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Addressing *Escherichia coli* contamination in the poultry sector: Insights into virulence, resistance, and nano-emulsion-based interventions

Shrouk, A. Raslan[1], Zeinab A.M. Mahdy[2], Ashraf, A. Abd El-Tawab[1]

[1]*Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*Bacteriology Department, Animal Health Research Institute - Banha Branch, Agriculture Research Center (ARC), Egypt*

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ABSTRACT

This study investigated the prevalence, virulence, antibacterial resistance, and potential control strategies for *Escherichia coli* isolated from poultry-related samples. Bacteriological examination confirmed the presence of *E. coli* in 36% of the samples, with the highest prevalence in poultry litter (60%), followed by chicken fillet (55%) and edible offal (45%). Lower contamination rates were observed in farm water and abattoir swabs (10% each). Virulence testing revealed that 20 strains were invasive based on Congo red dye uptake, with 35% forming strong biofilms and all exhibiting proteolytic activity. However, none of the strains displayed hemolytic activity. Antibacterial susceptibility testing demonstrated extensive multidrug resistance among the isolates, with complete resistance to ampicillin, ciprofloxacin, gentamicin, and cefamandole. Imipenem was the only antibiotic showing full efficacy (100% sensitivity). Given the alarming resistance patterns, plant-based nano-emulsions were evaluated as alternative antibacterial agents. Lemongrass nano-emulsion exhibited superior antibacterial activity against *E. coli*, with minimal inhibitory concentration (MIC) at 2.5% and minimal bactericidal concentration (MBC) at 5%, followed by garlic and onion nano-emulsions. Lemongrass nano-emulsion also demonstrated excellent physicochemical properties and low cytotoxicity (IC50 = 183.7 µg/mL). The effective results of lemongrass nano-emulsion show that it could be a suitable substitute for traditional antibiotics in managing multidrug-resistant *E. coli*.

1. INTRODUCTION

Escherichia coli, one of the most commonly studied bacteria all over the world, is known as fecal-borne bacteria. It is a Gram-negative bacterium that contributed to the Enterobacteriaceae family. It is a largely commensal bacterium that inhabits the gastrointestinal tracts of different animals and humans and is characterized by diverse phylogenetic characters (Rojas-Lopez et al., 2018). *Escherichia coli* is considered a commensal bacterium, but it may convert to be a pathogenic strain through acquiring a virulence gene that is carried either chromosomally or extra chromosomally (Sobhy et al., 2020). The acquisition of such a gene provides the organism with new characteristics and, frequently, a competitive advantage that leads to exacerbating the severity of this pathogen (Gomes et al., 2021). Although several pathogenic strains of *Escherichia coli* are isolated from clinical cases, it may also contaminate non-clinical sources like litter of poultry farms, drinking water systems, and inanimate surfaces (Otokunefor et al., 2018). That may represent a pronounced source of infection for food and subsequently humans.

The virulence of *E. coli* is reflected in its phenotypic traits, which arise from gene expression and include the production of toxins, adhesins, and cell surface factors like hydrophobicity. These traits, such as enterotoxin production causing diarrhea, fimbriae-mediated adherence, hemolysis, serum resistance, proteolytic activity, and Congo red binding, contribute to the bacteria's ability to infect hosts and evade immune responses. These phenotypic variations, often driven by specific gene clusters, allow *E. coli* to adapt to different host environments and cause diverse diseases (Shruthi et al., 2012; Pakbin et al., 2021; and Salam et al., 2024).

Development of poultry production and the desire of the investors to reduce the mortality rate and enhance the growth of poultry motivate them to the uncontrolled use of antibacterial agents, which raises the emergence of resistant bacteria, including commensal bacteria (Rasool et al., 2018). That subsequently led to hazardous effects on human health as a result of the presence of antibiotic residue in meat (Reig and Toldrá, 2008). Through this mechanism the antibiotic fosters the development of resistance genes in human gut bacteria, thus turning them into pathogens that lead to therapeutic failure and an increase of mortality rate (Abd El-Baky et al., 2020).

Enterobacteriaceae is mainly characterized by carrying a major resistance factor known as extended-spectrum β-lactamase (ESBL), which arises from the development of several enzymes like cefotaximase and oxacillinase that exhibit high resistance to penicillin and carbapenems. The variation between β-lactamases occurs due to changes in amino acid sequences, and some *E. coli* isolates may contain multiple variants. As a result, a single strain can harbor diverse types of ESBLs (Touati et al., 2006; Ullah et al., 2017).

The nature of *E. coli* and their resistance toward several antibiotics direct the researcher to use a green, safer alternative with antimicrobial effects that is safe for both humans and animals (Castro-Rosas et al., 2017; Das et al., 2021). Recently, plant essential oil concentrates have drawn the world's attention due to their antibacterial properties

* Correspondence to: rm5986447@gmail.com

without affecting the nutritional quality of food (Burt, 2004). However, several restrictions appear later in using such oils due to their synergistic effect with other chemical compounds in the food after prolonged preservation and their capability to change the organoleptic characters of the added food due to their higher concentrations to produce the intense antimicrobial effect (Jiang et al., 2009; Olatunde and Benjakul, 2018). Therefore, the encapsulation of essential oils by nanotechnology in various carrier systems represents an innovative, environmentally friendly solution that improves essential oil applications for food industry applications (Salvia-Trujillo et al., 2015; Chaudhari et al., 2021). Amongst nanometric systems, nano-emulsion displays superior effectiveness (Anwer et al., 2014) because it has at least one dimension below 100 nm (Hasan et al., 2020; Amiri et al., 2021).

Several researchers demonstrate the effectiveness of essential oil nano-emulsions as they facilitate the penetration of essential oil into the bacterial cells (Severino et al., 2015; Lu et al., 2018), as seen in the effect of citral essential oil nano-emulsion against *E. coli* (Lu et al., 2018), which represents a main component of lemongrass. Furthermore, garlic essential oil represents one of the most potent and broad-spectrum antibacterial agents that are greatly used against both Gram-positive and Gram-negative bacteria (Fufa, 2019), as it is rich in sulfur compounds, especially allicin (Bhatwalkar et al., 2021).

This research focused on detecting *E. coli* in diverse samples from poultry environments, including water, litter, abattoir swabs, chicken meat, and edible offal. It further investigated the virulence characteristics of these isolates and their susceptibility to different antibiotics. The research also assessed the antibacterial properties of lemongrass, garlic, and onion nano-emulsions against virulent *E. coli* strains.

2. MATERIAL AND METHODS

Ethical Approval

This study is based on ethical approval number BUFVTM 50-12-24

Sample collection:

A total of 100 samples represented as "water samples from poultry farms, litter samples, swabs from poultry abattoirs, chicken fillet, and chicken edible offal" 20 samples from each were collected from different farms, abattoirs, and shops in the Al-Qalyubia governorate. The samples were collected in a sterile container and transferred as soon as possible to the laboratory for bacteriological examination.

Preparation of samples:

For chicken fillet and chicken edible offal, the preparation occurs according to APHA (2001), in which 25 g of each sample were aseptically transferred into a sterile stomacher bag containing 225 ml of sterile 0.1% peptone water (Oxoid CM9); the contents were homogenized in a stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes, and the mixture was allowed to stand for 5 minutes at room temperature.

Then, one ml of prepared samples was inoculated into 9 ml of MacConkey's broth (Oxoid) and incubated at 37 °C for 24hrs for bacteriological examination.

The samples were inoculated into 9 ml MacConkey's broth (Oxoid) and incubated at 37 ºC for 24hrs.

Bacteriological examination and identification according to Markey et al. (2013).

A loopful from incubated broth was streaked over MacConkey's agar plate (Merck), Tryptone Bile X Glucuronide (TBX, Lab) agar, and incubated at 37oC for 24hrs. The suspected colonies, "pink colony in MacConkey's agar and blue colonies on TBX agar," were picked for morphological identification and culturing over nutrient agar (Lab) at 37 ºC for 24hrs. for biochemical examination. The following biochemical tests were done to confirm the isolated strain: indole test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, catalase test, oxidase test, sugar fermentation test, lysine hydrolysis, and motility.

Virulence activity for the isolated strains.

Invasiveness of Escherichia coli.

This is detected through culturing of isolated strains over Congo red agar. "The Congo red medium was prepared by adding 0.03% of Congo red dye to the trypticase soya agar (TSA) (Himedia)" and incubated for 72 hours at 25 ºC. Reaction was recorded at 18, 24, 48, and 72 hours. The appearance of red colonies within 72 hours was recorded as a positive reaction. Noninvasive strain did not bind the dye and remained white or grey even after 72 hours (Berkhoff and Vinal, 1986).

Biofilm formation

The ability of the isolated bacteria to produce biofilm was determined through culturing of bacteria into Congo red agar media "prepared with brain heart infusion broth (Himedia) 37 g/L, sucrose 50 g/L, agar No. 1 (Himedia) 10 g/L, and Congo red indicator 8 g/L" according to the method described by Freeman et al. (1989). The suspected colony was inoculated over CRA "Congo red agar" media and incubated at 37 ºC.for 24hrs, the ability of bacteria to produce a black colony with a dry crystalline consistency indicated biofilm production.

Hemolytic activity

The hemolytic activity of the isolated strains was detected through culturing of the pure colony of the strain on 5% sheep blood agar (LAB28). The development of clear zones around the colony after incubation for 24 hours at 37 ºC. indicates positive results (Siegfried et al., 1994).

Protease test

This involves inoculation of pure culture into skimmed milk agar (Oxoid) and incubation at 37 ºC. for 24hrs. The development of clearance zones around bacterial growth indicates positive results (Azeez et al., 2024).

In vitro antibiotic sensitivity test for invasive *E. coli* (n=20). The antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Bioanalyse) using the disk diffusion (Kirby-Bauer's) technique (Hudzicki, 2009). The following antibiotic discs were used amikacin (AK-30), ampicillin (AMP-10), ampicillin\sulbactam (SAM-20), aztreonam (ATM-10), cefamandole (MA-30), cefepime (CPM-30), ciprofloxacin (CIP-5), gentamicin (CN-10), imipenem (Ipm- 10), and tetracycline (TE-30). The interpretation of the results occurs following the CLSI guidelines (2023) as appear in table (1).

Table (1) The interpretation of the antibiotic discs following the CLSI guidelines (2023).

Antibiotic Class	Antibiotic (Code)	Disk Concentration	Resistant (≤ mm)	Intermediate (mm)	Sensitive (≥ mm)
Aminoglycosides	Amikacin (AK-30)	30 µg	16	17–19	20
	Gentamicin (CN-10)	10 µg	14	15–17	18
Beta-lactams	Ampicillin (AMP-10)	10 µg	13	14–16	17
	Ampicillin/Sulbactam (SAM-20)	20 µg	11	12–14	15
	Aztreonam (ATM-10)	10 µg	17	18–20	21
	Cefamandole (MA-30)	30 µg	14	15–17	18
	Cefepime (CPM-30)	30 µg	18	19–24	25
	Imipenem (IPM-10)	10 µg	19	20–22	23
Fluoroquinolones	Ciprofloxacin (CIP-5)	5 µg	21	22–25	26
Tetracyclines	Tetracycline (TE-30)	30 µg	11	12–14	15

Effect of plant-based nano-emulsion on Escherichia coli.

Preparation and characterization of essential oil nano-emulsions.

Garlic, onion and lemongrass nano-emulsions were prepared at the Nanomaterials Research and Synthesis Unit in Animal Health Research Institute, Dokki, Egypt. according to Rao and McClements (2011) with concentration 60% for garlic and onion nano-emulsion and 20% for lemongrass nano-emulsion.

Cytotoxicity assay

The cytotoxicity assay for garlic and onion nano-emulsions occurs based on the method described by (Skehan et al., 1990). While the lemongrass nano-emulsion according to the method described by (Mosmann, 1983).

In-vitro antibacterial activity and minimal inhibitory concentrations of the essential oil nano-emulsions.

This was detected by using the microdilution tube method described by Wiegand et al. (2008). In sterile 96 U-bottom well microtiter plate, a double fold serial dilution of the nano-emulsions is prepared by placing 100 µl of nano-emulsion over 100 µl of Muller Hinton broth "TM MEDIA" to obtain the final concentration " 30%,15%,7.5%,3.75%, and 1.8%" for both garlic and onion nano-emulsions and the concentrations "10%,5%,2.5%,1.25%, and 0.62%" for lemongrass nano-emulsion. Then place 100 µl of bacterial culture that was previously adjusted to be 0.5 McFarland over the prepared nano-emulsion wells and incubate the plate at 37 ºC for one day. The negative control well contains 100 µl of Muller Hinton broth. After incubation, the MIC was detected as the last well that contains turbidity and the MBC determined through culturing the suspensions on MacConkey agar, the lowest concentration that prevented bacterial growth was detected as the MBC of the nano-emulsion.

3. RESULTS

Bacteriological examination of examined samples.

The development of pink colonies in MacConkey's agar "lactose-positive bacteria" and blue colonies on TBX agar "B- glucuronidase positive" were indicated for presence of *E. coli* in the examined samples (Fig 1.). This confirmed by morphological identification as appear as gram-negative, rod-shaped bacteria under microscope and by biochemical tests include "oxidase negative, catalase positive, indole positive, MR positive, VP negative, able to ferment sugars giving yellow color, negative citrate and urea, positive lysine, and motile".

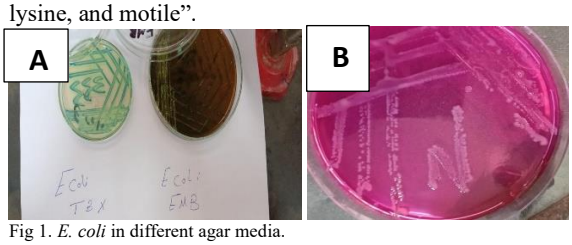

Fig 1. *E. coli* in different agar media.
A: *E. coli* in TBX agar appears as blue colonies and on EMB appear as Metallic green colonies.
B: E. coli in MacConkey's agar appears as pink colonies.

Prevalence of *E. coli* in examined samples.

The existence of *Escherichia coli* in the examined samples was summarized in table2. The highly infected samples were seen in litters of the poultry farms (60%), which represent a hotspot for *E. coli* proliferation, followed by 55% and 45% for chicken fillet and chicken edible offal, respectively. While the lowest prevalence was limited to both farm water samples and swabs from abattoirs, 10% for each.

Table (2). Existence of *Escherichia Coli* in the examined samples.

Samples	Number of examined samples	Positive samples	
		No.	%
Litters	20	12	60%
Chicken fillet	20	11	55%
Chicken edible offal	20	9	45%
Water from poultry farm	20	2	10%
Swabs from abattoirs	20	2	10%
Total	100	36	36%

Virulence activities for the isolated *Escherichia coli*

Invasiveness of E. coli: 20 strains were seen to be invasive and appear as brickle red color in the agar while 16 other strains unable to uptake dye and appear as white or gray color in the agar. The isolated invasive strains were detected only in examined chicken fillet, chicken edible offal, and litter as seen in Table 3.

Biofilm formation: The ability of invasive *E. coli* to form biofilm was assessed as seen in Table 4. From 20 invasive strains, only 7 (35%) strains "five isolates from chicken fillet and two from edible offal" were able to form strong biofilms that appeared as black colonies with a dry crystalline consistency, while the other 13 (65%) strains were unable to form biofilms and appeared as red colonies in the media.

Hemolytic activity: The isolated strains were unable to make hemolysis in the blood agar.

Protease test: All isolated invasive strains showed proteolytic activity in the skim milk agar that appeared as clearance zones around bacterial growth.

Table (3). Invasiveness of *Escherichia coli* based on Congo red dye test

Samples	Number of isolated Escherichia coli	No. Of strain taken dye and appear red "invasive"	No. Of strains unable to take dye "noninvasive"
Water from poultry farm	2	0	2
Litters	12	6	6
Swabs from abattoirs	2	0	2
Chicken fillet	11	9	2
Chicken edible offal	9	5	4
Total	36	20	16

Table (4). The virulence activity of invasive *E. coli* (n=20).

Strain	Hemolytic activity		Proteolytic activity		Biofilm formation					
	No.	%	No.	%	Strong		Medium		No biofilm	
					No.	%	No.	%	No.	%
E coli	0	0.0	20	100	7	35	0	0.0	13	65

In-vitro antibiotic sensitivity test for the invasive strains of *E. coli.*

The isolates exhibited extensive multidrug resistance. All strains were fully resistant to ampicillin, ampicillin/ sulbactam, ciprofloxacin, cefamandole, and gentamicin. And highly resistant (80%) to aztreonam, cefepime, and tetracycline. Imipenem is standing out as the only agent with full efficacy (100% susceptibility). Notably, amikacin showed 80% intermediate susceptibility (Table 5. Fig 2.)

Table (5). *In-vitro* antibiotic sensitivity test for the invasive E. coli strains (n=20).

Antimicrobial agents	Disk Concentration	Sensitive		Intermediate		Resistant		AA
		No.	%*	No.	%*	No.	%*	
Amikacin	30 µg	0	0	16	80	4	20	I
Ampicillin	10 µg	0	0	0	0	20	100	R
Ampicillin/sulbactam	20 µg	0	0	0	0	20	100	R
Aztreonam	10 µg	4	20	0	0	16	80	R
Cefamandole	30 µg	0	0	0	0	20	100	R
Cefepime	30 µg	0	0	4	20	16	80	R
Ciprofloxacin	5 µg	0	0	0	0	20	100	R
Gentamicin	10 µg	0	0	0	0	20	100	R
Imipenem	10 µg	20	100	0	0	0	0	S
Tetracycline	30 µg	0	0	4	20	16	80	R

No.: Number of isolates
%: Percentage concerning the total number of isolates (20)
AA: Antibiogram activity
R: Resistant S: Sensitive IS: Intermediate

Characterization of the used nano-emulsions "Garlic, Onion and Lemongrass".

The characterization of garlic, onion, and lemongrass nano-emulsions revealed that lemongrass exhibited the smallest particle size (49.3 nm) and lowest PDI (0.1036), followed by onion that showed particle size (202.9 nm) and PDI (0.28), then garlic had the largest particle size (420.7 nm) and highest PDI (0.432).

Cytotoxicity for nano-emulsions.

For lemongrass nano-emulsion cell viability remained high (64.97% at 20 %), with an IC50 of 183.7 µg/ml, indicating low cytotoxicity even at elevated concentrations. Also, onion showed excellent biocompatibility (>90% viability across most concentrations). While garlic showed moderate cytotoxicity at high concentrations (52.95% viability at 60 %) with an IC50 >60% for both onion and garlic nano-emulsions.

Minimal inhibitory concentrations of invasive *Escherichia coli* against the used nano-emulsions.

The examined *E. coli* shows MIC at 7.5%, 15%, and 2.5%, respectively, to the following nano-emulsions: garlic, onion, and lemongrass nano-emulsions Figure (3). While MBC was at 15%,30%, and 5% for garlic, onion, and lemongrass nano-emulsions, respectively.

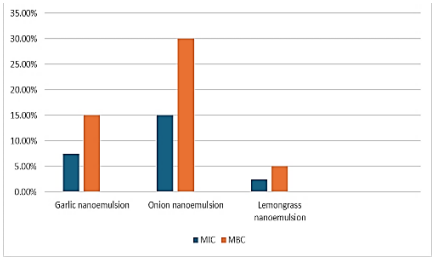

Fig (3) MIC and MBC of the used nano-emulsions against *Escherichia coli.*

4. DISCUSSION

The development of the poultry sector encounters multiple restrictions, including the spread of diseases that result in annual chicken mortality reaching 30%. There were several agents that cause diseases in poultry including microorganisms, parasites, other environmental stressors, and deficiency in feed elements. The primary microbial agents that cause sever disease in poultry is *Escherichia coli* (Islam et al., 2014). That inhabits the intestinal tract of birds and sheds in faeces where it concentrates in the litter and act as a source of infection to feed, water and other poultry by-products (Buabeng et al., 2019).

Escherichia coli represents one of the Enterobacteriaceae family that is able to ferment lactose in MacConkey agar and produce pink colony and its ability to produce glucuronidase directs the scientists to use TBX agar as a presumptive media to confirm its isolation, in which it appears as blue colonies, as mentioned by (Verhaegen et al., 2015). In addition, several biochemical tests were used to differentiate it from other lactose fermenting Enterobacteriaceae as illustrated by (Markey et al., 2013).

E. coli mainly showed higher prevalence in litter of poultry farms, about 60%, which seems to be lower than that detected by Martin et al., (1998) and Shepherd et al, (2007), who demonstrated that *E. coli* was found in 100% of litter samples and Islam et al. (2014) who found *E. coli* in 87.5% of examined litters. This is followed by that detected in chicken fillet and edible offal, where the prevalence of *E. coli* was 55% and 45%, respectively. This is nearly higher than that detected by Wardhana et al. (2021), who detected the presence of *E. coli* in 40% of examined chicken meat, while lower than that determined by Rahman et al. (2020) and Rahmahani et al. (2020), who detected the presence of *E. coli* in 63.5% and 96.7%, respectively, of examined chicken meat samples. The lower incidence of *E. coli* was restricted to the farm water samples and swab from the abattoir (10%), this was lower than the findings of Augusto et al. (2022), who detected *E. coli* in 15% of the examined poultry farm water samples. The contamination of water samples mainly occurs due to runoff either from poultry facilities or from excessive land application of poultry waste (Berry et al., 2007). The isolation of *Escherichia coli* from swabs in poultry abattoirs highlights significant contamination risks in food production chains, it was isolated from 10% of examined samples, which is lower than that detected by Tegegne et al. (2024), who isolated *E. coli* from 53% manuals and 60% of knife swabs at Addis Ababa Slaughterhouses.

Pathogenic potency of *E. coli* depends mainly on their ownership of virulent factors because bacteria normally contain multiple features that help them cause diseases, while the determination of these pathogenic elements occurs through several phenotypic tests like the ability to bind to Congo red dye, biofilm formation, protease test, and hemolytic activity. The recorded results showed the ability of 20 isolated strains of *E. coli* to bind with Congo red dye. The Congo red binding assay acts as an epidemiological marker help in differentiate between invasive and noninvasive *E. coli* infections, where dye binding refers to the presence of virulence gene (Reichhardt and Cegelski, 2018; Saha et al., 2020). This test is used in the isolates of poultry origin as a screening test to differentiate between colisepticaemic (invasive) and non-coliseptecaemic *E. coli* as recorded by Yadav et al. (2014) who found that 92.86% of examined isolates showed a Congo red binding ability, while 7.14 % of isolates did not bind Congo red dye up to 72 hours post inoculation.

The invasive strains were subjected to other virulence tests, as hemolytic activity, where all the examined strains were unable to exhibit hemolytic activity. While all the invasive strains make proteolytic activity, that may reflect the capability of the isolates to cause damage to intestinal cells and so it is greatly associated with the pathogenicity of *E.*

coli (Abed et al., 2016). It's a pivotal indicator that potentiates the virulence of extraintestinal disease caused by *E. coli* (Tapadero et al., 2019). The other virulence activity of *E. coli* that tests the capability of bacterial communities to adhere permanently to the hydrophobic surfaces for survival and persists infection is known as biofilm formation (Xavier and Foster, 2007). The examined strains showed that 7 strains (35%) able to form strong biofilm, while 13 strains (65%) were unable to form biofilm, this is higher than that recorded by Abd-Elall et al. (2023) who reported that out of 50 isolates of *E. coli* isolated from poultry farms tested for biofilm production, 92% (46/50) were able to produce biofilm, where 16% (8/50), 32% (16/50) and 44% (22/50) were strong, moderate and weak biofilm producers, respectively. This character was affected by several factors including different environmental variables together with culture constituents and biofilm promotion characteristics like curli and nonconjugative pili (Cergole-Novella, et al., 2015).

The antimicrobial susceptibility profile of the isolated strains was examined to align the resistance rate of the tested strains to different antibiotics that reflect the widespread antimicrobial misuse in farming. Based on the presented results, all the examined strains showed multidrug resistance to more than three antibiotics classes, as correlated with the study done by Adebowale et al. (2022) and Agusi et al. (2024) in *E. coli* isolated from poultry sources where 56.3% and 45%, respectively, of the examined strains exhibit MDR. The examined strains showed total resistance to ampicillin, ampicillin/sulbactam, and cefamandole that came nearly similar to the findings reported by Rahman et al. (2020). This may be explained by the fact that Food animals as well as retail meat act as reservoirs of ESBL and AmpC-producing *E. coli* (Belmar Campos et al., 2014; Maleki et al., 2015). Also, the total resistance toward ciprofloxacin and gentamicin was detected in examined strains that disagree with Tesfaheywet and Berhanu (2013), who found that the isolated *E. coli* showed high sensitivity to gentamicin (77%), and slightly agree with Racewicz et al. (2022), who detected resistance of isolated *E. coli* from cloacal swabs to ciprofloxacin by about (92%). The fully effective antibiotics in the examined strains was imipenem, which agrees with findings of Abd El-Baky et al. (2020), detected the highly sensitivity of the isolated strains to imipenem (80% sensitive).

The most critical worldwide health challenge that is exacerbating the treatment of *E. coli* is the development of bacterial resistance, so the redirection toward the use of natural products like plant extracts has increased recently. The use of plant essential oil nano-emulsions was intensifying its effect, as it facilitates its delivery to bacterial cells and also as its concentration will be safe toward host cells (Begum et al., 2024). The recent study is dedicated to using garlic, onion, and lemongrass nano-emulsion against isolated *E. coli*. The physiochemical characters of the used nano-emulsions showed that the superior nano-emulsion was lemongrass nano-emulsions, which demonstrated superior colloidal stability with uniform droplet distribution and long-term stability. This aligns with studies showing that citral-rich nano-emulsions maintain droplet sizes below 115 nm with minimal destabilization over 30 days (Pereira et al., 2021). And based on the cytotoxicity profile lemongrass keeps high cell viability (64.97% at 20% concentration), and an IC50 of 183.7 µg/mL highlights its low cytotoxicity, which agrees with (Pereira et al., 2021). Followed by onion nano-emulsion and then garlic nano-emulsion, which have cytotoxicity >90% and 52.95% viability at 60%, respectively.

The antibacterial activities of the used nano-emulsion illustrated that lemongrass nano-emulsion has the strongest antibacterial agent against *E. coli,* followed by garlic then onion nano-emulsions. This occurs as a result of the fusion of nano-emulsion droplets with the lipid layer of the cell wall of the microorganism and so its distribution due to the release of its stored energy. This fusion is further achieved by electrostatic attraction between the cationic charges of the emulsion and the anionic charge on the pathogenic particles (Begum et al., 2024). The high activity of lemongrass nano-emulsion against *E. coli* was also demonstrated by a previous study done by Gago et al. (2019), who found a reduction in *E. coli* growth by up to 10X the MIC. This occurs due to the bioavailability of the small droplets of the citral component of lemongrass to distribute inside the bacterial cell wall and so potentiate the cell lysis. In contrast, the garlic oil nano-emulsion showed lower antibacterial activity against *E. coli,* which came in the same line with the results reported by Hasssanzadeh et al. (2018) and Gabriel et al. (2022). The garlic's antimicrobial properties primarily derive from allicin along with its metabolites. The compounds exhibit antimicrobial activities by specifically blocking acetyl coenzyme A-synthetase, which stops lipid and fatty acid synthesis until cellular viability stops (Hassan and Mujtaba, 2019). Onion exhibits notable antibacterial activity due to its high content of bioactive compounds, particularly quercetin (a flavonoid) and allicin (a sulfur-containing compound), which work together to disrupt essential bacterial enzymes and cell processes of *Escherichia coli,* and so suppress its growth (Kabrah et al., 2016; Sharma et al., 2018).

5. CONCLUSIONS

This study highlights the significant challenges posed by *Escherichia coli* in the poultry sector, including its high prevalence in various poultry-related samples and its pathogenic potential. The findings underscore the role of *E. coli* as a major contributor to poultry diseases, with contamination risks extending to food production chains. The virulence of *E. coli* strains was confirmed by various phenotypic tests. The antibacterial resistance profile of the strains revealed alarming multidrug resistance patterns, reflecting widespread misuse of antibiotics in farming practices. This resistance complicates treatment options and emphasizes the need for alternative strategies to combat bacterial infections. Among the tested natural products, lemongrass nano-emulsions demonstrated superior antibacterial efficacy against *E. coli*, followed by garlic and onion nano-emulsions. These findings suggest that plant-based nano-emulsions could serve as promising alternatives to traditional antibiotics by leveraging their enhanced bioavailability and low cytotoxicity.

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