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(RESEARCH ARTICLE)

Prevalence of *E. coli serovars* in broiler farms: Biosecurity and the disinfectants sensitivity in Egypt

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Abstract

This study was conducted to evaluate the degree of biosecurity level with especial reference to *E. coli Spp* as an example to explain the expected causes and risk factors that leads to spread them in poultry flocks in Egypt and to evaluate its sensitivity to most common disinfectants used in Egypt. About 300 samples (100 cloacal swabs, 100 liver and intestinal samples,100 litter samples) were collected from 10 broiler farms with different age (at 0 old day, one week,2,4 and 6 weeks of age). The samples were investigated for *E. coli Spp* and subsequently identified based on biochemical and serological tests. The obtained results showed that 44 isolates were isolated (27±1.99%) ;(11±0.42%) and (7±0.72%) from cloacal swab; liver and litter, respectively. Mean prevalence of *E. coli spp.* was 15±1.22%. *E. coli* serotypes were: O78 (31.81%), O2: H6 (18.18), O1: H7 (15.9), O91: H21(11.36), O128: H2 (9.09), O26: H11(4.54) O146: H21, O124, O44: H18 and O153: H2. The most common serovars were O124, O44: H18 and O153: H2 (2.27%). In absence of organic matter; there was great statistical significant difference in the sensitivity of *E. coli* to the most common disinfectants*(P<0.05)* as Verkon- S® achieved 3 log reduction after 5-minute,Formalin and Phenique were achieved 4 log and 3log reductions respectively , Aldekol Des- Gda® achieved 4 log reduction, TH4®,Biosentry® 904 and Iodophore achieved 2 log reductions after 5 minutes.

Keywords: Broiler farms; *Escherichia coli*; Serovars; Biosecurity; Disinfection.

1. Introduction

The species *E. coli* includes a wide variety of strains, some of which may be responsible for severe infections. However, *E. coli* can become pathogenic through the acquisition of mobile genetic elements such as bacteriophages, pathogenicity islands, and plasmids. Among pathogenic *E. coli* strains are the Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains. The safety concern about foods of poultry origin increased in recent years because of the growing number of human infections with Shiga toxin-producing *Escherichia coli*. These infections result in illnesses such as mild diarrhoea, bloody diarrhoea, haemorrhagic colitis, and haemolytic uremic syndrome.

E. coli is a member of the family *Enterobacteriaceae*, which may, constitute a great hazard to poultry industry causing high mortality, loss of weight and reduction of egg production [1].

E. coli infection is one of the serious problems that cause a great threat to the profitability of birds' enterprises all over the word [2]. Although *E. coli* is a normal inhabitant of the intestinal tract of birds, under the influence of predisposing factors, like inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality,

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high mortality during rearing, reduced weight gain and condemnation of birds at the time of slaughter. Avian colibacillosis is a complex syndrome characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis being most typical [3].

The main clinical signs of naturally infected chicks with *E. coli* are reported as depression, loss of appetite, tendency to huddle respiratory distress, reduction of weight gain, dropped wing, closed eyes, cyanosis and laboured breathing [4].

Reducing bacterial and fungal populations is a major issue in poultry houses [5,6]. The presence of a high population of pathogenic bacteria in broiler grow-out houses can contribute in declining the wellness of the flock and lead to a sensitive production loss.

The principles of disease prevention and control within the poultry industry are based on flock management, biosecurity, preventive vaccination and sanitation [7]. The last step in a cleaning and disinfection program is the actual disinfection process that will further reduce pathogens in the facilities. To maximize the effectiveness of a cleaning and disinfection program, it is crucial to modify such a program based on the suspected pathogens that should be eliminated or reduced. In addition, specific disinfectants may be selected for certain known microbial contaminants following an infectious disease outbreak.

The aim of this study is

- To investigate the contamination of poultry farms with *E. coli* strains.
- To evaluate the degree of biosecurity level in some broiler farms with especial reference for *E. coli*.
- To determine the disinfectants sensitivity of *E. coli* to most common disinfectants in Egypt.

2. Material and methods

A total of 10 broiler houses were studied from November 2019 to July 2020.The farms were visited at different ages (one day old, week one, week 2, week 4 and week 6 of age). The data collected form the visited farms were description for their construction, bird species, stocking densities, traffic control, pest control, vaccination programmes, disinfection protocol and other managemental criteria. The evaluation process was carried out through filling out a designed questionnaire and taking samples for the isolation of bacterial pathogens.

2.1. Designed questionnaire

2.1.1. The biosecurity score was determined by the application the following Questionnaire:

Biosecurity parameters	Score(code)
Self-proofing (bird and house)	(yes=0.1,no=0)
Rodent and wild bird proofing	(yes=0.1,no=0)
Adequate ventilation area	(yes=0.1,no=0)
Adequate distance between farms and other poultry operations	(yes=0.1,no=0)
Hygienic disposal of carcass	(yes=0.1,no=0)
Self-sufficient	(yes=0.1,no=0)
Cleaning and disinfection	(yes=0.1,no=0)
Foot dips	(yes=0.1,no=0)
Traffic control	(yes=0.1,no=0)
Visitor restriction	(yes=0.1,no=0)

2.1.2. Correlation coefficient

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\, n\Sigma x^2 - (\Sigma x)^2 \,][\, n\Sigma y^2 - (\Sigma y)^2 \,]}}$$

2.2. Sampling

- Litter samples 10 gm each) were randomly collected from the commercial broiler farms. 100 samples were collected from the broiler farms (Triple litter samples).
- 100 Cloacal swab samples were collected from the broiler farms (three Cloacal swabs).
- Liver swab samples, 50 samples were collected from the broiler farms (three samples).
- Intestine swab samples, 50 samples were collected from the broiler farms (three samples).

Samples were collected aseptically and then brought to the laboratory in the Department of Veterinary Hygiene and Management, Faculty of Veterinary, Cairo University These samples were subjected to various bacteriological and biochemical examination in the laboratory.

2.3. Isolation and Identification of *E. coli*

Nutrient Broth (NB) and Nutrient Agar (NA) were used to grow the organisms from the collected samples before performing biochemical test according to the procedure describe by Cheesebrough [10]. Eosin Methylene Blue (EMB) agar medium was used for observing growth of *E. coli.* Suspected isolates of *E. coli* organisms were identified according to MacFaddin [11].

2.4. Serological identification of of *E. coli*

The isolates were serologically identified according to Kok *et al*. [12] by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan**)** for diagnosis of the Enteropathogenic types.

2.5. Preparation of tested *E. coli*

2.5.1. Propagation of the selected bacterial isolate

The bacterial isolates **(*E. coli* O153:H2:** STEC strain harbouring virulence genes,**)** were propagated using pour plate method. A loopful was transferred from all bacterial strains that was stored onto nutrient slopes into 10 ml nutrient broth and incubated at 37°C for 20-24 h. [8,9].

2.5.2. Preparation of source of organic matter

5% stock solution of yeast suspension (5 g of dried yeast was added to 100 ml of sterile distilled water); the yeast suspension was dispensed into 5 ml tubes, sterilized by autoclaving for 20 min at 121°C.

2.6. Evaluation of the efficacy of chemical disinfectants

2.6.1. Selected disinfectants

- Potassium proxy Monosulphat (Verkon- S® 1:120)
- Aldehyde / QUACS disinfectant (Aldekol des- Gda®) (0.4%).
- Quaternary ammonium compound disinfectant (biosentry® 904™) (0.4%).
- Quaternary ammonium compounds and glutaraldehyde (TH4® 1 ml of TH4® solution was added to 100 ml distilled water, pH 8.7).
- Formalin (2.5%, pH 7.9).
- Iodophore1 % in water
- Phenique 3% in water.

2.6.2. Method of evaluation

The laboratory evaluation of the efficacy of the chemical disinfectants was carried out using modified use-dilution test [13]. The test was repeated twice; once in the presence of organic matter and the second time in the absence of the organic matter. Bacterial suspension was prepared and propagated. 10 ml of the tested chemical disinfectant were

poured into a sterile test tubes, 0.1 ml of the bacterial suspension (1–2 x 10^8) was added and shaken thoroughly to give the chance for micro-organism to come in contact with the disinfectant. At time interval 1, 5, 10 and 30 min from original zero-time 1 ml of disinfectant-bacterial mixture were taken into tube containing 9 ml of in-activator (Tween 80 3%) in nutrient broth, mix thoroughly. One ml from in-activator tubes was used for the bacterial count using pour plate method [14]. The numbers of survival bacteria on each plate were counted. The calculation was carried out using the following formula: Log (average CFU/ drop vol.) (dilution factor) (Vol. scrapped into/ surface area) [8,9].

2.6.3. Statistical Analysis

The data were analyzed by the student t test and One-Way analysis of variance (ANOVA) according to Winer *et al*.,[15].

3. Results and discussion

3.1. Prevalence of *E. coli* at different ages of broiler farms

The incidence of *E. coli* in one day old living diseased chicks, 1week, 2week, 4week and 6 weeks of age were10% ,14% 12%,5.7% and 10.1%, respectively (Table 1 and Figure 1).

Table 1 Showing Prevalence of *E. coli* at different ages.

Age / isolates	Mean ±SD %				
	0 day	1week	2week	4week	6week
E. coli	10 ±0.99%	14±1.22%	12±1.69%	5.7±0.99%	10.1±0.88%

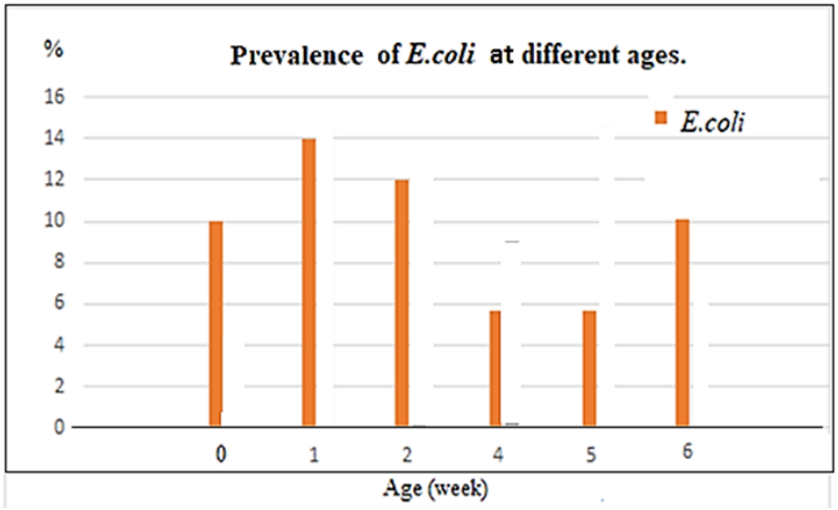

Figure 1 Prevalence of *E. coli* at different ages

3.2. Prevalence of *E. coli* in broiler farms

The obtained results showed that 44 *% E. coli* were isolated from 10 broiler poultry houses (27%) ;(11%) and (7%) from cloacal swab; Liver and Litter, respectively, as shown in Table 2 and Figure 2.

3.2.1. Prevalence of E. coli in different breeds

Mean prevalence of *E. coli spp.* was 15 **%** in Cobb, Ross or Sasso breeds, Tapan *et al.*, [16] detected colibacillosis from different farms. the highest isolation rate of *E. coli* from yolk sac (52.6%) and heart blood (38.4) in one day old -4week, and the highest percentage of *E. coli* isolation was from pericardial fluid (35.8%) followed byheart blood (33.4%) in older age (4-7 week). AbdElatif [17**]** examined 150 samples taken from five broiler chickens revealed the isolation of *E. coli* with percentage of78.7%, where the isolation from apparently healthy chickens with percentage of 72.0%and clinically diseased chickens with percentage of 85.3%, respectively. From the isolated *E. coli*, 208 strains recovered from different organs of chickens relieved that158 strains can be serotyped serologically and belonged to different serogroups.

Table 2 Showing mean incidence of *E. coli* in different samples of broilers poultry farms**.**

Types of samples	No. of samples	*E. coli* spp.
Cloacal swab	100	27±1.99%
Liver	50	4±0.12%
Intestine	50	7±0.30%
Litter	100	7±0.72%
Total %	300	15±1.22%

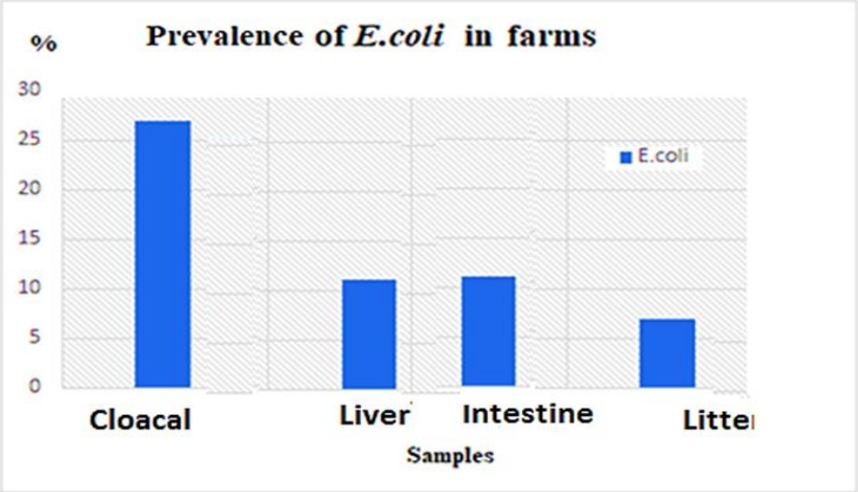

Figure 2 Mean Prevalence ±SD % of *E. coli* in different samples of broilers poultryfarms**.**

3.3. Prevalence of *E. coli* serotypes isolated from the studied farms

Table 3 Showing Prevalence of *E. coli* serotypes isolated from the studied farms

Serotypes	Number of isolates	Percentage
O78	14	31.81
O2: H6	8	18.18
O1: H7	7	15.9
O91: H21	5	11.36
O128: H2	4	9.09
O26: H11	2	4.54
O146: H21	1	2.27
O124	1	2.27
O44: H18	1	2.27
O153: H2	1	2.27
Total	44	-

O78 (31.81%), O2: H6 (18.18), O1: H7 (15.9), O91: H21(11.36), O128: H2 (9.09), O26: H11(4.54) O146: H21. The percentage of isolated *E. coli* serotypes O124, O44: H18 and O153: H2 was 2.27%.

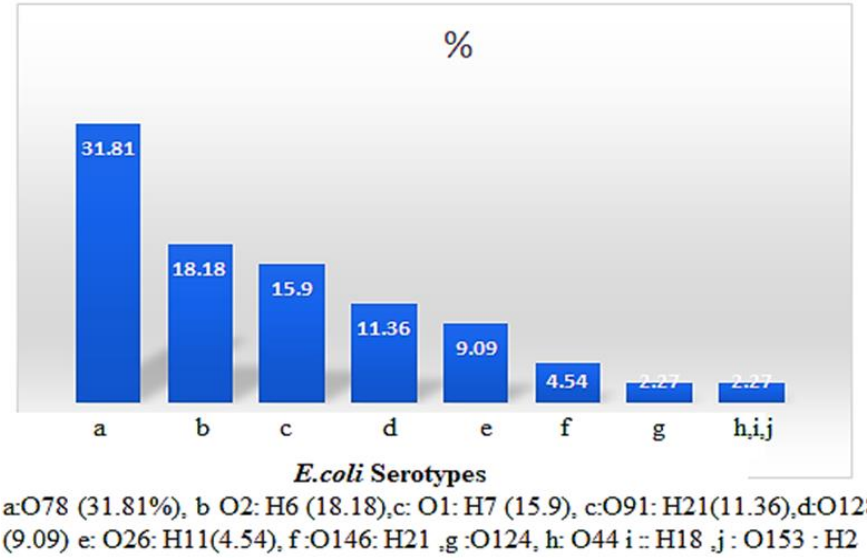

Figure 3 Prevalence of *E. coli* serotypes isolated from the studied farms

The detected *E. coli* serogroups in our study were; O78 (31.81%), O2: H6 (18.18), O1: H7 (15.9), O91: H21(11.36),O128 : H2 (9.09),O26: H11(4.54) O146: H21.The percentage of isolated *E. coli* serotypes O124, O44: H18 and O153 : H2 was 2.27%,as shown in Table 3 and Figure 3.

The most commonly detected *E. coli* serogroups serotyped by Tapan et al., [16] were O44 (11.3%), O158 (11.3%), O125(7.5%), O103 (9.4%), O63 (7.5%), O91(8.8%), while 50 strains were un- typed.

These results go hand to hand with the previous studies of [18,19,20,21] who reported that serogroups O44, O158, O114 and O91 were traditionally associated with colibacillosis in poultry.

3.4. The biosecurity scores in relation to mortality % and prevalence of isolation

Table 4 Showing Biosecurity score, Prevalence of *E. coli* in the studied farms

Biosecurity	Mort. %	Prevalence of isolate
		E. coli
0.6	12	43.7%
0.4	15	81.3%
0.8	8	68.7%
0.5	15	50%
0.4	20	75%
0.8	6	43.7%
0.4	20	25%
0.5	15	25%
0.7	12	56.3%
0.7	12	68.7%

3.4.1. The Correlation coefficient between biosecurity level and Mortality rate

Form Table 4 and fig,4a we found that there was strong association concerning level of applied biosecurity in broiler farms and mortality rate (significant negative *P<0.05*) **(R=** 0.8545) , its means that the mortality rate ' will be reduced significantly *(P<0.05)* if the satisfactory biosecurity applied to such farms.

3.4.2. The Correlation coefficient between biosecurity level and E. coli spread

Form Table 4 and fig,4b we found that there was a very strong association concerning level of applied biosecurity in broiler farms and *E. coli* spread (significant negative *P<0.05*) The value of R is NaN. This is a strong negative correlation, which means that high X variable scores go with low Y variable scores (and vice versa).

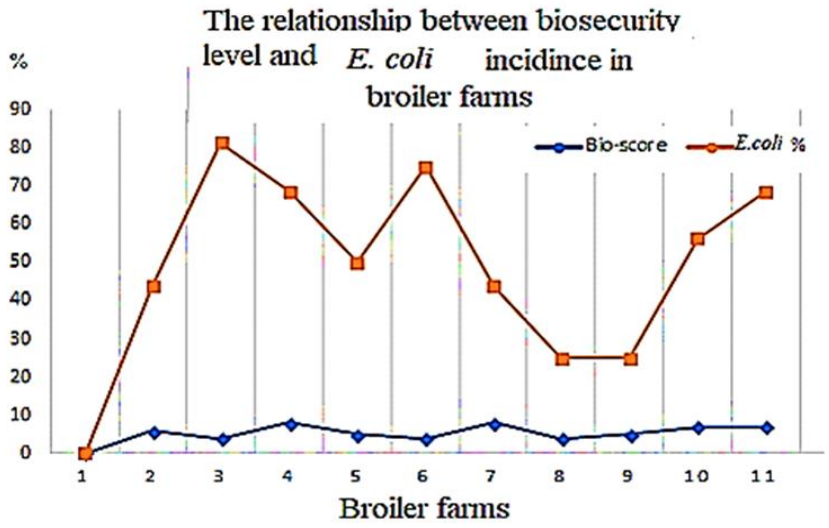

Figure 4a Biosecurity score, Prevalence of *E. coli* in the studied farms

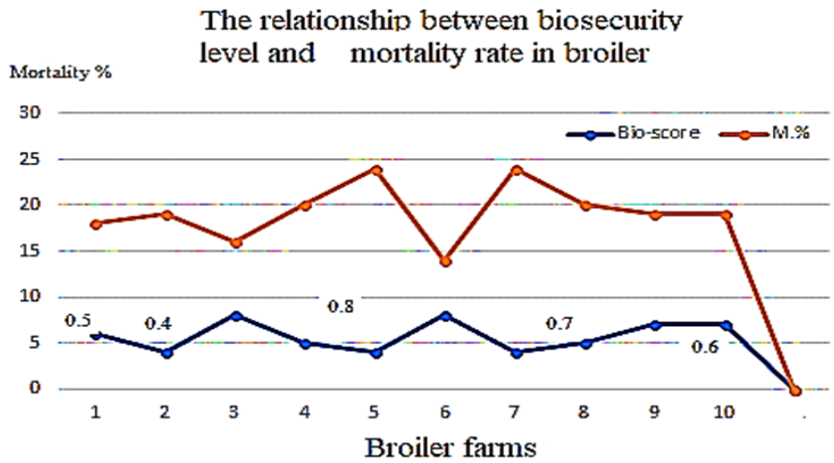

Figure 4 b Biosecurity score and Mortality rate due to spread of *E. coli* in the studied farms

3.5. Evaluation of the efficacy of chemical disinfectants

3.5.1. Evaluation of the efficacy of chemical disinfectants against E. coli in the absence of organic matter

In the absence of organic matter,Verkon- S®Aldekol Des- Gda® ,TH4®, Biosentry® 904™ and Formalin ® achieved 100% efficacy against *Escherichia coli* after 10 min ($p<0.05$) while Iodophore™ and Phenique were achieved 3 log reduction against *Escherichia coli* after 30 min ($p<0.05$),as shown in Table 5 and Figure 5.

Table 5 The Mean viable colony count (cfu/ml) of *E. coli* after contact time with the tested disinfectants in the absence of organic matter.

Disin. / contact time	Initial count	1 min	5 min	10 min	30 min
Verkon- S®	1.5×10^8	log6[a]	log5[a]	log4[a]	log4[a]
Aldekol Des- Gda®	3.6×10^8	log6[a]	log4[a]	log3[a]	log3[a]
TH4®	3.6×10^8	log7b	log6[a]	log4[a]	log4[a]
Biosentry® 904™	3.6×10^8	log7b	log6[a]	log4[a]	log4[a]
Iodophore™	1.5×10^8	log5a	log5a	log5 a	log5a
Formalin	1.2×10^8	log5a	log4a	log4a	log4a
Phenique	1.5×10^8	log5a	log5a	log5a	log5a

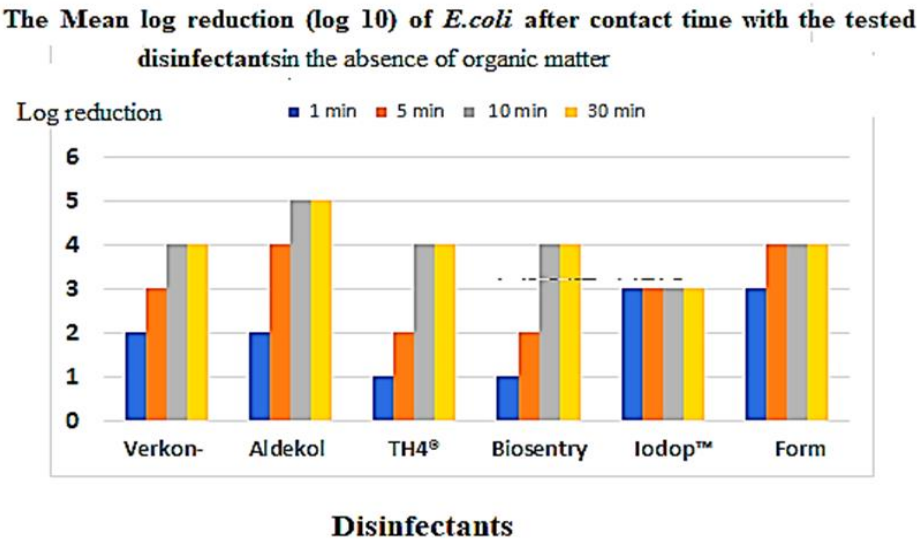

Figure 5 The Mean viable colony count (cfu/ml) of *E. coli* after contact time with the tested disinfectants in the absence of organic matter.

3.5.2. Evaluation of the efficacy of chemical disinfectants in the presence of organic matter

In the presence of organic matter, Verkon- S®achieved 3 log reductions after 5 min, but Aldekol Des- Gda®and Iodophore were achieved 3 log reductions after 30 min against *Escherichia coli (*P<0.05) without any log reduction after words (Table 6 and Figure 6).

On the other hand, Formalin achieved 4 log reductions after 5 min against *Escherichia coli (*P<0.05) without any log reduction after words. TH4® was achieved 2 log reductions after 5 min against *Escherichia coli (*P<0.05) while Biosentry® 904™ and Phenique were 2 log reductions after 10 min without any log reduction after words.

Table 6 The Mean viable colony count (cfu/ml) of *E. coli* after contact time with the tested disinfectants in the presence of organic matter.

Disinfectant/ contact time	Initial count	1 min	5 min	10 min	30 min
Verkon- S®	1.5×10^8	$log6^a$	$log5^a$	$log5^a$	$log5^a$
Aldekol Des- Gda®	3.6×10^8	$log6^a$	$log6^a$	log6	$Log5^a$
TH4®	3.6×10^8	$log7^b$	$log6^a$	$log6^a$	$log6^a$
Biosentry® 904™	3.6×10^8	$log7^b$	$log7^b$	$log6^a$	$log6^a$
Iodophore™	1.5×10^8	$log7^b$	$log7^b$	$log7^b$	$Log5^a$
Formalin	1.2×10^8	log^{5a}	log^{4a}	log^{4a}	log^{4a}
Phenique	1.5×10^8	$log6^a$	$log6^a$	$log6^a$	$log6^a$

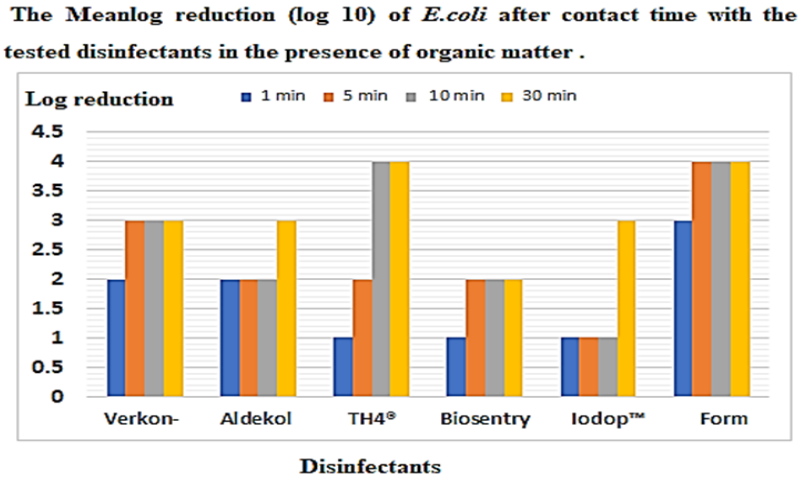

Figure 6 The Mean log reduction (Log 10) of *E. coli* after contact time with the tested disinfectants in the presence of organic matter.

In case of Formalin, it achieved 3 log reduction after one min while Phenique achieved one log reduction after one min without any log reduction after words Mohamed [22] concluded that all used disinfectants were affected by presence of organic matter except formalin solution. He found that the best reduction in total bacterial count were obtained with 10% formalin solution followed by Creolin 3% while lower efficiency was recorded when iodophor preparation was applied. Formaldehyde and phenolic compound were effective in presence of organic matter. The poultry houses and equipment should be fogged with formaldehyde solution which might be repeated after placing the litter. Ka-Oud [23] found that 1% formalin failed to produce its germicidal action on pathogenic *E. coli* of an artificially infected litter after 30 minutes, while in a concentration of 5% it destroyed *E. coli* within the same exposure time. *E. coli* strains were susceptible to in-use concentrations of formaldehyde, benzalkonium chloride and a formulation of peracetic acid and hydrogen peroxide M (24).

4. Conclusion

From the findings we can concluded that the most prevalent *E. coli* serovars in broilers were: O78 , O2: H6 , O1: H7 , O91: H21, O128: H2 , O26: H11, O146: H21, O124, O44: H18 and O153: H2 . There was a strong association concerning level of applied biosecurity and mortality rate, its means that the mortality rate will be reduced significantly if a satisfactory biosecurity applied to such farms. Also, the variables, such as application rate, disinfectant type, time of exposure, and the presence or absence of organic matter, are important considerations when including a chemical

disinfectant application into a sanitation program. The potassium peroxymonosulfate, nascent oxygen, formalin and phenol products provided the best *E. coli* reductions in the laboratory trials.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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