination between glutaraldehyde and QACs. It looks like ALDEKOL. (McDonnell and Russell, 2001) stated that glutaraldehyde and QACs have a great activity against bacteria and their spores, fungi and viruses.

Many approaches are used to eliminate or prevent growth of *E. coli* and/or other bacterial pathogenic agents contaminating fertile egg shell including disinfectant (Motola *et al.,* 2020). So, the aim of this study is to evaluate the efficacy of well known disinfectant glutaraldehyde (ALDEKOL GDA) on experimentally *E. coli* infected egg shell simulating field condition.

2. Material and Methods

2.1. Fertile Eggs

This study was conducted on 160 specific pathogen free embryonated chicken egg (SPF ECE). Ten eggs were randomly chosen, tested and farther examined for the freedom of any contamination then the remaining 150 was divided into five equal groups 30 egg in each.

2.2. Disinfectant used

Aldekol GDA® :It consist of glutaraldehyde 24.8% - Quaternary Ammonium combination 2.4%, which is composed of the active ingredients Aldehyde / QACS combined was used as fine direct spray on eggs in concentration of 0.5% in distilled water as recommended by the producer.

2.3. Pathogenic bacteria

E. coli O78 strain isolated and identified by Amer *et al.* (2020) was used for shell contamination.

2.4. Antibiotic susceptibility test

Applying the disc agar diffusion test, the antibiotic susceptibility of the identified isolates was established according to (Watts, 2008 and CLSI, 2016). Single and similar colonies on solid media plate were mixed in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard. The Muller Hinton agar plates, measuring 9 cm in diameter, were streaked with the bacterial suspension using sterile swab sticks and rotating the plate to confirm even distribution. The inoculated plates were left to dry at room temperature for 10 minutes and then The agar's surface was covered with antibiotic discs. The plates were left for the pre-diffusion time at room temperature before aerobic incubation at 37°C for 16-18 hours. The isolates' growth inhibition zones were categorized as sensitive, moderate, and resistant, with measurements taken to the closest millimeter based on (CLSI, 2016).

Bacterial culture preparation

From enriched *E. coli* 24 hrs broth culture (Oxoid®) prepared in a 1.0×10^8 cfu/ml L flasks, incubated overnight at 37°C and prepare a working broth with cellular density equivalent to MacFarlan tube 0.5 (10^8 cfu/ml) (Motola *et al.,* 2020).

2.5. Disinfection of the eggs

Glutaraldehyde/ Quaternary ammonium chloride combination (Aldekol GDA®) was used in concentration of 0.5 ml/liter of sterile distilled water and sprayed directly on treated egg groups.

2.6. Experimental design

A total number of 150 fertile SPF embryonated chicken eggs were divided into five equal groups (30 eggs per each group) as showed in table (1) all SPF eggs were examined at seven days of age for check viability and 10 days of embryonated life treatment per each group takes place as follow:

Group (1) be non-infected non-treated (control negative), while group (2) considered non-infected treated (treatment control). Three groups (90 eggs) were held overnight at 37°C, and dipped for 5 minutes in the *E. coli* working broth culture with the same temperature. The infected eggs were drained at 37°C for 2 hours, and then divided into 3 groups 30 eggs per each; group (3) infected non-treated group, and those of groups (4) and (5) were direct wet spray disinfected using (Aldekol GDA®) one time for group (4), while group (5) was re-sprayed twice once as group (4) and the second time 2 hours later. Ten minutes after treatment all egg groups were separately incubated at 37.5°C with daily observation. Starting from second day post treatment till hatching all egg groups were candled for dead in shell embryos which were subjected for bacterial examination for reisolation of *E. coli* from egg shell, liver and yolk sac.

Table 1: Showing management of experimental groups

Group	Infection with *E. coli* (dipping)	Treatment with disinfectant	
		Once	Twice
1	-	-	-
2	-	√	-
3	√	-	-
4	√	√	-
5	√	√	√

2.7. Microbiological analysis

Three eggs from each group were utilized to test the contaminated eggs, including the negative control group, for the presence of *E. coli* immediately 5 min and 10 min after treatment. The edible parts (yolk + albumen) and the shells (together with the membrane) were both subjected to the tests.

Next, using sterile tools to create a tiny hole, the egg was transferred using a sterile pipette to a sterile sampling bag. The membrane and the remaining egg shell were put in a separate sampling bag. Each group's samples were thoroughly mixed by crashing, and then 10 g of samples were homogenized 1: 10 (w/v) with sterile physiological saline to create a 1: 10 solution that was then serially diluted up 10^{-8}. MacConkey Agar was used to inoculate the dilutions. Biochemical and serological testing were used to identify the typical colonies after incubation (Anon, 1995; Harrigan, 1998). The same microbiological examination was carried out on dead in shell embryo liver, shell membrane and egg yolk.

2.8. Reisolation of pathogenic *E. coli*

All egg groups were incubated at 37°C followed by daily candling to detect non-fertile, died embryo. At the hatching time hatched chicks, died in shell each group were cultured to detect the presence of *E. coli* in liver, yolk sac, lung and heart.

3. Results and Discussion

Breeding houses in Egypt are either furnished with deep litter systems or with slatted floors in addition to deep litter systems (Bhadhauria, 2016). Al-Shammari *et al.* (2015) found that bacteria and spoilage organisms on the eggshell in deep litter systems had fifteen times more than battery cage systems and twenty to thirty times more bacteria on the egg shell than slatted floor houses. So a sanitizing agent is required to prevent chick infection. In the present study we found *E. coli* isolate is resistant to some commonly used antibiotics in poultry as shown in table (2), Resistance was detected to 17 of the 26 antibiotics commonly used in poultry Similar results were reported by (Guerra *et al.,* 2003 and Gyles, 2008). This gives rise to great need to have other approaches that control *E.coli* in order to prevent this pathogenic organism rather than treatment failure due to resistance of this

microorganism. Thus, Disinfectants have an important role to prevent the severity of contamination of hatching eggs with antibiotic resistant *E. coli*.

Table 2: Showing antibiogram of *E.coli* O78

Antibiotics	Zone of inhibition (mm)	Result
Ciprofloxacin (CIP) 5	30	S
Neomycin (N) 30	17	S
Kanamycin(K) 30	15	S
Colistinsulphate(CT) 10	12	S
DoxycyclineHydrochloride(DO) 30	9	R
Colistin (CL) 10	17	S
Enrofloxacin (Ex) 5	30	S
Sipramycin (SP) 100	7	R
Norfloxacin (NX) 10	27	S
Gentamycin (GEN) 10	16	S
Apramycin (APR) 15	15	R
Amikacin (AK) 30	19	S
Amoxicillin (AML) 10	No Zone	R
Strepomycin(S) 10	No Zone	R
Cefotaxime/ Clavulanic acid (CE) 30	No Zone	R
Cefotaxime(CTX)30	No Zone	R
Ceftazidime/ clavulanic acid(CZC) 40	No Zone	R
Metronidazole(MTZ)5	No Zone	R
Amoxicillin(AMP)10	No Zone	R
Clindamycin(DA)2	No Zone	R
Flurofenicol(FFC)30	No Zone	R
Sulphamethizole (SM)100	No Zone	R
Tetracycline(T)30	No Zone	R
Ampicillin(AM)10	No Zone	R
Erythromycin(E)15	No Zone	R
Trimethoprime/Sulohamethaxozole(SXT)25	No Zone	R

Where **(S) means **Sensitive** and **(R)** means **Resistant**

Poultry producers are particularly concerned about microbial contamination of hatching eggs because it results in poor hatchability and poor performance of one day old chicks (Willinghan *et al.,*

1996; Fasenko *et al.,* 2009). Therefore, good hatching egg sanitization is essential to attain a high rate of hatchability and guarantee the production of high quality chicks. In this study, Results demonstrated in table (3) showed that total bacterial counts were reduced as a result of treatment with Aldehyde – QAC based disinfectant in comparison with the bacterial count in both infected and infected no-treated group, this could be explained in that severe bacterial contamination and subsequent growth can result in diminished hatchability, poor chick quality, and higher mortality if hatching eggs are not sanitized before incubation, this was matched with (Youseif *et al.,* 2001 and Nasr *et al* 2018) who found that Aldekol® (glutaraldehyde and QACs) was very effective on gram negative pathogenic bacteria.

Table 3: Total bacterial count from egg shell swabs post treatment by disinfectant

Dilutions	Total bacterial count in group 3 after 5 minutes	Total bacterial count in group 3 after 10 minutes	Total bacterial count in group 4 after 5 minutes	Total bacterial count in group 4 after 5 minutes
5	>300	>300	>300	260
6	>300	>300	300	240
7	>300	>300	240	200
8	>300	>300	200	160

Table (4) showed that control negative group 1 hatchability was not affected which indicate good experimental condition, on the other hand infected group 5 (infected and disinfected twice) showed highest hatchability (93.3%) followed by group 4 (infected and disinfected once) which showed 73.3% hatchability while group 3 (infected non treated group) showed the lowest hatchability (10%), group 2 hatchability was not affected which indicated that disinfectant with the used dilution rate has no negative impact on hatchability this results was parallel with (Youseif *et al.,* 2001 and Hrnčár *et al.,* 2021) who found that using Aldehyde – QAC based disinfectant combination has no negative impact on hatchability. Improved hatchability which reached 28 (93.3 %) in infected - Aldekol GDA treated twice (Gr 5) as compared with 10% in infected non-treated group (Gr 3) as illustrated in table (4) and (5) this may be due to disinfection power induced by used disinfectant against *E. coli*, this results was matched with (Youseif *et al.,* 2001 and Hrnčár *et al.,* 2021) who found that Aldehyde – QAC based disinfectant has great disinfection power against pathogenic gram negative bacteria.

Table 4: Hatchability % in different groups

Groups	No. of live chicks	No. of dead in shell	Hatchability %
1	30	zero	100%
2	30	zero	100%
3	3	27	10 %
4	22	8	73.3 %
5	28	2	93.3 %

Disinfection twice improves hatchability about 20% this also could be due to disinfection power of used disinfection when used twice rather than one time this results was parallel with results found by (Jabbar *et al.,* 2020). who stated that disinfection power increased against pathogenic bacteria when using disinfection more than one time. On the other hand it was found that hatchability was severely affected in group infected with *E. coli* (10%) this may be due to negative effect of *E. coli* in hatchability, this was parallel with (Nolan *et al.,* 2020).

Table 5: Embryonic mortalities in SPF ECE experimentally infected with E. coli and disinfected with ALDEKOL GDA

Groups	Dead in shell embryonated chicken egg			Total mortality	Mortality%
	Early	Med	Late		
1	0	0	0	0	0%
2	0	0	0	0	0%
3	20	7	0	27	90%
4	4	4	0	8	73.3%
5	0	2	0	2	93.3 %

Rapid egg sanitization eliminated bacteria contaminating egg shell's surface before they could enter through egg shell pores. Egg shell permeability should be considered in our concept when choosing any method and time of egg disinfection. According to (Oviasogie *et al.,* 2016), hatching eggs are highly susceptible to exposure to a wide variety of microorganisms, including fungus and bacteria, which could ultimately result in economic losses (Bailey *et al.,* 1998; Kim *et al.,* 2007). *Escherichia coli, Salmonella spp., Pseudomonas, Aspergillus* and *Staphylococcus* are a significant pathogen groups that have been found to be prevalent in hatch cabinets in significant quantities (Berrang *et al.,* 1995) and Kizerwetter-Świda & Binek, 2008).

Table (6) showed that Isolation of *E. coli* from group 3 egg shell, yolk sac and liver was the highest followed by group 4 that treated with disinfectant once while group 5 was the lowest, this may be due to effect of disinfectant on *E. coli* this also was parallel with (Nasr *et al* 2018) who found that aldehyde combined with QAC strongly disinfectant *E .coli* and eliminate its hazard effect. Isolation of *E. coli* from different parts of fertile eggs in group 3 indicate pathogenicity of used microorganism in current experiment for embryonated chicken egg, this results was parallel with (Mousa-Balabel *et al.,* 2017) who found that entero pathogenic *E. coli* causing embryo mortalities and was re-isolated from dead- in shell embryos.

Table 6: Bacterial isolation from SPF-ECE from different groups from dead in shell embryo and post hatching

Groups	Organs	No. of positive samples for isolation of *E.coli*
1	Egg shell	Zero
	Yolk sac	Zero
	Liver	Zero
2	Egg shell	Zero
	Yolk sac	Zero
	Liver	Zero
3	egg shell	30
	yolk sac	30
	liver	30
4	Egg shell	8
	Yolk sac	8
	Liver	8
5	Egg shell	1
	Yolk sac	2
	Liver	2

4. Conclusion

From these results, it could be concluded that, ALDEKOL GDA disinfectants display a high rate of hatchability and reduce embryonic mortalities and production of high quality chicks. Hatchability improved when ALDEKOL GDA disinfectants re-sprayed twice. ALDEKOL GDA were effective disinfectants, as reduce total bacterial counts and eliminate *E. coli* from fertile egg taking in consideration that this study must be complemented in further experiment with other parameters includes microbial counts, and post hatching studies in terms of microbial trials for re-isolation of tested pathogens from all groups under investigation to ensure health and survival.

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Author's Contribution

All authors equally participated in design, experimental procedure, writing, revised, and reviewing the manuscript.

Ethical approval

The ECE experiments were carried out following the standard procedures of animal care and handling and under approval of the Medical Research Ethical Committee (MREC) at the National Research Centre, Dokki, Cairo, Egypt with registration no; 24712012022.

Conflict of interest

The authors have declared no conflict of interest.

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