Relationship between poultry biosecurity assessments and *Escherichia coli* prevalence in poultry farms

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Introduction

Biosecurity is a program aimed to keep birds safe from disease-causing microorganisms and considered the most affordable and effective method of disease control. Biosecurity as an integrated aspect of farm operations, understanding of characteristics and management techniques, including biosecurity precautions and disease management (Fagrach *et al.*, 2023). Biosecurity includes many health-management measures designed to prevent pathogens from entering farms (external biosecurity) and spreading within a flock (internal biosecurity) (Damiaans *et al.*, 2018).

Escherichia coli species are a commensal species in the lower gastrointestinal tract of chickens. In dry and dusty environments, E. coli can survive for a very long time outside of the bird's body. Water and feed contamination have the ability to be the cause of E. coli infections. (Baranwal et al., 2019). So, it acts as an indicator of the environmental quality of poultry farms. Although most strains of E. coli are harmless, some can become virulent and pathogenic. It is a serious public health problem since food and water are common sources of transmission for pathogenic E. coli. Avian pathogenic E. coli (APEC) is popular term for pathogenic E. coli that can infect birds of various ages and species causing colibacillosis (Ammar et al., 2015). The virulence characteristics of the E. coli strain, the host's health, and further risk factors such as stress all influence the severity of systemic colibacillosis in broilers. Pericarditis, air sacculitis, perihepatitis, peritonitis, and other extra-intestinal disorders are symptoms of colibacillosis. Poor biosecurity procedures enhance the prevalence of colibacillosis, which causes significant economic losses in the poultry industry in Egypt and many other nations due to high rates of morbidity and mortality as well as higher treatment and prevention costs (Dawadi

ABSTRACT

A crucial first step in preventing the introduction and spread of harmful microorganisms in poultry farms is biosecurity. The objectives of this investigation were to assess biosecurity measures and the prevalence of different E. coli species in commercial chicken farms in Sharkia province, Egypt. Sensitivity of E. coli strains was identified, and their susceptibility to antibiotics and disinfection was evaluated as well. Four farms provided 21 samples each, for a total of 84 samples. Three samples of each were collected for water, feed, litter, cloaca, wall, hand, and foot boots. All the studied farms obtained a "poor biosecurity" grade, meaning that their overall biosecurity score, which is comprised of 40% external and 36.54% internal biosecurity, was less than 50%. Escherichia coli species were found in 79.76% of the samples. It was isolated from water, feed, litter, cloaca, walls, hands, and foot boots with percentages of 91.6; 83.3; 91.6; 100; 58.3; 58.3 and 75%, respectively. Biosecurity level had significant negative correlations with the isolated E. coil species. Escherichia coli O119 was the most common serotype in litter and the cloaca, while E. coli O1 was the most prevalent serotype in walls and hands. Additionally, serotypes for E. coli O26, O159, O128 and O78 were included. According to the patterns of antibiotic sensitivity, amikacin, nitrofurantoin, gentamycin, and levofloxacin showed intermediate sensitivity, whereas E. coli O119 bacteria were highly sensitive to ciprofloxacin. In the absence and presence of organic matter, Virocid, Cid 2000 and finally Virkon S were the most efficient against E. coli O119. According to this study, there were differences in the investigated farms' biosecurity levels in relation to the occurrence of Escherichia coli. To reduce the introduction and persistence of E. coli in poultry farms, several biosecurity practices and management, including stringent cleaning and disinfection measures should be applied.

et al., 2021; Nguyen et al., 2022; Xu et al., 2022).

For more than 50 years, the subtherapeutic dosages of antibiotics are used as feed additives for growth promotion, maintaining animal health and for disease prevention in poultry industry (Hasan *et al.*, 2011). The main drugs' groups that used to prevent colibacillosis in the parent flocks were cephalosporin and fluoroquinolone in broilers (Kuznetsova *et al.*, 2020). Semisynthetic penicillins such as amoxicillin, oxytetracyline, and tetracycline are commonly used to decrease the morbidity and mortality of avian colibacillosis. On other hand, the widespread usage of antibiotics may have adverse impacts on both human and animal health as well as the environment, as shown by the high degree of antibiotic resistance in avian diseases (El-Saadony *et al.*, 2022; Kamil *et al.*, 2023).

Cleaning and disinfectants are critical components of the biosecurity strategy as the main objective of disinfectant programs is to kill or minimize the populations of disease-causing agents and prevent their spread between flocks. Disinfectants, which are frequently employed in poultry farms, may be the poultry industry's last line of defense against infections such as E. coli. The efficacy of disinfectant may be affected by disinfectant concentration, water pH, temperature, contact surfaces, and exposure time. Furthermore, if disinfectants are applied without first thoroughly cleaning the facility, the disinfectant's effectiveness may be impaired owing to the presence of organic matter. In poultry farms, the commonly disinfectants are oxidizing agents, chlorhexidine compounds, quaternary ammonium compounds, halogens and phenolics, Hydrogen peroxide was demonstrated to have good disinfection power against E. coli (Aksoy et al., 2020; Kaoud et al., 2022). The objective of this study was to assess the biosecurity measures in place and determine how prevalent E. coli is in chicken farms in the Sharkia governorate, Egypt. Additionally,

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the sensitivity of the detected *E. coli* strains to common antibiotics and disinfectants was evaluated.

Materials and methods

Study area

The targeted population of this study was four commercial broiler poultry farms distributed in Sharkia governorate, Egypt. All surveyed broiler farms used an open deep litter system (open type) with one or two floors. All farms were climate-controlled, naturally ventilated through hopper-style windows and equipped with electrical fans for use in hot weather. Lighting was provided by the sun during the day and by electric lighting at night. Furthermore, all other information included: location, farm area, stocking density of birds, kind of farmed poultry species, mortality rate, cycle duration, storage of poultry feed, type of floor, water sources, ventilation & lighting system were recorded (Table 1).

Questionnaire development

To evaluate the level of biosecurity in the broiler farms under investigation, a biosecurity questionnaire was created based on biosecurity practices used in chicken farms. Biosecurity framework was categorized into: External biosecurity included access to the farm, distance from nearest farm, distance from water source, disposal of dead birds, manure disposal and management, drinking water origin, rodent control, bird proofing, visitor restriction and vehicles. Internal biosecurity is evaluated by several things such as conditions of chicks placing : birds density at one day, concrete floor, management of ill birds, water sanitation, types of drinkers, foot bath dip, contact of workers with other flock, cleaning and disinfection of farm between flocks, cleaning and disinfection of equipment and vehicles, cleaning and disinfection of footwear before and after visit, hand hygiene before and after poultry handling ,utilization of farm cloths & foot wear and disinfection of worker cloths.

Data collection and management

Clear, understandable, and comprehensive questionnaires that help collect all information regarding biosecurity precautions and farm characteristics were used for data collection. Finally, a face-to-face interview with farm owners was conducted. To score biosecurity, variables in the questionnaire were divided into external (10) and internal biosecurity components (13). The questionnaire responses earned a score of 0 (total lack of preventative measures) or 1 (complete existence of preventive measures) (Maduka *et al.*, 2016). The biosecurity score (BS) percentag-

es were then computed and compared to the conventional biosecurity grade "Good" if the farm's BS was greater than 50% and "Poor" if the farm's BS was less than 50% (Ismael *et al.*, 2021).

Samples and sampling procedures

A total of 84 samples were collected equally and randomly from four commercial broiler chicken farms (n=21 each farm) at Sharkia governorate, Egypt. Water, feedstuff, litter, cloaca, wall, hand, and foot boot samples were obtained in threes from each poultry farm from November 2021 to July 2022.

The top layers of litter, 100 g of properly mixed feed, and 100 ml of water drinkers were aseptically taken from the poultry farms under investigation. Sterile swabs were used to collect samples from the cloaca, wall, hands, and foot boot while maintaining the aseptic procedures previously used (Abunna *et al.*, 2016; Abdi *et al.*, 2017). Immediately after sampling with a minimum of delay, all samples were aseptically transported to the laboratory for further investigation.

Isolation and identification of Escherichia coli

Twenty-five ml / g of each examined water, homogenized feed stuff and litter samples were mixed with 225 ml of pre-enrichment broth (B.P.W), while swabs samples were incubated in nine ml B.P.W (Cruichshank *et al.*, 1975).

After processing samples and addition of pre-enrichment broth, all samples were incubated at 37°C for 6 hours. A loopful of the 24 hourscultured enriched MacConky broth was streaked onto Eosine Methylene Blue agar and incubated at 37°C for 24 hours (Quinn *et al.*, 1994). Typical colonies of suspected *Escherichia coli* were picked and identified using morphological characters and biochemical reactions (Koneman *et al.*, 1997; Ahmad *et al.*, 2022).

Serological identification of Escherichia coli

Eight biochemically identified *E. coli* isolates were subjected to slide agglutination method for serological identification (Sojka *et al.*, 1961; Dou *et al.*, 2016) at The National Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

Antibiotic sensitivity of Escherichia coli

The isolated *E. coli* were tested for their antibiotics resistance using disc diffusion method according to methods previously explained (Cherkaoui *et al.*, 2020). The antibiotics were chosen in accordance with

Table 1. Topographical examination of investigated poultry farms.

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Categories	Farm I (El-Salheya)	Farm II (El-kattara)	Farm III (Kafr Saqr)	Farm IV (Awlad Saqr)
Location	El-Salheya	El-kattara	Kafr Saqr	Awlad Saqr
Farm area / m ²	650 m ²	700 m ²	750 m ²	780 m ²
Stocking density of bird / m ²	8-10 birds	6-8 birds	8-10 birds	10-12 birds
Total capacity of the farm	5500 birds	4500 birds	6000 birds	8000 birds
Reared Spp.	Arbo Acres	Arbo Acres	Sasso	Ross
Cycle duration	40 days	40days	50 days	60 days
Distance between farms	20 m	50 m	150 m	200 m
Mortality rate/ cycle (%)	5%	5-10%	10-15%	15%
Time interval between cycles	1 month	Not fixed	Not fixed	Not fixed
Cleaning & disinfection between flock	Chlorine & iodine	Phenol & chlorine	Formalin	Formalin
No. of windows / side	7	8	10	12
Types of Floors	Cement	Muddy	Muddy	Cement
Type of water source	Private (Underground)	Private (Underground)	Public	Public

the National Committee for Clinical Laboratory Standards' instructions (NCCLS, 2002). Thirteen antibiotics were tested (BioMerieux F6980 Marcy Etoite France) includes ciprofloxacin (15µg), amoxicillin +clavulanic acid (30µg), gentamycin (10µg), sulbactam+Ampicillin (10µg), nitrofurantoin (300 µg), fusidic Acid (10µg), ceftazidime (30µg), amikacin (30µg), levofloxacin (5µg), penicillin (10µg), cefotaxime (30µg), erythromycin (15µg) and ceftriaxone (30 µg). The diameter of the inhibitory zone was used to measure *E. coli*'s sensitivity to several antibiotic discs, and the results were compared to an antibiotic susceptibility testing sheet. Interpretation of the zones of growth inhibition's size with respect to Aditi *et al.* (2017).

Disinfectant efficacy against identified Escherichia coli

The efficacy of three chemical disinfectants compressing Virocid (Glutaraldehyde, 0.5%), Virokin S (Potassium peroxymonosulfate and Sodium chloride 0.5%) and Cid 2000 (Hydrogen peroxide and acetic acid,2%) are chosen to applied singly on *E. coli* isolates at different contact time (1, 5, 10, 20, 30, 45, 60 minutes) in the absence and presence of organic matter (2% yeast) as described before (Pilotto *et al.*, 2007; Aidaros *et al.*, 2022). Disinfectant efficacy was determined by the absence of microbial growth on plates of selective media at 37°C for 24-48 hours.

Statistical Analysis

Chi-square test and Pearson correlation were run to test differences and relations among four farms through biosecurity level. The occurrence of *Escherichia coli*. P < 0.05 statistically considered significant. All analyses were performed by SPSS version 24.0 (IBM. Corp., Armonk, NY) (McHugh, 2013).

Results and Discussion

A crucial first step in stopping the entrance and spread of harmful microorganisms that start diseases in chicken farms is implementing biosecurity practices.

Assessment of biosecurity status in the investigated poultry farms

Table 2 shows the assumed biosecurity scores (%) in the four investigated poultry farms. The suitable biosecurity levels in the investigated chicken farms may be justified based on the increased risk of infection with a certain disease, pathogenicity, and in connection to the applied biosecurity level. External biosecurity levels evaluated poultry farms by certain things such as access to the farm, distance from nearest farm,

Table 2. The assumed score of biosecurity levels in the examined poultry farms

Biosecurity variables		El Salhava Farm	El-Khattara	Vofe Soon Form	Awlad Saqr	Total score	
		EI- Saineya Farm	Farm	Kair Saqr Farm	Farm	No.	%
External biosecurity: (No.=10)							
Access to the farm		1	1	1	1	4	100
Distance from nearest farm.		0	0	1	1	2	50
Distance from water source.		1	1	0	0	2	50
Disposal of dead birds		0	0	0	0	0	0
Manure disposal & management.		1	0	0	0	1	25
Drinking water origin		1	1	1	1	4	100
Rodent control		1	1	0	1	3	75
Bird proofing		0	0	0	0	0	0
Visitors restriction		0	0	0	0	0	0
Vehicles (allowed to enter farm)		0	0	0	0	0	0
	No	5	4	3	4	1	16
Sub-total external biosecurity	%	50	40	30	40	2	40
Internal Biosecurity: (No.=13)							
Birds density at day 1 (chicks/m2)		0	0	1	1	2	50
Floor built with concrete		1	0	0	0	1	25
Management of ill birds		1	1	0	0	2	50
Water sanitation		0	0	0	0	0	0
Type of drinkers		1	1	1	1	4	100
Foot bath dip		0	0	0	0	0	0
Contact of workers with other flock		1	1	1	1	4	100
Cleaning and disinfection of farm between flocks		1	1	1	1	4	100
Cleaning and disinfection of equipment and vehicles	1	1	0	0	2	50	
Cleaning and disinfection of footwear before and after	0	0	0	0	0	0	
Hand hygiene before and after poultry handling		0	0	0	0	0	0
Utilization of farm cloths and footwear		0	0	0	0	0	0
Disinfection of worker cloths		0	0	0	0	0	0
Sub total internal biogeomity	No	6	5	4	4	19	
Sub-total internal biosecurity	%	46.15	38.46	30.76	30.76	36	5.54
	No.	11ª	9ª	7ª	8ª	3	35
10(a)	%	47.8	39.1	30.4	34.8	38	3.04

 abc Means within the same row carrying different superscripts are significant. Chi square is no significance $\chi 2$ (3) = 1.614, p= 0.656

distance from water source, disposal of dead birds, manure disposal and management, drinking water origin, rodent control, bird proofing, visitor restriction and vehicles. Internal biosecurity is used in several things such as conditions of chicks placing: birds density at one day, concrete floor, management of ill birds, water sanitation, types of drinkers, foot bath dip, contact of workers with flock, cleaning and disinfection of farm between flocks, cleaning and disinfection of equipment and vehicles, cleaning and disinfection of footwear before and after visit, hand hygiene before and after poultry handling, utilization of farm cloths and foot wear as well as disinfection of worker cloths.

Our findings revealed that complete biosecurity was attained by 38.04% of the farms studied. The entire biosecurity was divided into 40% external biosecurity and 36.54% internal biosecurity. The first farm, located in El- Salheya, had the greatest degree of external biosecurity (50%) and internal biosecurity (46.15%). The second farm in El-Khattara had an external biosecurity level of 40% and an internal biosecurity level of 38.46%. The third farm in Kafr Saqr had an external biosecurity level of 30% and an internal biosecurity level of 30.76%. External biosecurity was determined to be 40% and internal biosecurity was 30.76% at the fourth farm tested, which was in Awlad Sagr (Table 2). There was no significant variance in biosecurity levels across the farms studied, according to these data. These results support the findings of Van Limbergen et al. (2018), who discovered that visitors and workers had the lowest level of education. Improved overall biosecurity on broiler farms in Europe may be facilitated by the training of broiler producers and their workers. These results were nearly similar to these previously cited (Tilli et al., 2022). Our findings contradicted those of Gelaude et al. (2014), who found significant variance in the levels of biosecurity on broiler farms in Belgium, with internal biosecurity scores ranging from 54 to 87% and external biosecurity values from 55 to 72%. In a recent study, it was shown that among inspected chicken farms, the external biosecurity score (59.55%) was lower than the internal biosecurity score (65.18%). Material supply and disease management received the best marks, while manure and carcass removal received the lowest marks. These preliminary findings revealed that, despite the necessity of biosecurity, many biosecurity measures are poorly implemented. Location, ventilation, immunization status, and feeder and drinker cleaning are the most critical risk factors and biosecurity measures. However, isolation, cleanliness, and movement restricagent disseminating external biosecurity measures, such as access control, vehicle disinfection and animal control, in addition to internal biosecurity measures, such as disinfection of house premises, demonstrated high biosecurity compliance, thereby representing an important phase in biosecurity implementation. In fact, adequate cleaning and disinfection processes have become essential for preventing disease transmission (Tilli et al., 2022).

tion were recognized as the most important factors in limiting disease

Prevalence of Escherichia coli species

Table 3 shows 67 out of 84 samples were positive for E. coli in all assessed farms, for a percentage of 79.76%. In El- Salheya, El-Khattara, Kafr Saqr, and Awlad Saqr farms, the percentages of E. coli species were 71.4 (15/21), 76.2 (16/21), 90.47 (19/21) and 80.95% (17/21). The third farm had the most E. coli (90.47%), followed by the fourth farm (80.95%), and the second farm (76.2%). The first farm, on the other hand, had the lowest value with a percentage of 71.4%. There was no statistically significant relationship between E. coli prevalence and the investigated farms. Different types of samples collected from the assessed poultry farms were further examined for E. coli isolation. It was isolated from water, feed, litter, cloaca, walls, hands, and foot boots with percentages of 91.6, 83.3, 91.6, 100, 58.3, 58.3 and 75%. These findings were remarkably identical to those reported earlier (Blaak et al., 2015), who identified E. coli species in rinse and run-off water (21/26; 81%). Furthermore, pathogenic E. coli was discovered in greater quantities in polluted water than in our data (Kunert et al., 2015). In another study, the usage of ground water and near to farms was major risk factors related with the prevalence of avian pathogenic Escherichia coli (APEC) in broiler chickens in Jordan. Furthermore, the usage of ground water and the proximity of farms were significant risk factors for the presence of avian pathogenic Escherichia coli (APEC) in broiler chickens in Jordan (Ibrahim et al., 2019). According to Gazal et al. (2021), E. coli strains found in water samples were negative for extra intestinal pathogenic E. coli (EXPEC), and chicken feed was not a source of E. coli bacteria. This highlights the significance of using biosecurity to prevent E. coli from spreading to new production cycles. These findings were almost identical to those reported before (Kushal et al., 2020), who indicated that good biosecurity measures, that involve water disinfection and

Table 3. Occurrence of E. coli species isolated from the examined poultry farms.

Source	No. of samples/ each farm	El- Salheya Farm Positive samples		El-Khattara Farm Positive samples		Kafr Saqr Farms Positive samples		Awlad Saqr Farms Positive samples		Total		
										Total No. of	Positive samples	
		No.	%	No.	%	No.	%	No.	%	samples	No.	%
Water	3	3	100	3	100	3	100	2	66.6	12	11	91.6
Feed stuffs	3	1	33.3	3	100	3	100	3	100	12	10	83.3
Litters	3	3	100	2	66.6	3	100	3	100	12	11	91.6
Cloaca	3	3	100	3	100	3	100	3	100	12	12	100
Walls	3	3	100	1	33.3	2	66.6	1	33.3	12	7	58.3
Hands	3	1	33.3	1	33.3	2	66.6	3	100	12	7	58.3
Foot boots	3	1	33.3	3	100	3	100	2	66.6	12	9	75
Total	21	15ª	71.4	16ª	76.2	19ª	90.47	17ª	80.95	84	67	79.76

Chi square is no significance association between infection of E. coli among the investigated farms $\chi^2(3) = 2.581, p = 0.461$

Table 4. Efficiency of disinfectants against E.coli 119 in the absence and presence of organic matter.

Disinfectant treatment		Contact time (minute)				
(Trade name)	Conc. (%)	In absence of organic matter	In presence of organic atter			
Virocid	0.5	Less than one min	20 min			
Virkon S	0.5	30 min	30 min			
Cid 2000	2	20 min	20 min			

visitor and vehicle entrance restrictions, may assist in reducing the prevalence of avian colibacillosis in broiler farms. *Escherichia coli* pathogens might thus be maintained in the litter. Our findings differed with those of Oliveira *et al.* (2020), who found a reduced prevalence of *E. coli* recovered from litter in avian pathogenic *Escherichia coli* (APEC), and Saha *et al.* (2020), who discovered that bird droppings had the highest percentage of avian pathogenic *Escherichia coli* (APEC) isolates (33.33%), followed by cloacal swabs (17.82%), handler's swabs (10.34%), water (9.20%), and feedstuffs (5.17%). In another investigation, 71.05% of *E. coli* isolates were recovered from chicken cloacal swabs (Nguyen *et al.*, 2021). Previous research found that *E. coli* isolates were common among poultry workers (Aworh *et al.*, 2021).

In respect to the hygienic aspects, E. coli is a frequent pathogenic bacteria found in poultry ecosystems resulting in billions of dollars in yearly colibacillosis losses (Fancher et al., 2020). Meanwhile, Fancher et al. (2021) stated that avian pathogenic E. coli (APEC) represents a significant economic and welfare problem for the chicken industry. Prophylactic feeding with antibacterial growth promoters was the principal method of combating avian pathogenic E. coli (APEC). Escherichia coli prevalence was unaffected by season, flock age, or sample type. Avian pathogenic E. coli causes poor performance, early mortality, and ultimate production loss. Awawdeh et al. (2022) suggested in their study that measures like having a shower facility on the farm, shortening the distance between the car park and the sheds, and/or creating a buffer area close to each shed where people can change into protective clothing might reduce the risk of avian pathogenic Escherichia coli (APEC) carriage and potential infection. To reduce the occurrence of E. coli inside the poultry farm, proper biosecurity implementation, such as limiting the number of people entering the house, thorough cleaning and disinfection processes between production cycles, the use of transition zones, and pest management, must be provided (Tilli et al., 2022).

The correlation between biosecurity levels and *E. coli* collected from poultry farms studied. There were significant negative correlations between biosecurity level and *E. coli* (p = 0.05).Biosecurity measures are critical in protecting flocks from colibacillosis since it has been well proven that effective biosecurity measures and proper flock management reduce disease transmission (Bernd *et al.*, 2020).

According to the data in Fig. 1, eight *E. coli* isolates have been subjected to serotyping. *E. coli* O119 was found to be the most frequent serotype of *E. coli* isolated from litter and cloaca, and *E. coli* O1 was found to be the most common serotype of *E. coli* isolated from walls and hands (25% for both). Furthermore, *E. coli* O26, *E. coli* O159, *E. coli* O128 and *E. coli* O78 were serotyped in 12.5% of samples. The isolates of *E. coli* were obtained from the cloaca, feed, water, and foot boot. Previously reported data (Amer *et al.*, 2015) indicated that the most prevalent serotypes were O125, O114, and O44, followed by O78, O86, O158, O127, O91, O25, and O119.



Antibiotic sensitivity testing of Escherichia coli 0119 in poultry farms

The sensitivity patterns revealed that all *E. coli* 0119 strains were significantly sensitive to ciprofloxacin (++). Amikacin, nitrofurantoin, gentamycin, and levofloxacin showed intermediate sensitivity (+). On the other hand, all *E. coli* strains had shown 100% resistance to fusidic acid, amoxicillin and clavulanic acid, ceftazidime, sulbactam and ampiciilin, cefotaxime, penicillin, ceftriaxone, and erythromycin (Fig. 2). Other previously published research; Matin *et al.* (2017) validated these findings, reporting that *E. coli* isolates were responsive to ciprofloxacin, gentamicin, and chloramphenicol but resistant to ampicillin and cephalexin based on inhibitory zone. Sabdoningrum *et al.* (2020) discovered that ciprofloxacin influenced *E. coli*, but that these strains were resistant to oxytetracyline, gentamycin, and enrofloxacin at the same dosage. Another study indicated that *E. coli* was particularly sensitive to ciprofloxacin, enrofloxacin, and gentamicin (Ahmad *et al.*, 2022).



Fig 2. Patterns of antibiotic resistance for *E. coli* O119 that were identified from the examined poultry farms.

Assessment of the efficacy of certain disinfectants on Escherichia coli O119

The research results shown in Table 4, demonstrated that in the absence of organic matter, the time required to eliminate E. coli 0119 following application of Virocid (0.5%) was less than one minute and 20 minutes in the presence of organic matter. In the absence or presence of organic matter, Virokin S (0.5%) was able to kill E. coli 0119 in 30 minutes. In the absence or presence of organic matter, E. coli 0119 was killed after 20 minutes in Cid 2000 (2%). Based on these findings, it was determined that Virocid (0.5%) was the most effective against E. coli 0119 in the absence of organic matter, with a contact time of less than one minute, followed by Cid 2000 (2%), with a contact duration of 20 minutes. Virkon S (0.5%), on the other hand, had greater contact time (30 minutes). After the presence of organic matter, Viocid (0.5%) and Cid 2000 (2%) were efficient against E. coli 0119, with both killing it after 20 minutes. However, Virkon S (0.5%) had the least effect on E. coli 0119 when the contact duration was 30 minutes in the presence of organic matter. Cid 2000 (2%), with a contact time of 20 minutes, came in second. As previously stated, when organic matter was removed all disinfectants performed better (Gosling, 2018). In chicken flocks, Virkon-S treatment resulted in a considerable decrease in E. coli species (Kaoud et al., 2022).

Conclusion

All examined broiler farms earned grade "poor biosecurity farms. Several biosecurity measures, including cleaning and disinfection have been found to be protective factors in reducing the introduction and persistence of *E. coli* on poultry farms. Many pathogens such as *E. coli* can be avoided with thorough cleaning and disinfection as well as preventive antibiotics. Visitors and workers had the lowest level of education, so good education of broiler farmers and their staff may assist to enhance overall biosecurity on broiler farms.

Fig. 1. Serotypes of E. coli species recovered from the examined poultry farms.

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Conflict of interest

The authors declare that they don't have any conflict of interest.

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