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Effect of Various Disinfectants on *E. coli* Isolated from Water Pipes in Broiler Farms at Giza and Dakahlia Governorates, Egypt

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Abstract

In poultry, *Escherichia coli* infections lead to substantial deaths and financial losses for producers each year. This study aimed to investigate the prevalence of *E. coli* from water pipes and drinkers in broiler farms, to characterize the isolated *E. coli* strains in terms of serotypes, biofilm production degree, presence of the *adrA* gene. The gene "*adrA*" encodes for the adhesion regulating protein A. *adrA* is a protein that plays a role in regulating the adhesion and biofilm formation of *E. coli* bacteria., and the efficacy of various disinfectants on *E. coli* biofilms. A total of 100 swab samples were collected from drinking water pipes and drinkers from different broiler farms in Giza and Dakahlia governorates in Egypt. Out of them, 18 *E. coli* serogroups were identified in 50 positive samples, and the most predominant serogroup was O91, which showed the highest incidence (20%), followed by serotypes O78 (18%), and O26 (8%). Microtiter-plate test for determination of biofilm production for 50 *E. coli* isolates were performed. PCR was done for the detection of the virulence gene *adrA* in the 13 strong biofilm *E. coli* isolates, and the results revealed that 100% were positive for the virulence gene. The effect of disinfectants on *E. coli* was studied by using ZnO nanoparticles, acidifiers, and quaternary ammonium dioxide. This study found a high prevalence of *E. coli* in water samples, identified various *E. coli* serotypes, observed biofilm production, and determined the effectiveness of different disinfectants on *E. coli* isolates.

KEYWORDS
Biofilm production, *E. coli*, *AdrA* gene, ZnO nanoparticles, Egypt.

INTRODUCTION

Egyptian poultry production has developed from agricultural activity in recent years into an industry. The Arab Republic of Egypt has seen an increase in the production of broiler chicken meat in response to the increasing demand for high-quality protein. The overall number of poultry consumed in Egypt will rise in 2026 to 14% more than it was in 2017 (Terwisscha van Scheltinga *et al*., 2021).

Avian pathogenic *E. coli* results in avian colibacillosis in poultry (Renzhammer *et al*., 2020). A physiological state known as a biofilm is one in which bacterial cells adhere to surfaces permanently and are encased in a matrix formed from proteins, polysaccharides, and nucleic acids called the self-secreted extracellular polymeric matrix (Sanchez-Vizuete *et al*., 2015). The biofilm matrix defends the implanted cells from the adverse effects of antimicrobials, sterilizers, and immune cells, making it hard to remove biofilm-related infections in the food production process. *E. coli* can transition from its individual, freely floating form (planktonic) to a collective, adherent form called biofilm. This transition serves as a protective measure to avoid being carried away by water flow. Additionally, biofilm cells exhibit significantly greater resistance to various environmental stresses compared to their planktonic counterparts, with a roughly 1000-fold increase in resistance may change from planktonic to biofilm form to prevent being washed away by water flow or because biofilm cells are around 1000 times more resistant than planktonic cells (Jefferson, 2004).

Sessile bacteria, which are present in the biofilm, are in a stationary or dormant growth phase. Bacteria in biofilms exhibit extraordinary resistance to environmental stressors, including antibiotics. Since that 60–80% of human microbial infections are brought on by bacteria forming as biofilms, this makes biofilms a serious public health issue (Høiby *et al*., 2010).

Resistance to disinfectant and the ability to form biofilms are two crucial traits that support the persistence of bacteria in food processing environments and the contamination of food products (Sun *et al*., 2019). Disinfectants are commonly used in the animal production industry to minimize or eliminate a load of parasites and infectious organisms in buildings and equipment used for sheltering or transporting animals. There is a growing concern that using disinfectants could promote antibiotic and disinfectant resistance (Maertens *et al*., 2020).

It is crucial to choose the right processes to ensure drinking water safety since biofilm recovery following ineffective treatment could result in populations of bacteria that are resistant to the subsequent disinfection process (Simões *et al*., 2004). Mechanical removal of biofilms is advised before water disinfection to obtain effective chlorine-based disinfection. In addition, due to the specificity of metabolic interactions between bacteria with

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various physiological requirements, studies about the composition of microbial consortia and the physiological activities in the related biofilms should be taken into consideration (Farkas *et al*., 2013).

Nanoparticles (NPs) are an innovative alternative therapy against common pathogenic microorganisms overlapping antibiotic resistance problems in organisms (Losasso *et al*., 2014). In various contexts, microbial growth is controlled using zinc oxide (ZnO), silver, and titanium dioxide. However, ZnO is a more significant disinfectant because it has the most powerful photocatalytic effect and is more biocompatible than titanium dioxide. Additionally, ZnO has better durability, greater selectivity, and heat resistance, it can be used to combat a diversity of microorganisms, such as *E. coli*, *S. aureus*, and *C. albicans* (Liu *et al*., 2009).

Antibacterial effects with low toxicity to mammalian cells against a wide range of infectious agents (Mohan and Renjanadevi, 2016).

Quaternary ammonium compounds (QACs), which have a variety of uses, are now the most promising antiseptic compounds. QACs are applied as prophylactic and therapeutic antiseptics, preservatives for surface clearances and disinfectants, medications, cosmetics, deodorants, etc. These compounds have a strong antimicrobial effect on a range of microorganisms, including bacteria, fungi, and some viruses. QACs have an antibacterial action in addition to being detergents, enabling the combination of cleaning and disinfection. QAC-based cleaners are non-toxic, highly effective against both gram-positive and gram-negative germs, and have good detergent qualities (Jennings *et al*., 2015). Understanding how disinfectants affect *E. coli* biofilm in water pipes can inform strategies for maintaining clean and safe water system. The present study aimed to detect *E. coli* in water sources, to investigate the ability of the isolated *E. coli* to form biofilm and to study the effectiveness of disinfectants.

MATERIALS AND METHODS

Collection of samples

One hundred swab samples were collected from drinking water pipes and drinkers in different broiler farms at Giza and Dakahlia governorates in Egypt, during the period from 2021 to 2022. A total of 100 swab samples were collected; 50 samples from Giza and 50 samples from Dakahlia, each of them included 25 swabs from water pipes and 25 swabs from drinkers. After sampling each location, the swabs were carefully inserted into a labeled, sterile collection tubes to preserve the sample's integrity. The labeled collection tubes were stored in a cool and insulated container during transport to the laboratory to maintain sample viability at an appropriate temperature (4°C) until further analysis.

Isolation and identification of E. coli isolates (Quinn et al., 2002)

Typically, within a 24-hour timeframe, the samples are subjected to an enrichment step using buffered peptone water broth at 37°C in aerobic conditions for 24 hours. For the selective colonization of *E. coli*, two differential media, MacConkey's agar and Eosin Methylene Blue agar were used. A loopful of each sample's broth was inoculated onto these plates, and they were then incubated at 37°C for 24 hours. Following the incubation, suspected *E. coli* colonies were isolated and preserved for future testing.

The samples were subjected to a battery of biochemical tests, including the indole reaction, methyl red test, Voges Proskauer test, citrate utilization test, catalase test, sugar fermentation test, oxidase test, and urea agar test.

Serological identification of E. coli isolates (Schouler et al., 2012)

The Animal Health Research Institute used the slide agglutination test and conventional monovalent and polyvalent anti-sera to type *E. coli* isolates. *E. coli* antisera. In nutritional agar media, only fresh bacterial cultures from 24-hour colonies were used. Using a kit provided by Safin Antisera Co., the isolates were serotyped using antisera against the *E. coli* somatic (O) antigens (Germany).

Microtiter-plate test for determination of biofilm production

Each of the three wells of a sterile 96-well flat-bottomed plastic tissue culture plate was filled with 200 µl of the bacterial suspension. according to Stepanović *et al*. (2000). Positive control wells only had broth in them plates. For 24 hours, the covered were heated to 37°C. Then, 250 µl of sterile physiological saline was used to wash each well three times after aspirating its contents.

All non-adherent bacteria were eliminated by vigorously shaking the plates. After 30 minutes, 200 µl of crystal violet 0.1% were used to stain the plates for 5 min. Plates were thoroughly washed in deionized water to remove any remaining stains, and then they were allowed to dry at 40°C for 15 minutes. 200 µl of 95% methanol was added to each well to measure the amount of biofilm. The plates were emptied after 15 minutes, then they were left to dry.

After setting the optical density (OD) of the negative control to zero, the optical density of stained adherent bacteria was measured using an ELIZA reader (model: dawn R4, serial no: 610000079) at the wavelength of (OD 620 nm).

The test was repeated three times, averages of the data were calculated, and the standard deviation was determined. OD readings from the fixative, dye, and sterile media were averaged and subtracted from all test results to account for background absorbance.

All tested OD values were subtracted from the mean OD value that was determined from the media control well. These OD values were regarded as a measure of the bacteria that adhere to surfaces and produce biofilm. According to the following equations, the data were used to categorize the strains as non-, weak, moderate, or strong biofilm producers:

Optical density ODc < OD ≤ 2 x Optical density ODc weakly adherent to surface

2 x ODc < OD ≤ 4 x ODc moderately adherent to surface

4 x ODc < OD strongly adherent to surface

Molecular detection of the E. coli adrA gene using PCR

Extraction of DNA

According to the result of biofilm production by the method of the microtiter-plate test, isolates were selected to detect the *adrA* gene as, the gene "*adrA*" encodes for the Adhesion Regulating Protein A. *AdrA* is a protein that plays a role in regulating the adhesion and biofilm formation of *E. coli* bacteria.

DNA Purification and Extraction

Using the PCR Template Kit (Roche Diagnostics, Mannheim, Germany), DNA was extracted from heat-inactivated pure cultures as described by the manufacturer. A NanoDropTM 1000 spectrophotometer was used to measure the amount and quality of DNA (Thermo Fisher Scientific, Wilmington, NC, USA).

Agarose gel electrophoresis with modification specific for *E. coli*

The PCR protocol for amplifying the *adrA* gene involves a series of temperature and time steps. Amplified segment (bp) 1113. Primers sequences 5'-3'; F: ATGTTCCCAAAAATAATGAA, R: TCATGCCGC CACTTCGGT GC. Initially, the DNA sample was heated to 94 C to denature the double-standard DNA into single strands. This was followed by a shorter denaturation step at the same temperature. The temperature was then lowered to 50°C for the annealing step, allowing the primers to bind to the target sequences. The extension step occurs at 72°C, where DNA polymerase synthesizes new DNA strands. This cycle of denaturation, annealing, and extension was repeated for 35 cycles. Finally, a final extension step at 72°C for 10 minutes ensures complete extension of any remaining incomplete DNA strands. The protocol was referenced by Bhowmick *et al.* (2011).

Determination of minimum inhibitory concentration (MIC) of Disinfectants

Three types of disinfectant were used; Zinc oxide nanoparticles (synthesis was done by Nanomaterial Research and Synthesis unit at Animal Health research Institute -ARC, Quaternary ammonia dioxide, Acidifier (Bio- PH) 1ml/liter.

The process of preparing Zinc oxide Nanoparticles

First, glycerol was added to $ZnCl_2$ (65 grams/100 ml) aqueous solutions with a certain mole ratio of glycerol to Zn^{2+} (3.3:1). The $ZnCl_2$-glycerol solution was then added dropwise to an alkaline solution (50%) of NaOH at room temperature while being continuously mechanically mixed to get a final pH value of 12. The reaction then continued for five minutes. After the process, a white emulsion was produced. The white emulsions were centrifuged after being cleaned twice with ethanol and water, respectively (6000 rpm, 10 min). After being dried in an oven at 80°C, ZnO nanoparticles were formed (Wang *et al*., 2018).

The sized and charge investigation of synthesized NPs was detected by NANOTRAC-WAVE II Zeta sizer (MICROTRAC, USA).

Minimum inhibitory concentration determination

We further screened 13 *E. coli* isolates, which are strong biofilm producers, to evaluate the antimicrobial efficacy of the three disinfectants, which were zinc oxide nanoparticles, acidifiers, and quaternary ammonium dioxide, and then the minimum inhibitory concentration for *E. coli* bacteria were compared between them.

The microdilution method was used to determine the lowest inhibitory concentration of ZnO-NPs, acidifiers, and QACs in *E. coli* (Alekish *et al*., 2018). Bacterial cultures were suspended overnight in MH broth with turbidity adjusted to 0.5 McFarland, producing a suspension with about 10^8 CFU/ml (Andrews, 2001). The MIC measured by the minimum inhibitory concentration (MIC) was determined using a 96-well microtiter plate with 12 wells. Each well was filled with 200 µl of MH broth culture. The first wells received 200 µl of a stock solution containing ZnO-NPs, acidifier, and QACs. Subsequently, the ZnO-NPs were serially diluted in each well using a 2-fold dilution method, resulting in concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, and 0.97 µg/ml. This setup allowed for the assessment of the MIC of ZnO-NPs against the tested microorganisms. The microbial suspension was then added in a volume of 200 µl to each well. Following a 24-hour incubation period at 37°C, the microplate's ZnO Nanoparticle, Acidifier, and QAC concentration in the well with no apparent bacterial cell growth was considered to be the MIC. A positive control contained MH Broth medium with the assessed bacterial concentrations, while a negative control just contained the inoculated broth utilized in the investigation.

We used a reference strain of *E. coli* bacteria from Animal Health Research Institute (*E. coli* NCIMB 50034) to compare the efficiency of different disinfectants on the reference strain and strong biofilm *E. coli* bacteria.

RESULTS

E. coli prevalence in examined samples

E. coli infection occurrence in broiler chickens from water pipes and drinkers in broiler farms in Giza and Dakahlia governorates.

20 positive *E. coli* samples from water pipes and 10 from drinkers in Giza, 10 positive samples from water pipes and 10 from drinkers in Dakahlia., the incidence of total positive *E. coli* isolates is 50% equal to 50 isolates.

E. coli serotypes isolated from water pipes and drinkers in broiler farms

Polyvalent and monovalent *E. coli* antisera were used to serotype the isolated *E. coli* and identify its serotype. 18 *E. coli* serogroups were identified in 50 positive samples, The distribution of *E. coli* serotypes along with the corresponding number of isolates and percentages. Among the identified serotypes, O91 exhibited the highest prevalence, accounting for 20% of the total isolates. O78 and O103 followed closely, representing 18% and 6% of the isolates, respectively. Additionally, serotypes O26, O159, and O166 each comprised 8% of the isolates. Serotypes O144, O158, O27, O128, and O126 were less prevalent, each constituting 4% of the isolates. The remaining serotypes (O125, O129, O6, O28, O55, O142, and O123) were each found in only 2% of the isolates. This distribution of *E. coli* serotypes sheds light on their varying prevalence and highlights O91, O78, and O103 as the most commonly encountered serotypes in the study samples.

Biofilm production degree

Following the Microtiter-plate test for *E. coli* positivity, fifty samples were examined to determine the degree of biofilm production which were found to be 13 samples out of 50 (26%) with strong biofilms, 17 isolates out of 50 (34%) had intermediate biofilms, 10 isolates out of 50 (20%) showed weak biofilms and 10 isolates out of 50 (20%) did not produce biofilm (Table 1).

Table 1. Biofilm production rate in *E. coli* isolates.

	Number of examined *E. coli* isolates (n.= 50)	Percentage (%)
Strong biofilm	13	26
Moderate biofilm	17	34
Weak biofilm	10	10
No biofilm	10	10

Detection of adrA gene of E. coli

These results showed that 1113 bp amplification of the *adrA* gene of all examined isolated serotypes from the extracted DNA of *E. coli* (Fig. 1).

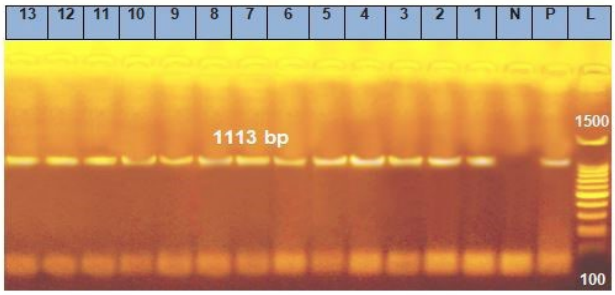

Fig. 1. PCR Agarose gel electrophoresis amplicons after amplification of 1113bp specific for *adrA* gene, Lane p: positive control (*E. coli*: NCIMB 50034) Lane L: DNA ladder (100-1500bp), N: negative control

Identifying the most sensitive biofilm-forming E. coli isolates and testing their resistance to zinc oxide nanoparticles and commercial disinfectants

Zinc oxide nanoparticles were more effective disinfectants than peroxide and acid together on *E. coli* biofilm, however quaternary ammonium dioxide was an effective disinfectant more than zinc oxide nanoparticles or peroxide and acid.

The results showed that zinc oxide nanoparticles exhibited higher antimicrobial activity against *E. coli* biofilms compared to peroxide with acid. QACs, on the other hand, demonstrated the most effective disinfectant activity among the tested agents (Table 2).

Table 2. Results for MIC for different disinfectant.

Number of Reference strain	ZnO-NPs	Acidifier	QACs
	31.25 (µg/ml)	31.25 (µg/ml)	31.25 (µg/ml)
1	500	500	7.81
2	250	500	3.9
3	125	500	3.9
4	125	250	3.9
5	125	250	3.9
6	125	250	3.9
7	125	500	15, 62
8	125	250	31.25
9	125	250	1.95
10	250	250	1.95
11	500	500	7.81
12	125	250	3.9
13	125	500	3.9

DISCUSSION

The present study aimed to investigate the prevalence and characteristics of *E. coli* in swab samples collected from drinking water pipes and drinkers in broiler farms in Giza and Dakahlia governorates in Egypt. The bacteriological examination of samples revealed the presence of *E. coli*. This finding highlights the importance of monitoring the quality of drinking water pipes in poultry farms to prevent the transmission of pathogenic bacteria to the birds and potential risks to human health. The incidence of *E. coli* in broiler farms from the Giza and Dakahlia governorates in this study was 50%. These results agree with that of Aberkane *et al*. (2023), who reported that *E. coli* was isolated from 675/938(72%) examined poultry farms between 2014 and 2020. The highest prevalence of *E. coli* was 98.3% in 2016 and the lowest prevalence of *E. coli* was 45.8% in 2017, while in other years, the prevalence ranged from 63.6% to 73.9%. The highest prevalence of *E. coli* among poultry farms in Egyptian governorates was 98.3% in 2016 (175/178).

The serological identification of the isolated *E. coli* strains using polyvalent and monovalent antisera revealed the presence of various serogroups. The most prevalent serogroup was O91, followed by O78 and O26. These results nearly go hand to hand with the previous studies of Abd El Tawab *et al*. (2015). The diversity of *E. coli* serotypes in the water pipes samples indicates the potential for the presence of multiple sources of contamination, emphasizing the need for effective control measures to prevent the spread of pathogenic strains.

Biofilm production is an important virulence factor in *E. coli*, contributing to its ability to persist and cause infections. In this study, the microtiter-plate test was used to assess the biofilm-forming capacity of the *E. coli* isolates. The results demonstrated that a significant proportion of the isolates (26%) exhibited strong biofilm production, while 34% showed intermediate biofilm formation, and 20% displayed weak biofilm formation. Additionally, 20% of the isolates did not produce biofilm. These findings indicated the presence of *E. coli* strains with varying degrees of biofilm-forming ability, which can have implications for their survival and persistence in the poultry farm environment.

In this study, around 80% of the *E. coli* isolates were able to produce a biofilm; these results agreed with those of Kot *et al*. (2016), who stated that 81.1% of *E. coli* strains could produce biofilm. However, they differ from those of Marhova *et al*. (2010), who found that just 24% of the examined *E. coli* strains produced biofilm in vitro.

The molecular detection of the *adrA* gene, responsible for cellulose synthesis and biofilm formation in *E. coli*, confirmed the presence of this gene in all examined serotypes. This finding further supports the biofilm-forming capacity of the isolated strains and provides molecular evidence for the observed biofilm phenotypes. The presence of the *adrA* gene suggests that the isolates possess the genetic machinery necessary for biofilm formation, which can contribute to their persistence and resistance to disinfection procedures. In the current investigation, PCR analysis of the isolates revealed that 100% of strains possessed the *adrA* gene. This result was in line with the findings of Yin *et al*. (2018), who reported that the prevalence of *adrA* gene was approximately around 75% across all bacteria able to create biofilms.

In this study, thirteen strong biofilm producer *E. coli* strains were tested to evaluate the efficacy of different disinfectants. To evaluate the efficacy of different disinfectants against strong biofilm-forming *E. coli* isolates, minimum inhibitory concentrations (MICs) were determined for zinc oxide nanoparticles, acidifiers, and quaternary ammonium dioxide (QACs). The results showed that zinc oxide nanoparticles exhibited higher antimicrobial activity against *E. coli* isolates compared to peroxide with acid. QACs, on the other hand, demonstrated the most effective disinfectant activity among the tested agents. These findings suggest that QACs can be considered a suitable disinfectant for controlling *E. coli* in poultry farm environments. This result is in line with Sivaranjani *et al*. (2022), the Calgary biofilm apparatus was used to test the adherent population of cells from 12 of the strong biofilm-forming isolates in evaluating the antibacterial activity of four commonly used disinfectants. The formulation of each of the selected disinfectants contained one or more active ingredients. Two of the many active ingredients utilized in the creation of the solution known as Virkon are peroxygenase and surfactants. The bacteria are destroyed by rupturing the cell wall by oxidizing sulfur bonds in proteins and enzymes. Virocid is a synergistic mixture of two different QACs, glutaraldehyde, and isopropanol that functions as a broad-spectrum disinfectant. Although glutaraldehyde and functional thiol and amine groups of proteins interact synergistically, QACs start the bactericidal activity by interacting with negatively charged cell membranes. According to the findings, at low concentrations, Virocid and Virkon had exceptional efficiency against the planktonic and biofilm cells of almost test strains, most likely because of the synergistic combination of several ingredients.

The effectiveness of 28 different disinfectants, individually or

in combination, was tested against avian *E. coli* strains. In vitro, testing was done both with and without serum as the source of organic material. The most effective antibacterial agents against almost strains examined included povidone-iodine (releasing 1% of the available iodine), 1% potassium permanganate, 70% ethanol, 2% chlorhexidine digluconate, and three commercial formulations based on quaternary ammonium compounds + formaldehyde or cresol derivates. These formulations decreased bacterial populations by more than 106 times (6-$\log_{10}$) Despite the existence of organic matter. Among the substances studied individually, these commercial compounds, along with ethanol and chlorhexidine, may be beneficial for implementing environmental controls to prevent *E. coli* infection (Martínez-Martínez *et al*., 2016).

Further testing for resistance to four different marketable disinfectants was done on 12 of these isolates (7 systemic + 5 cecal) by Sivaranjani *et al*. (2022). Each disinfectant, which ranged from 0.0016- 0.0031% for Virocid, 0.125-0.25% for Virkon, 1.9-3.8 ppm for the quaternary ammonium disinfectant DDAC, and 0.031-0.063% for H_2O_2, could kill and decreased the microbial activity for planktonic cells of almost *E. coli* isolates at small doses. The minimum bacterial concentration (MBC) values of all tested disinfectants were either identical to the matching MIC values or one serial dilution higher. We believed that these results could have been slightly confusing for MIC determination and needs antimicrobials to be exposed for 24 hours.

According to Alekish *et al*. (2018), ZnO-NPs had MIC and MBC values of 3.9 µg/ml and 7.81 µg/ml against *S. aureus* respectively. ZnO-NPs had an antibacterial effect against *E. coli* MIC and MBC were 31.25 µg/ml and 62.5 µg/ml, respectively. These findings resemble earlier research by Ibrahem *et al*. (2017). Also, these results are consistent with earlier findings showing that ZnO-NPs made Gram-positive bacteria more responsive and susceptible than Gram-negative bacteria (Liu *et al*., 2009). In general, it has been determined that the size and concentration of ZnO-NPs have a significant efficacy on their inhibitory activity (Palanikumar *et al*., 2014).

Reference strain that was used as a control strain exhibited MIC of the original stock towards three different disinfectants, these results explained that the reference strain was sensitive to zinc oxide nanoparticles, acidifier, and quaternary ammonium dioxide disinfectants (Table 2) and showed the MIC value at 31.25 µg/ml concentration, this result in the line with Alekish, M., *et al*. (2018) reported that ZnO-NPs have antibacterial activity against multidrug-resistant *E. coli* that was isolated from ovine subclinical mastitis when used at concentrations of 31.25 µg/ml.

When ZnO particles are reduced in size to the nanometer range, they begin to show notable antibacterial properties. Once within the bacterial cell, nanosized ZnO interacts with the surface and/or the core of the bacteria and then exhibits specific bactericidal mechanisms. The postulated mechanism of action of zinc oxide is the generation of reactive oxygen species, which enhances membrane lipid peroxidation, results in membrane leakage of reducing sugars, DNA, and proteins, and decreases cell viability (Sirelkhatim *et al*., 2015).

The cell membrane is the point of action for quaternary ammonium compounds, which interact with phospholipids to cause leaking and coagulation of the cytoplasm (Vijayakumar and Sandle, 2019).

By altering the pH, acidifiers in feed slow the growth of harmful bacteria and reduce microbial competition for host resources. Below pH 5, the growth of most pH-sensitive bacteria, including *E. coli*, *Salmonella*, and Clostridium perfringens, is reduced while acid-tolerant ones persist.

The development of bacterial *E. coli* and *S. aureus* monospecies biofilms was inhibited on nano-ZnO and nano-ZnO/Ag composite-enabled surfaces in a dose-dependent manner; thinly coated nano-ZnO surfaces were less inhibitory than densely coated ones, and the decrease of biofilm development was accompanied by antibacterial action against planktonic cells. Human keratinocytes did not significantly reduce yeast biofilm formation, and they were not cytotoxic to the surfaces (Rosenberg *et al*., 2020).

According to Shakerimoghadda *et al*. (2017), stronger biofilm-forming uropathogenic *Escherichia coli* (UPEC) isolates can be inhibited from forming biofilm by ZnO-np at the MIC level. Almost 20% of the isolates with strong biofilms fully inhibited biofilm development, compared to 14% and 16% of the isolates with moderate or weak biofilms, respectively. The authors added that, in UPEC isolates with strong biofilms, the sub-MIC concentration of ZnO-np significantly reduces flu gene expression but was unable to prevent biofilm formation This result agrees with our results which showed that the effect of ZnO-np had a low effect on the isolates with strong biofilm.

This study provides valuable insights into the prevalence, serotypes, biofilm-forming capacity, and resistance patterns of *E. coli* isolated from drinking water pipes and drinkers are sources in broiler farms. The results emphasize the need for continuous monitoring of water quality and the implementation of effective disinfection strategies to prevent the transmission of pathogenic strains and minimize the risk of poultry-related infections. The findings regarding the efficacy of different disinfectants can inform the development of targeted interventions for controlling *E. coli* biofilms in poultry farm settings.

CONCLUSION

The findings of the study highlight the significant prevalence of *E. coli* in water samples from broiler farms, indicating a potential risk of waterborne *E. coli* infections in these environments. The identification of multiple *E. coli* serotypes, with serogroup O91 being the most widespread, underscores the diverse nature of the pathogen present. Furthermore, the study revealed varying abilities of the isolated *E. coli* strains to produce biofilms, supported by the presence of the biofilm-related *adrA* gene. The evaluation of disinfectant effectiveness demonstrated that zinc oxide nanoparticles exhibited the highest efficacy in controlling *E. coli* biofilms, followed by quaternary ammonium dioxide, while the combination of peroxide and acid showed lesser effectiveness. These findings emphasize the importance of selecting appropriate disinfection strategies to combat *E. coli* biofilm contamination in water systems within broiler farms. Overall, the study contributes valuable insights into the potential health risks and management strategies associated with *E. coli* in water sources, providing essential information for future preventive measures and interventions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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