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Environmental Surveillance of *Escherichia Coli* in Chicken Water Systems: Correlations Between Biofilm Formation And Antibiotic Resistance

Aya Nasser Ibrahim[1], Hanan S. Khalefa[1]*, Zeinab Ahmed[2] and Sherief T. Mubarak[1]

[1]*Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, 12211, Egypt.*

[2] *Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.*

Abstract

THE water supply system with inadequate maintenance and poor water quality management can lead to significant microbial risks. Biofilm-forming strains of *Escherichia coli* (*E. coli*) have a substantial negative impact on both animal health and water quality. This study aimed to characterize biofilm-associated *E. coli* in poultry water systems by assessing their prevalence, the molecular characteristics of biofilms, and susceptibility to antimicrobial agents. Thirty biofilm samples were collected from the water supply lines. Total viable count, coliform count, fungal count, and total *staphylococcus* spp. Were all analyzed as part of the microbiological analyses. *E. coli* was isolated using selective media, and its molecular identity was verified by amplifying the *16s RNA* gene. Biofilm-related genes (*FimH* and *LuxS*) were identified by PCR, and biofilm development was measured using tissue culture plate methods. *E. coli* was detected in 63.3% of samples, and genes linked to biofilms are significantly present (*FimH* in 68.4% and *LuxS* in 73.6%). Phylogenetic analysis indicated that groups A and D were predominant. The isolates tested showed variation in biofilm formation, with 47.4% identified as strong producers, 21% as moderate producers, and 31.6% as weak producers. Cefepime demonstrated the greatest resistance (78.9%), whereas azithromycin, gentamicin, and amoxicillin-clavulanic acid displayed significant susceptibility. Correlations were observed between antibiotic resistance profiles, biofilm formation, and the presence of biofilm-associated genes. Our findings indicate the persistence of biofilm-forming multidrug-resistant *E. coli* in chicken's farms water systems, highlighting the importance of enhancing water hygiene and monitoring to reduce public health risks.

Keywords: *E. coli*, Biofilm, Microbial Ecology, Water Hygiene, Antibiotic resistance.

Introduction

Drinking water distribution systems in commercial poultry farms create favorable conditions for the development of biofilms, which protect bacteria from environmental stress, disinfectants, and antimicrobial agents [1, 2]. Once established, these biofilms can act as persistent reservoirs of microbial pathogens that threaten animal health and food safety [3,4]. *Salmonella*, *Listeria*, and *Escherichia coli* are commonly found in chicken waterline biofilms [4].

Among the bacterial species frequently detected in poultry production systems, *E. coli* is of particular significance. Although many strains are commensals, pathogenic variants can cause colibacillosis in poultry and contribute to significant economic losses [5, 6]. More importantly, *E. coli* serves as an indicator and reservoir of antimicrobial resistance (AMR) genes that are transferable to other bacteria, including those infecting humans [7], and the presence of ARGs in biofilms poses significant health risks to both humans and animals [8]. Therefore, the contamination of poultry environments with multidrug-resistant (MDR) *E. coli* is not only a veterinary concern but also a public health challenge within the One Health framework [9]. Understanding these interactions is essential for developing strategies to monitor and mitigate the impact of antibiotic-resistant bacteria in water supply systems [10].

*Corresponding authors: Hanan S. Khalefa, E-mail: hanansaad04@gmail.com, Tel.: 01125702638
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The ability of *E. coli* to persist and disseminate in poultry environments is strongly linked to its capacity for forming biofilms. Biofilm-associated genes, such as *luxS* and *fimH*, play crucial roles in quorum sensing and adhesion, enabling bacterial communities to colonize surfaces and resist antimicrobial treatments [11–13]. *FimH* encodes the major adhesin of type 1 fimbriae, a well-characterized determinant of initial surface attachment and microcolony formation, facilitating early biofilm development on abiotic surfaces in water systems [14]. These traits enhance survival within poultry waterlines, creating continuous exposure risks for chicken and opportunities for zoonotic issue through contaminated meat, handlers, and the wider environment [15].

Several studies have investigated AMR in *E. coli* isolated from poultry farm environment [16–17]. However, limited attention has been given to the role of water supply systems as hidden sources of biofilm-forming and drug-resistant strains. Understanding this reservoir is critical, as waterlines may serve as a point of entry for resistant bacteria into the food chain, found in agricultural runoff, and can endanger human and wildlife populations [18, 19]. Given these concerns, this study aimed to investigate and characterize the antibiotic resistance profiles and biofilm-forming capabilities of *E. coli* strains isolated from biofilm samples collected from waterline systems of different chicken farms.

Material and Methods

Sampling and Sample Preparation

Thirty biofilm samples were collected from the inlet sections of the water supply lines at different times from 15 chicken poultry farms, including eight broiler farms, five layer farms, and two broiler breeder farms. The sampling sites were classified according to water source as follows: (1) untreated water (wells, n = 6), (2) treated water (groundwater after treatment, n = 8), and (3) tap water (public water, n = 16). Samples were collected using sterile sponge sticks under aseptic conditions. The sponge stick method involved swabbing the internal surface of the water lines near the water entry point with a 5 × 2 × 2 cm sponge that was pre-moistened with 10 mL of sterile saline. Approximately 20 cm² of the internal water line surface was swabbed to collect the biofilm sample, as described by Ibrahim et al. [20] and Maes, [21]. The samples were immediately placed in sterile containers, transported in an icebox at 4°C, and processed within 24 h of collection. In the laboratory, the samples were vortexed for two minutes to achieve thorough homogenization. A single dilution from a series of tenfold dilutions was analyzed as outlined by Maes, [21].

Bacterial Count and Isolation

The spread plate method was used as recommended by the International Organization for Standardization, ISO [22]. A 0.1 mL dilution of each selected sample was spread on four different microbiological media plates. Total viable colonies (TVC) were determined using nutrient agar plates incubated at 37°C for 24 h. The total coliform count (TCC) was determined using MacConkey agar, which was incubated at 37°C for 24 h. To determine the total fungal count (TFC) of yeast and mold, Sabouraud's dextrose agar supplemented with chloramphenicol was incubated at 30°C for 48 h, as described by Ibrahim et al. [23]. *Staphylococcus* species (TSC) were isolated on Mannitol salt agar and incubated at 37°C for 18–72 hours. Approximately 30 to 300 colony-forming units (CFUs) can be counted on a plate according to Messer et al. [24].

Isolation and identification of E. coli from biofilm water samples

All biofilm samples were tested for the presence of *E. coli.* To isolate *E. coli.* species, samples were enriched with tryptic soy broth (Oxoid, Basingstoke, UK) for 18–24 h at 37°C. A loopful of each tube exhibiting turbidity was streaked onto MacConkey Lactose Agar (Oxoid, Basingstoke, UK) plate. In accordance with Zinnah et al. [25], lactose-fermenting pink and smooth colonies were streaked onto Eosin Methylene Blue (EMB; Oxoid, Basingstoke, UK) agar plate. After 24 h of incubation at 37°C, *E. coli.* colonies appeared metallic green. Suspicious colonies were selected for further identification based on morphological characteristics. Biochemical techniques, such as the urease test, Voges-Proskauer and citrate consumption tests, methyl red, and indole synthesis, were used to identify the bacterial species.

Molecular identification, phylogrouping of E. coli strains, and detection of biofilm-encoding genes

Molecular identification was performed by amplifying the *E. coli* using *16S rRNA* gene, as described by Wang et al. [26] (Table 1). Phylotyping of different *E. coli.* strains was performed using the Clermont method based on the presence or absence of three specific genes (*chuA*, *yjaA*, and *TspE4.C2*). The phylogenetic grouping method can assign *E. coli.* strains into four phylogroups: A (*chuA-/TspE4.C2−*), B1 (*chuA-/TspE4.C2+*), B2 (*chuA+/yjaA+*), and D (*chuA+/yjaA−*) according to Clermont et al. [27]. Biofilm-encoding genes (*FimH* and *LuxS*) were investigated in *E. coli* strains using polymerase chain reaction (PCR) (Table 1).

Antibiotic sensitivity test

Antimicrobial susceptibility testing was conducted using the disc diffusion technique on Muller–Hinton agar plates (HiMedia, India) with

standardized antibiotic-impregnated discs: cefepime (CPM, 30 µg), tetracycline (TE, 30 µg), azithromycin (AZM, 15 µg), gentamicin (GEN, 10 µg), ampicillin (AMP, 10 µg), and amoxicillin/clavulanic acid (AMC, 20/10 µg). The assay was performed in compliance with the protocol described in the protocol guidelines according to the Kirby-Bauer method [30]. A bacterial inoculum was prepared from a freshly grown 24-hour culture and adjusted to a density of 1.5×10^8 colony-forming units per cubic centimeter, equivalent to the 0.5 McFarland turbidity reference. The inoculated plates were incubated at 35°C for 20–24 h to allow sufficient growth. The zones of inhibition were measured and interpreted according to the most up-to-date criteria for antimicrobial susceptibility assessment by Clinical and Laboratory Standards Institute [CLSI, 31].

Tissue culture plate method

The quantitative method described by Christensen et al. [32], which is regarded as the gold standard for biofilm detection, was adopted in this study. All 19 pure isolates from agar plates were inoculated into 10 mL of Trypticase Soy Broth supplemented with 1% glucose and incubated at 37°C for 24 h. After incubation, the cultures were diluted 1:100 with freshly prepared medium, and 200 µL of each diluted sample was transferred into individual wells of sterile, flat-bottomed 96-well polystyrene tissue culture-treated plates. The control strains underwent identical incubation, dilution, and inoculation procedures, whereas the negative control wells contained only sterile broth. The plates were then incubated at 37°C for 24 h. After incubation, the contents of each well were gently removed by tapping, and the wells were washed four times with 0.2 mL of Phosphate-Buffered Saline (PBS, pH 7.2) to eliminate non-adherent bacteria. Biofilms attached to the well surfaces were fixed using 2% Sodium Acetate and stained with 0.1% Crystal Violet stain. Excess stain was removed with deionized water, and after air-drying the microplate at room temperature, 150 µL of 95% ethanol was added to each well to solubilize the dye bound to adherent cells. The plates were immediately covered to minimize evaporation and maintained at room temperature for at least 30 min without agitation. This procedure facilitated the elution of dye from bacteria attached to both the base and walls of the wells, allowing for indirect quantification of biofilm biomass. Floating biofilms (pellicles) formed at the air–liquid interface in static cultures have also been noted as a distinct biofilm type [43]. The optical density (OD) of the stained biofilm was measured at 570 nm using a microenzyme-linked immunosorbent assay auto reader. All experiments were performed in triplicate and repeated three times. Biofilm production was interpreted according to the criteria described by Stepanović et al. [33].

Statistical analysis

A statistical analysis of the data was performed using the Statistical Analysis System, USA (Version 18). Bacteriological counts were displayed along with their mean and standard error (SE). Tukey's post-hoc test was used to compare significant means ($p< 0.05$). The "Heatmap" R package (version 4.2.2, R Foundation for Statistical Computing) was used to group isolates based on their AMR, and biofilm traits.

Results

Bacterial analysis of different biofilm samples revealed significant variations in microbial counts across untreated, treated, and tap water samples. For the Total Viable Count (TVC), treated water showed the highest log 10 bacterial load 8.10 ± 0.16 CFU/ml . This value was significantly greater than the counts observed in both untreated water, which measured 6.70 ± 0.90 CFU/ml, and tap water, which recorded 6.25 ± 0.27 CFU/ml. The *p*-value of 0.01 indicates that these differences are statistically significant. Similarly, the Total Coliform Count (TCC) was highest in treated water 7.20 ± 0.20 CFU/ml, followed by untreated water 6.18 ± 0.95 CFU/ml, and lowest in tap water 5.33 ± 0.28 CFU/ml, with significant differences (p=0.046). For Total Fungal Count (TFC), treated water showed the highest count 6.51 ± 0.56 CFU/ml, significantly higher than both untreated 3.71 ± 0.88 CFU/ml, and tap water 4.49 ± 0.34 CFU/ml, (p=0.007). However, the Total Staphylococcus Count (TSC) did not differ significantly among the samples (p = 0.517).

According to our results in Table 3, 63.3% of water samples tested positive for *E. coli*, with tap water showing the highest contamination level at 68.7%. Phylogenetic analysis of 19 isolates indicated that 37% belonged to group A, 32% to group D, and 26% to group B2, with 5% untypeable. Furthermore, a significant proportion of the positive samples (73.6%) contained the *Lux* gene, and 68.4% had the *Fim* gene, both associated with biofilm formation as shown in Figure 1. These findings raise concerns regarding the effectiveness of current water treatment methods, particularly in tap systems, as biofilm-forming *E. coli* strains may resist disinfection.

The antimicrobial susceptibility profile shown in Figure 2 reveals varying levels of resistance among the tested antibiotic. CPM (Cefepime) showed the highest resistance rate at 78.9%, with no susceptible isolates, indicating poor effectiveness. In contrast, AZM (Azithromycin), GEN (Gentamicin), and AMC (Amoxicillin-clavulanic acid) demonstrated high susceptibility rates of 84.2%, suggesting that they are the most effective against the tested organisms. TE (Tetracycline) and AMP (Ampicillin) had moderate susceptibility rates of 68.42% and 73.68%, respectively, but still showed notable resistance.

Intermediate resistance was observed only for CPM (21.1%), AZM (5.26%), and AMC (15.78%).

Among the tested isolates, the ability to form biofilms varied, with 47.4% classified as strong producers, 21% as moderate producers, and 31.6% as weak producers. This distribution suggests that nearly half of the isolates possess a high potential for biofilm formation, which may contribute to increased resistance to antimicrobial agents and persistence in the water systems. These findings highlight the importance of monitoring and controlling biofilm-forming bacteria in environmental and clinical settings.

Among the 19 *E. coli* isolates, the most common phylotype was group A (seven isolates), followed by group D (six), group B2 (five), and one untyped isolate. The ability to form biofilms varied, with strong producers being the most prevalent (9 isolates), followed by weak (6) and moderate (4) producers. The *Lux* gene was present in 14 isolates, whereas the *Fim* gene was present in 13, indicating a high potential for biofilm formation. In terms of antibiotic resistance, cefepime (CPM) showed the highest resistance (15 isolates), followed by tetracycline (TE), ampicillin (AMP), gentamicin (GEN), and azithromycin (AZM). These results suggest a strong correlation between biofilm-forming ability, gene presence, and multidrug resistance, particularly in isolates of phylotypes A and D.

The correlation heatmap illustrates the relationships between antibiotic resistance profiles, biofilm formation, and the presence of biofilm-associated genes (*Lux* and *Fim*) in *E. coli* isolates. Cefepime (CPM) showed weak or negative correlations with biofilm (-0.05), Lux (-0.02), and Fim (-0.35), suggesting a limited association with biofilm-forming traits. Azithromycin (AZM) displays moderate positive correlations with Tetracycline (TE) (0.43) but strong negative correlations with Gentamicin (GEN) (-0.73), Ampicillin (AMP) (-0.50), Amoxicillin-Clavulanic acid (AMC) (-0.73), biofilm (-0.17), Lux (-0.25), and *Fim* (-0.07), indicating that resistance to Azithromycin may inversely relate to biofilm formation and gene presence. The observed trends suggest variable interactions between resistance and biofilm-related traits.

Discussion

Regular water quality testing is crucial as it helps detect harmful substances that may negatively impact animal well-being or performance [1]. In poultry farming, water serves multiple vital functions: sustaining hydration, regulating body temperature, supporting digestion, delivering medications and vaccines. Moreover, water quality directly influences product quality and breeding efficiency [34]. One of the key contributors to this issue is microbial buildup within drinker lines, which gradually develops owing to the accumulation of dirt, rust, drug residues, minerals, and algae. These conditions foster biofilm formation in water distribution systems [10]. In poultry systems, biofilms present a serious challenge as they act as reservoirs for both pathogenic and opportunistic microorganisms, including *Acinetobacter*, *Campylobacter*, *Escherichia coli*, *Enterobacter*, *Salmonella*, and *Pseudomonas* [35]. Their presence in drinking water systems (DWS) complicates farm management by causing issues such as clogged water lines and filters, leaking drinkers, and increased moisture levels in litter [4].

Bacteriological assessment of water samples revealed significant differences between untreated, treated, and tap water. Treated water surprisingly recorded the highest total viable count (TVC) at 8.10 ± 0.16, significantly higher than that of untreated water (6.70 ± 0.90) and tap water (6.25 ± 0.27), (p=0.01). This may be attributed to the persistence of biofilm-associated microorganisms, which diminish the apparent effectiveness of disinfection. Factors such as limited disinfectant penetration, short contact times, and uneven distribution of disinfectants throughout the waterline system could allow the survival of entrenched bacterial populations [36]. Furthermore, residual chlorine levels are often inadequate to completely disrupt established biofilms or manage high microbial and organic loads. Under optimal conditions, such as favorable temperature and nutrient availability, biofilm regrowth occurs rapidly in poultry waterlines [37]. In contrast, Maharjan et al. [38] reported that maintaining a continuous low level of residual disinfectant, combined with thorough cleaning between flocks, can effectively reduce microbial persistence. Additionally, studies have demonstrated a strong association between the presence of biofilms and elevated aerobic plate counts (APC), with notable variations observed across different poultry farms [4].

In drinking water distribution systems (DWDS), the final storage tank represents the last point of containment before water reaches consumers, serving as a critical barrier for maintaining public water quality. However, this section often experiences reduced hydraulic flow, stable thermal conditions, and extended water retention times, which collectively promote nutrient accumulation and microbial proliferation. These factors contribute to an increased risk of biological contamination, as evidenced by recent studies reporting microbial presence in final-stage storage tanks [39].

This study demonstrates that poultry water systems serve as important reservoirs for biofilm-forming and antimicrobial-resistant *E. coli*. The recovery of *E. coli* from 63.3% of biofilm samples confirms that drinking waterlines represent a favorable niche for bacterial persistence and proliferation in poultry production. Similar findings

have been reported in other poultry and livestock environments, emphasizing the role of water supply systems in pathogen transmission [16-17]. Among the 19 *E. coli* isolates, phylogroups A, D, and B2 were the most prevalent, with 7 (37%), 6 (32%), and 5 (26%) isolates, respectively. Phylogenetic analysis revealed a predominance of group A isolates, followed by groups D and B2. Previous studies have shown that phylogroups D and B2 often harbor virulence-associated genes, while group A is generally considered commensal. The detection of biofilm-forming and multidrug-resistant isolates within both commensal and pathogenic phylogroups suggests that waterlines may facilitate the persistence and dissemination of diverse *E. coli* lineages, some with zoonotic potential [40].

Antimicrobial susceptibility testing revealed alarming resistance rates, with 78.9% of isolates resistant to cefepime, followed by resistance to ceftriaxone, ciprofloxacin, and tetracycline. These findings are consistent with the widespread occurrence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* in poultry reported globally [41]. Resistance to critically important antimicrobials for human medicine highlights the public health risk of poultry-associated *E. coli*, as resistant strains may be transferred to humans through direct contact, the food chain, or the environment [42]. Conversely, the continued effectiveness of azithromycin, gentamicin, and amoxicillin–clavulanic acid provides potential options for treatment, although their overuse must be carefully avoided. The observed resistance profile aligns with the broader trends of higher resistance in environmental and wastewater-associated *E. coli* to third- and fourth-generation cephalosporins, with variable susceptibility to other antibiotic classes depending on local selection pressures and the biofilm lifestyle [43].

In the study, biofilm related genes *Lux* and *Fim* were found in 74% and 68% of tested isolates, indicating a significant potential for biofilm formation, particularly in treated and tap water samples of *E. coli*. This prevalence highlights public health risks and the shortcomings of current water treatment methods. The presence of these genes enhances bacterial resilience against disinfectants, necessitating biofilm-targeted strategies that disrupt quorum sensing and remove biofilm matrices [44]. Routine monitoring of *LuxS* and *FimH*, alongside traditional methods, could improve detection of high-risk biofilm-forming populations, and molecular surveillance might identify samples with high levels of biofilm-capable *E. coli* for targeted interventions [45].

Correlation analysis further demonstrated significant associations between biofilm-forming ability, carriage of *luxS* and *fimH*, and multidrug resistance. This supports the hypothesis that biofilm formation contributes not only to bacterial persistence but also to the maintenance and horizontal transfer of resistance genes within microbial communities [46]. Such findings emphasize the critical link between biofilm ecology and AMR epidemiology in poultry production systems. From a One Health perspective, the persistence of MDR, biofilm-forming *E. coli* in poultry water systems is of major concern. Waterlines act as hidden reservoirs that can perpetuate infection cycles in flocks, reduce antimicrobial efficacy, and ultimately increase the likelihood of zoonotic spillover to humans [47]. The presence of resistant strains in water systems may also facilitate their spread to farm workers, surrounding environments, and the wider food chain [48].

Conclusion

This study demonstrates that poultry waterlines serve as important reservoirs for biofilm-forming *E. coli* carrying antimicrobial resistance determinants. The high prevalence of *luxS* and *fimH* genes, together with strong biofilm-forming capacity, underlines the adaptive mechanisms that enable persistence in poultry environments. The detection of multidrug resistance, including resistance to critically important antimicrobials, highlights a serious risk of transmission of resistant strains from poultry to humans through the food chain, occupational exposure, or environmental contamination. Addressing this challenge requires integrated approaches combining improved farm hygiene, continuous AMR surveillance, and the exploration of novel antibiofilm and antimicrobial strategies. These interventions are vital to protect poultry productivity and, more importantly, to safeguard public health within the One Health framework.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Cairo University, Egypt (No approval is required since the samples were taken from the waterline and not from the chicken).

TABLE 1. Primer names, target genes, oligonucleotide sequences, and the product size used in PCR

Target gene	Oligonucleotide sequences (5′–3′)	Product size (bp)	References
Molecular identification of *E. coli*			
16S rRNA	F: CCCCCTGGACGAAGACTGAC R: ACCGCTGGCAACAAAGGATA	401	[26]
Phylogroup encoding genes			
ChuA	F:GAC GAA CCA ACG GTCAGG AT R:TGC CGC CAG TAC CAAAGA CA	279	[27]
yjaA	F:TGA AGT GTC AGG AGA CGC TG R:ATG GAG AAT GCG TTC CTC AAC	211	
TspE4	F:GAG TAA TGT CGG GGCATT CA R:CGC GCC AAC AAA GTATTA CG	152	
Biofilm encoding genes			
FimH	F: TGC AGA ACG GAT AAG CCG TGG R: GCA GTC ACC TGC CCT CCG GTA	508	[28]
Luxs	F: ATG CCG TTG TTA GAT AGC TTC A R: GAT GTG CAG TTC CTG CAA CTT C	513	[29]

TABLE 2. Microbial quality parameters (total viable count (TVC), total coliform count (TCC), Total Fungal count (TFC), and Total *Staphylococcus* Count of biofilm samples collected from three water sources in the survey farms.

Bacterial count	Water source	Mean±SE log 10 (CFU/ml)	Minimum	Maximum
TVC	Untreated Water	6.70 ± 0.90^{ab}	5.17	8.94
	Treated Water	8.10 ± 0.16^{a}	7.3	8.46
	Tap Water	6.25 ± 0.27^{b}	5.17	7.85
	p value	0.01		
TCC	Untreated Water	6.18 ± 0.95^{b}	4.5	8.76
	Treated Water	7.20 ± 0.20^{a}	6.6	7.93
	Tap Water	5.33 ± 0.28^{c}	4.3	7.48
	p value	0.046		
TFC	Untreated Water	3.71 ± 0.88^{b}	2	6.69
	Treated Water	6.51 ± 0.56^{a}	3.95	7.61
	Tap Water	4.49 ± 0.34^{b}	1.79	5.79
	p value	0.007		
TSC	Untreated Water	5.74±0.99	4.1	8.51
	Treated Water	5.97±0.21	5.3	6.9
	Tap Water	5.05±0.58	0	7.29
	p value	0.517		

TVC: Total viable count; TCC: Total coliform count; TPC: Total Pseudomonas Count; TSC: Total Staphylococcus Count; TFC: Total Fungal Count CFU/ml: Colony forming unit per milliliter.
[a, b, ab] the mean values with different superscript letters in the same row differ significantly at $p \leq 0.05$.

TABLE 3. Prevalence of *E. coli* and Biofilm-Associated Genes (*Lux* and *Fim*) in Water's biofilm Samples.

Water source	Total samples	Positive *E. coli* (%)	*16S rRNA* gene	Biofilm-Associated Genes	
				Lux	*Fim*
Untreated Water	6	3 (50)	3	2	2
Treated Water	8	5 (62.5)	5	4	3
Tap Water	16	11 (68.7)	11	8	7
Total	30	19 (63.3)	19	14/19 (73.6)	13/19 (68.4)
**P* value (chi-square)	0.717				

*The result is not significant at $p < .05$.

TABLE 4. Characterization of *E. coli* Isolates Based on Phylotype, Antibiotic Resistance, Biofilm Formation Ability, and Presence of Biofilm-Associated Genes *(Lux, Fim)*

Samples	Phylotype	Antibiotic resistance	Biofilm formation ability	Biofilm gene (Lux, Fim)
2	B2	CPM, TE	Weak	Absent
3	B2	CPM, TE, AMP	Moderate	Absent
4	B2	CPM	Weak	Absent
5	D	TE, GEN, AMP	Moderate	Fim
6	A	CPM, AZM, TE, GEN, AMP	Weak	Lux
7	D	CPM, TE, AMP	Weak	Lux, Fim
9	D	CPM, AZM, TE, GEN, AMP	Moderate	Lux, Fim
10	D	CPM	Strong	Lux, Fim
16	untyped	-----	Strong	Lux, Fim
28	D	-----	Weak	Lux, Fim
32	A	----	Strong	Lux, Fim
36	A	CPM	Strong	Lux
40	B2	CPM	Strong	Lux, Fim
46	D	CPM	Strong	Lux, Fim
47	A	CPM	Strong	Lux, Fim
50	A	CPM	Strong	Lux, Fim
55	A	CPM	Moderate	Lux, Fim
64	B2	CPM	Strong	Lux, Fim
66	A	CPM	Weak	Absent

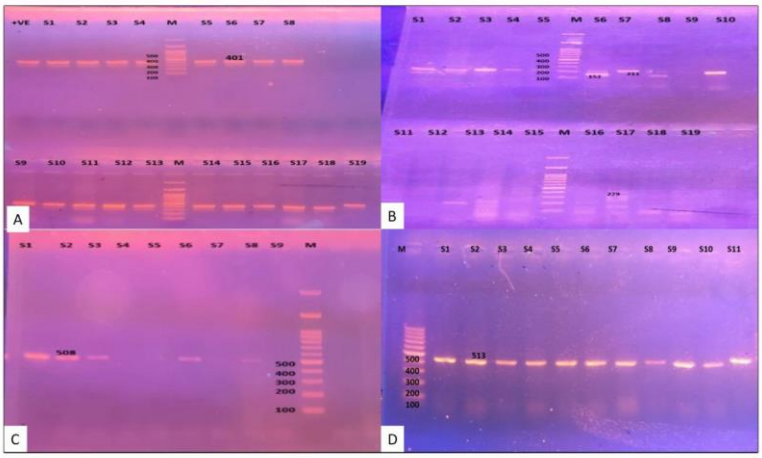

Fig. 1. Gel electrophoresis image confirming the molecular characterization of *E. coli* isolates from biofilm samples. A) Confirmation of 19 *E. coli* isolates based on 16S rRNA gene analysis; B) Phylogenetic grouping of the 19 isolates, showing distribution among different phylogroups A, D, and B2. C) Detection of the Fim gene, with positive samples at 508 bp. D) Detection of the Lux gene associated with biofilm formation, with *E. coli-positive* samples at 513 bp.

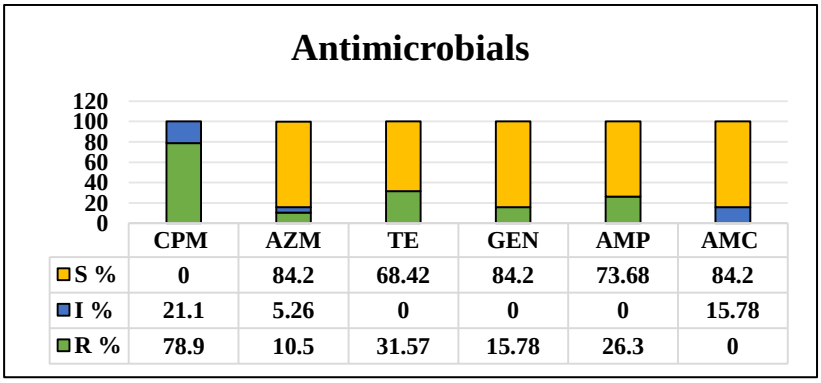

Fig. 2. Bar chart illustrating the antimicrobial susceptibility profile of 19 *E. coli* isolates against six antibiotics: CPM (cefepime), AZM (azithromycin), TE (tetracycline), GEN (gentamicin), AMP (ampicillin), and AMC (amoxicillin-clavulanic acid).S (susceptible), I (intermediate), and R (resistant).

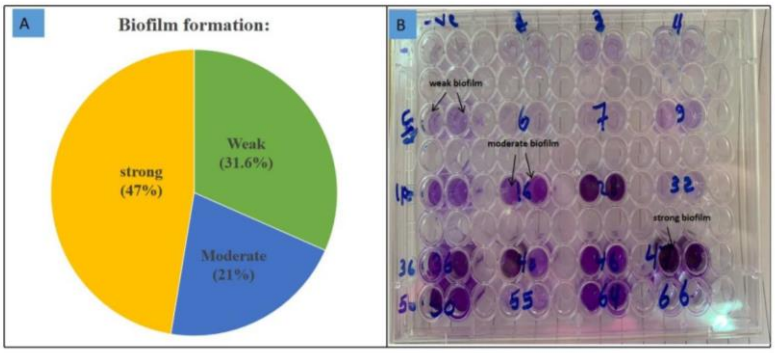

Fig. 3. Assessment of biofilm formation among *E. coli* isolates. (A) Pie chart illustrating the distribution of biofilm-forming capacity among the samples. (B) Representative image of a 96-well microtiter plate showing the crystal violet-stained biofilms. The wells were labeled and classified based on the staining intensity as weak, moderate, or strong biofilm producers.

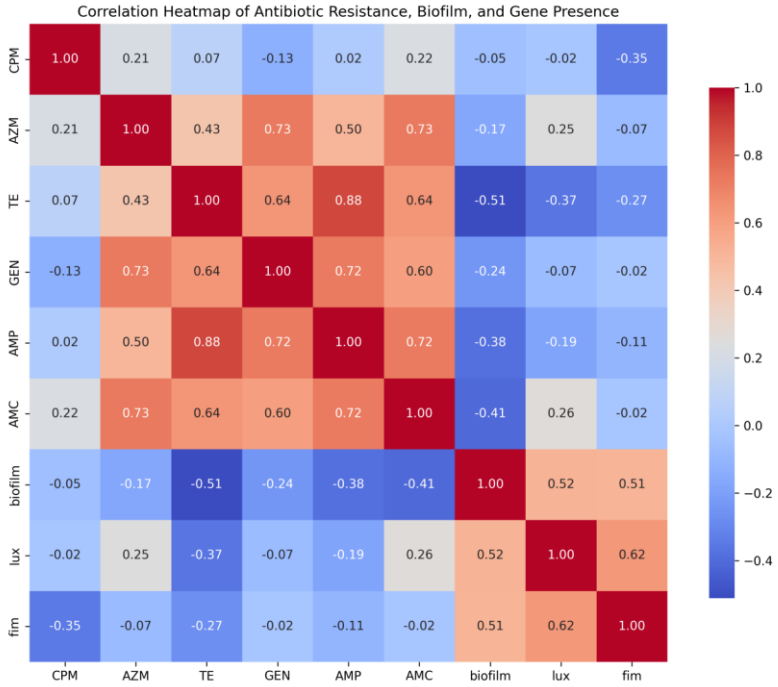

Fig. 4. Interpretation of Correlation Heatmap: -Strong positive correlation between Lux and Fim (gene presence), suggesting co-occurrence. A moderate positive correlation was observed between biofilm and Lux, indicating that biofilm strength may be related to gene presence. A negative correlation was observed between some antibiotics (e.g., Cefepime 30) and biofilm, suggesting that resistance may reduce biofilm formation. Most antibiotics showed a low correlation with each other, indicating independent resistance mechanisms.

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المراقبة البيئية لبكتيريا الإشريكية القولونية في أنظمة مياه الدواجن :العلاقات بين تكوين الأغشية الحيوية ومقاومة المضادات الحيوية

اية ناصر ابراهيم [1]، حنان سعد خليفة[1]*، زينب احمد [2] و شريف توفيق مبارك[1]

[1] قسم الصحة والرعاية البيطرية، كليه الطب البيطري، جامعه القاهرة، مصر.

[2] قسم الامراض المشتركة، كليه الطب البيطري، جامعه القاهرة، مصر.

الملخص

يمكن أن يؤدي نظام إمداد المياه، عند غياب الصيانة الجيدة وسوء إدارة جودة المياه، إلى مخاطر ميكروبية كبيرة. تُعد السلالات المنتجة للأغشية الحيوية من *E. coli* ذات تأثير سلبي كبير على صحة الطيور وجودة المياه. هدفت هذه الدراسة إلى توصيف E. coli المرتبطة بتكوين الأغشية الحيوية في أنظمة مياه الدواجن من خلال تقييم انتشارها، والخصائص الجزيئية للأغشية الحيوية، ومدى حساسيتها للعوامل المضادة للميكروبات. تم جمع 30 عينة من الأغشية الحيوية من خطوط إمداد المياه، وأُجريت تحاليل ميكروبيولوجية شملت العد الكلي للبكتيريا الحية، وعدد الكوليفورم، وعدد الفطريات، وعدد بكتيريا *Staphylococcus* spp. تم عزل E. coli باستخدام الوسائط الانتقائية والاختبارات البيوكيميائية، وتم التحقق من هويتها الجزيئية عبر تضخيم جين S rRNA16. كما تم تحديد الجينات المرتبطة بتكوين الأغشية الحيوية (مثل FimH و LuxS) باستخدام تقنية PCR، وتم قياس تطور الأغشية الحيوية باستخدام طريقة الألواح النسيجية. تم الكشف عن *E. coli* في 63.3% من العينات، وكانت الجينات المرتبطة بالأغشية الحيوية موجودة بنسبة كبيرة (FimH بنسبة 68.4% و LuxS بنسبة 73.6%). أظهرت التحليلات الوراثية أن المجموعتين A وD كانتا الأكثر انتشارًا. أظهرت العزلات تفاوتًا في قدرتها على تكوين الأغشية الحيوية، حيث تم تصنيف 47.4% منها كمنتجين أقوياء، و21% كمنتجين متوسطين، و31.6% كمنتجين ضعفاء. أظهرت مقاومة عالية للمضاد الحيوي سيفيبيم (78.9%)، بينما أظهرت أزيثروميسين وجنتاميسين وأموكسيسيلين-كلافولانيك فعالية ملحوظة. لوحظت علاقات بين أنماط مقاومة المضادات الحيوية، وتكوين الأغشية الحيوية، ووجود الجينات المرتبطة بها. تشير نتائجنا إلى وجود مستمر لسلالات *E. coli* متعددة المقاومة والمكونة للأغشية الحيوية في أنظمة مياه الدواجن، مما يبرز أهمية تعزيز ممارسات النظافة المائية والمراقبة البيئية للحد من المخاطر الصحية العامة.

الكلمات الدالة: الإشريكية القولونية، الغشاء الحيوي، نظم صحة المياه، مقاومة المضادات.