

Antimicrobial potential of essential oils and blue-green Algae against *E. coli* isolated from diseased broiler

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ABSTRACT

Enterobacteriaceae have a significant importance as an infectious agent of chickens causing substantial morbidity and mortality in addition to economic losses. In the present work, the prevalence of *Enterobacteriaceae* was investigated in many broiler chickens farms in Beni-Suef Governorate. One hundred and seventeen samples were obtained aseptically from the affected organs including heart, liver, yolk sac, air sac and pericardium of diseased and recently dead broilers. Collected samples underwent bacteriological investigation, which revealed that 95 isolates were isolated with a percentage of 81.2% including 90 *E. coli* isolates (76.9%) and 5 *Proteus* species (4.3%). In-vitro antimicrobial sensitivity test results indicated that *E. coli* isolates exhibited complete resistance against ciprofloxacin (100%), reduced resistance against enrofloxacin (82%), chloramphenicol (76%), streptomycin (74%), cefotaxime (60%), sulfamethoxazole-trimethoprim and ceftazidime (46% for each), while showed high susceptibility to amikacin (92%), fosfomycin (76%), doxycycline (74%), amoxicillin-clavulanic acid (70%), ertapenem (66%) and colistin (60%). Moreover, multi-drug resistance was determined in 78% of tested isolates. The antibacterial effect of some essential oils (clove, cinnamon, oregano and eucalyptus oil) and blue green algae was evaluated against the growth of MDR *E. coli* isolates and the results revealed that clove, cinnamon and oregano EOs completely inhibited the growth of all examined *E. coli* isolates (100%) at concentrations of 1000 ppm, 500 ppm and 250 ppm, respectively. Whereas eucalyptus EO showed no bactericidal effect on all tested *E. coli* isolates at concentrations up to 1500 ppm. Moreover, blue green algae showed no bactericidal effect on all tested *E. coli* isolates at concentrations up to 10%. Finally, molecular characterization of resistance and virulence genes declared that all isolates carried *bla*_{TEM} *sul1*, *fimH*, *iss* and *iutA* (100%), while *qnrS* was detected in 60% of tested isolates.

Introduction

Globally, poultry meat is considered an economic source of good-quality protein. Bacterial infections frequently threaten the poultry industry's vast production. *Escherichia coli* is one of the most common bacterial pathogens affecting the global poultry industry is virulent or avian pathogenic *E. coli* (APEC) (Tenaillon *et al.*, 2010). *E. coli* is a normal inhabitant in the intestine of humans, poultry and animals (Levine, 1987). Moreover, extra-intestinal APEC causes colibacillosis disease in poultry at different ages (Ahmed *et al.*, 2013). Colibacillosis disease causes different generalized and localized diseases such as perihepatitis, colisepticemia, airsacculitis, omphalitis, pericarditis, salpingitis, peritonitis, arthritis, swollen head syndrome, coligranuloma, panophthalmitis and cellulitis (Dziva and Stevens, 2008; Kim *et al.*, 2020) and consequently causing significant economic losses, including increased mortality, decreased production, medical costs and increased carcass condemnation rates (Kabir, 2010). Moreover, *E. coli* is one of the important and common bacterial pathogens that contribute to approximately 30% or more of chicken deaths (Radwan *et al.*, 2020). In chicken husbandry, long-term usage of antimicrobials for therapy and growth promotion in chickens resulted in antimicrobial resistance (Singer & Hofacre, 2006). Overuse and indiscriminate use of antimicrobials can result in spreading of MDR *E. coli* strains (Miles *et al.*, 2006; Gyles, 2008; Islam *et al.*, 2021) that can be transmitted to people by direct contact with infected birds or via food (Founou *et al.*, 2016). Furthermore, it is important to identify multi-drug resistant (MDR) isolates to select the appropriate antibiotic by laboratory examination. Recently, there is a growing interest in substituting natural, safe and efficient substances for synthetic antimicrobials. These are mostly herbs, essential oils (EOs) and spice extracts (Radwan *et al.*, 2018), where many significant properties of EOs including antioxidant, anti-inflammatory and antibacterial that promote livestock growth have been demonstrated by the researchers (Sakkas & Papadopoulou, 2017). The antibacterial activity of EOs may be attributed to their high hydrophobicity which allows them

to pass through microbial cell membranes and cause damage or loss of function of cellular proteins, lipids and organelles, ultimately leading to bacterial cell death (Bakkali *et al.*, 2008). Recently, the spreading of resistance genes in *E. coli* emerged as a significant concern for humans, animals, birds and environmental systems (Mohamed *et al.*, 2014; Poirel *et al.*, 2018; Islam *et al.*, 2022). Plasmids are the primary vectors that disseminate genes of resistance across the population of bacteria (Radwan *et al.*, 2020). *E. coli* strains frequently harbored antimicrobial resistance genes, among them β -lactams (*bla*_{TEM}) resistance encoding gene (Parvez *et al.*, 2016) and sulfonamides (*sul1*) resistance encoding gene (Parvin *et al.*, 2020). Several virulence genes have been found to be considerably associated with APEC pathogenesis (Ewers *et al.*, 2005), including adhesion factors; *fimH* that contributes to adherence to the upper respiratory tract of poultry (Wooley *et al.*, 2000). As well as *iss* gene, this is related to increased serum survival in poultry, which was recognized as a vital feature for pathogenic *E. coli* (Ellis *et al.*, 1988). The *iutA* gene is responsible for encoding for siderophores that facilitate the growth of *E. coli* on media with a low concentration of iron (Garénaux *et al.*, 2011). Therefore, the current work aimed to conduct bacterial identification and genotypic characterization of *E. coli* recovered from diseased broilers, as well as evaluation of the antibacterial effect of some EOs and blue green algae on the growth of MDR *E. coli*.

Materials and methods

Ethical approval

The present study was approved by Veterinary Medicine, Beni-Suef University Veterinary Animal Care, Use and teaching Committee (VACUTC).

Samples

A total of 117 pooling samples were collected from euthanized dis-

eased and freshly dead broilers of different ages (1-30 days) in the period between June and November 2024 from various poultry farms in Beni-Suef Governorate. These broilers were exposed to clinical and post-mortem investigations. These samples were collected under aseptic condition from internal organs lesions; yolk sac, heart and liver were collected from young ages (1-15 days) suffering from omphalitis and enteritis while samples including air sac, pericardium and liver were collected from ages (15-30 days) suffering from chronic respiratory disease (CRD).

Bacteriological examination

A loopful of each sample was inoculated into tryptone soya broth (TSB) aseptically (Oxoid)® and incubated at 37°C for 24 h. Next, a loopful from TSB was streaked onto tryptone soya agar and incubated at 37°C for 24 h, followed by application of Gram staining and oxidase test. The Gram negative, oxidase negative bacilli were streaked onto MacConkey agar (purple) (Merc)® and incubated at 37°C for 24 h. Suspected lactose fermenting colonies (yellow) were streaked onto EMB (Hi media)® agar medium and incubated at 37°C for 24 h and *E. coli* isolates were identified biochemically by the following tests; IMViC, urease as well as their reaction on TSI (Collee et al., 1996).

Antimicrobial susceptibility testing

Fifty *E. coli* isolates were examined for their sensitivity to 13 various antimicrobial agents of the most common antimicrobials used in the field of broiler chickens' production and human medication representing 8 antimicrobial classes by the disc diffusion method on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI, 2024). Antimicrobial discs included amoxicillin-clavulanic acid (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ertapenem (10 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), fosfomycin (200 µg), chloramphenicol (30 µg), colistin (10 µg), doxycycline (30 µg), amikacin (30 µg), streptomycin (10 µg) and sulfamethoxazole-trimethoprim (25 µg) (Hi media)®. Multidrug resistance (MDR) also was recorded.

Table 1. Primers of resistance and virulence genes used in PCR.

Gene	Primer sequence (5'-3')	Amplified product	Reference
<i>bla</i> _{TEM}	ATCAGCAATAAACCCAGC CCCCGAAGAACGTTTTTC	516 bp	(Colom et al., 2003)
<i>Sul1</i>	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433 bp	(Ibekwe et al., 2011)
<i>qnrS</i>	ACGACATTCGTCAACTGCAA TAAATTGGCACCCCTGTAGGC	417 bp	(Robicsek et al., 2006)
<i>Iss</i>	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	(Yaguchi et al., 2007)
<i>fimH</i>	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508 bp	(Ghanbarpour and Salehi, 2010)
<i>iutA</i>	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAAATCG	300 bp	(Yaguchi et al., 2007)

Results

Prevalence of *E. coli* isolates

Out of 117 collected samples, 90 *E. coli* isolates had been isolated with a percentage of 76.9%.

Antimicrobial susceptibility of recovered *E. coli* isolates

The antimicrobial susceptibility testing of *E. coli* isolates showed complete resistance against ciprofloxacin (100%), reduced resistance against enrofloxacin (82%), chloramphenicol (76%), streptomycin (74%), cefotaxime (60%), sulfamethoxazole-trimethoprim and ceftazidime (46%)

Detection of the antibacterial effect of some essential oils and blue green algae on the growth of MDR *E. coli* isolates

Preparation of oils

Essential oils: prepared as 10000 ppm in dimethyl sulfoxide (DMSO) as original solution.

Clove, cinnamon, eucalyptus and oregano oils (MULTIPHARMA co. For veterinary and agricultural development) were filter sterilized and mixed with the prepared TSA medium at concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 63 ppm for clove oil, cinnamon and oregano oil and 1500 ppm, 1250 ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 63 ppm for eucalyptus oil.

Preparation of blue green algal extract

One gram of dried algal powder was suspended into 10 ml D.W., leaved for 24 h and then centrifuged at high speed, the aqueous extract was mixed with the TSA medium at concentrations of 1%, 3%, 5%, 7% and 10% and then was autoclaved.

Effect of EOs and blue green algae on antimicrobial resistance against antibiotics

Four MDR *E. coli* isolates tested for the effect of oregano, cinnamon, clove and blue green algae on the antimicrobial resistance at concentrations of 63 ppm, 250 ppm, 500 ppm and 10%, respectively (Collee et al., 1996; CLSI, 2024).

Detection of some resistance and virulence genes of *E. coli* isolates

Polymerase chain reaction (PCR) was conducted to identify three resistance genes (*bla*_{TEM}, *sul1*, and *qnrS*) and three virulence genes (*fimH*, *iss*, and *iutA*) in 5 MDR *E. coli* isolates using their specific forward and reverse primers as demonstrated in Table 1.

for each) while showed high susceptibility to amikacin (92%), fosfomycin (76%), doxycycline (74%), amoxicillin-clavulanic acid (70%), ertapenem (66%) and colistin (60%). Multi-drug resistance was detected in tested isolates (n=50) with a percentage of 78%; they resisted at least 3 antimicrobials of various classes and reached to resist 7 out of 8 tested antimicrobial classes (Table 2).

Antibacterial effect of EOs and blue green algae on MDR *E. coli* isolates

Cinnamon oil completely suppressed the growth of all tested *E. coli* isolates (100%) at concentrations of 1000 ppm and 500 ppm, while at concentrations of 250 ppm, 125 ppm and 63 ppm showed no bactericidal effect on all tested isolates.

Table 2. Antimicrobial susceptibility of different *E. coli* isolates (n=50)

Antimicrobial type	Symbol	Disc content (µg)	Tested isolates (n= 50)					
			Resistant		Intermediate		Sensitive	
			No	%	No	%	No	%
Amoxicillin-clavulanic acid	AMC	30	4	8	11	22	35	70
Ceftazidime	CAZ	30	23	46	8	16	19	38
Cefotaxime	CTX	30	30	60	2	4	18	36
Ertapenem	ETP	10	2	4	15	30	33	66
Enrofloxacin	ENR	5	41	82	0	0	9	18
Ciprofloxacin	CIP	5	50	100	0	0	0	0
Fosfomycin	FOS	200	12	24	0	0	38	76
Chloramphenicol	C	30	38	76	1	2	11	22
Colistin	CL	10	3	6	17	34	30	60
Doxycycline	DO	30	0	0	13	26	37	74
Amikacin	AK	30	0	0	4	8	46	92
Streptomycin	S	10	37	74	3	6	10	20
Sulfamethoxazole- trimethoprim	COT	25	23	46	0	0	27	54

% was calculated according to the number of the tested isolates.

Clove oil completely inhibited all tested *E. coli* isolates growth (100%) at concentration of 1000 ppm, while at 500 ppm inhibited the growth of 26.6% of examined *E. coli* isolates, whereas at concentrations of 250 ppm, 125 ppm and 63 ppm showed no bactericidal effect on all tested isolates.

Oregano oil completely inhibited all examined *E. coli* isolates growth (100%) at concentrations of 1000 ppm, 500 ppm and 250 ppm while at concentrations of 125 ppm and 63 ppm showed no bactericidal effect on all tested isolates.

Eucalyptus oil and blue green algae showed no bactericidal effect on all tested *E. coli* isolates at all tested concentrations; (1500 ppm, 1250 ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 63 ppm for eucalyptus oil and 1%, 3%, 5%, 7% and 10% for blue green algae).

Effect of EOs and blue green algae on antimicrobial resistance against antibiotics

Clove EO treatment of resistant isolates resulted in change in 2 isolates to be sensitive to chloramphenicol and 2 isolates became intermediate sensitive to chloramphenicol. Moreover, oregano EO treatment resulted in change in one isolate to become intermediate sensitive to amoxicillin-clavulanic acid. Whereas, cinnamon EO and blue green algae treatment caused no change in antimicrobial resistance.

Prevalence of resistance genes (*bla_{TEM}*, *sul1* and *qnrS*)

All tested isolates (n=5) harbored *bla_{TEM}* and *sul1* genes (100%) while 3 isolates harbored *qnrS* gene (60%) (Fig. 1).

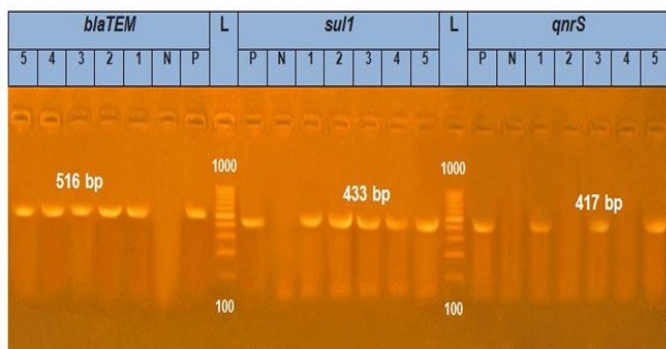


Fig. 1. PCR amplification of *bla_{TEM}*, *sul1* and *qnrS* resistance genes at 516, 433 and 417 bp fragments, respectively. Lanes 1-5 demonstrated positive amplification of *bla_{TEM}* and *sul1* genes. Lanes 1, 3 and 5 demonstrated positive amplification of *qnrS* gene. P (Positive control) and N (Negative control).

Prevalence of virulence genes (*fimH*, *iss* and *iutA*)

All tested isolates (n=5) harbored all tested virulence genes (100%) (Fig. 2).

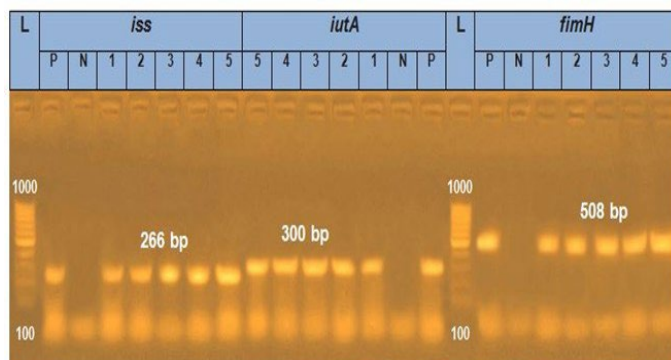


Fig. 2. PCR amplification of *iss*, *iutA* and *fimH* virulence genes at 266, 300 and 508 bp fragments, respectively. Lanes 1-5 demonstrated positive amplification of *iss*, *iutA* and *fimH* genes. P (Positive control) and N (Negative control).

Discussion

Colibacillosis is the most significant disease detected in chickens which is caused by *E. coli* (Saif et al., 2003). Also, salpingitis, acute colisepticemia, pulmonary colibacillosis, omphalitis and swollen head syndrome are among the systemic and localized infections resulted from APEC infection. The most frequent form of colibacillosis is an early respiratory disease that primarily affects broiler chickens and turkeys aged 3 to 12 weeks. It is followed by a systemic infection with fibrinous lesions (pericarditis, perihepatitis and airsacculitis) and ultimately, deadly septicemia. The infection is exacerbated by predisposing agents such as environmental variables, viral or mycoplasma infection (Barnes et al., 2003).

In the present work, the total prevalence of *E. coli* was 76.9%, where 90 isolates were recovered. Nearly similar results were reported by Baraka et al. (2021) and Hassan et al. (2020); 77.5% for each, Abd Elatif et al. (2019); 77.7% and Habak et al. (2020); 77.64%. While, lower results were determined by Amer et al. (2013); 22%, 39% by Bushen et al. (2021), 41.5% by Radwan et al. (2014), 44.58% by Shoab et al. (2016), 45.5% by Radwan et al. (2016), 48.13% by Kamboh et al. (2018), 48.98% by Yahya et al. (2021), 53% by Senthamilselvan et al. (2024), 53.8% by Ali et al. (2019), 60.81% by Tigabie et al. (2023), 60.97% by Halfaoui et al. (2024) and 70% by Khalaf et al. (2020). Meanwhile, higher *E. coli* isolation was recorded by Kamel et al. (2024); 80.5% and 83.7% by Mezalira et al. (2019). The high-

est prevalence was obtained by Bezerra *et al.* (2016) with a percentage of 95.9%.

In poultry farms, antibiotics serve as both growth promoters and therapeutic agents. However, overuse possesses a serious risk as it leads to the emergence and dissemination of antimicrobial-resistant strains of both pathogenic and commensals bacteria, which can then enter the food chain and infect humans (Apata, 2009; Kariuki *et al.*, 1999; Suleiman *et al.*, 2013). In-vitro antimicrobial sensitivity testing of veterinary pathogens can offer a beneficial direction for choice of effective therapeutic treatments (Blanco *et al.*, 1997). Also, identification of MDR isolates is very important.

In the current study, isolates of *E. coli* were examined for their sensitivity to 13 various antimicrobial agents. The isolates showed complete resistance against ciprofloxacin (100%), reduced resistance against enrofloxacin (82%), chloramphenicol (76%), streptomycin (74%), cefotaxime (60%), sulfamethoxazole-trimethoprim and ceftazidime (46% for each), while showed high susceptibility to amikacin (92%), fosfomycin (76%), doxycycline (74%), amoxicillin-clavulanic acid (70%), ertapenem (66%) and colistin (60%). Additionally, MDR was found in 78% of tested isolates.

The complete resistance against ciprofloxacin in the current work was confirmed by the results of the previous studies (Radwan *et al.* (2019), Kamel *et al.* (2024) and Bezerra *et al.* (2016)) as they recorded resistance against ciprofloxacin with percentages of 95.9%, 94.19% and 91.4%, respectively. Whereas a lower resistance against ciprofloxacin was determined by Salem *et al.* (2023); 82.1%, Abd Elatiff *et al.* (2019); 81% and Limbachiya *et al.* (2022); 71.67%, whereas, complete sensitivity to ciprofloxacin was recorded by Sarba *et al.* (2019). The high resistance reported in the current study against streptomycin, enrofloxacin, chloramphenicol, sulfamethoxazole-trimethoprim, cefotaxime and ceftazidime was nearly similar to higher rates of resistance that were recorded by Barka *et al.* (2021), Radwan *et al.* (2019), Salem *et al.* (2023) and Tigabie *et al.* (2023).

Regarding the result of colistin sensitivity, it was confirmed by investigations of Abd Elatiff *et al.* (2019) and Kamel *et al.* (2024) who declared that resistance against colistin was 62% and 62.5%, respectively. Meanwhile, higher sensitivity to colistin recorded by Tahir *et al.* (2021); 92.2%, Limbachiya *et al.* (2022) and Mohamed *et al.* (2023); both reported 100% sensitivity to colistin, Whereas, Younis *et al.* (2017) found 100% resistance of *E. coli* against colistin.

Additionally, in the present work, MDR was found in 78% of tested *E. coli* isolates. Lower results were recorded by Bushen *et al.* (2021) and Yahya *et al.* (2021) as they recorded MDR in 52.5% and 63.64% of tested isolates, respectively. Meanwhile, higher prevalence of MDR were reported by Bezerra *et al.* (2016) and Moawad *et al.* (2018); (98.2%, for each), Tigabie *et al.* (2023) and Barka *et al.* (2021); 83.9% and 87.9%, respectively. Moreover, Kamel *et al.* (2024); Mohamed *et al.* (2023) and Radwan *et al.* (2019) detected MDR in 100% of tested isolates.

In recent decades, antimicrobial resistance (AMR) in both nonpathogenic and pathogenic organisms had increased because of the improper usage of antibiotics (Phillips *et al.*, 2004). Several herbs and spices are now highly valued for their antimicrobial properties (Shan *et al.*, 2007). Oregano oil contains a high level of carvacrol and thymol essential oils. Those two compounds have demonstrated effectiveness in suppressing bacterial growth and decreasing biofilm development, consequently, oregano exhibited strong antibacterial and antifungal properties (Caputo *et al.*, 2022; Lombrea *et al.*, 2020; Polito *et al.*, 2022). Moreover, clove oil has antibacterial and antioxidant characteristics due to its free hydroxyl group which suppresses some enzymes and disrupts cell membrane (Peng *et al.*, 2022; Souza *et al.*, 2022). As well as the antibacterial activity of cinnamon oil is mainly attributed to the presence of a high concentration of cinnamaldehyde that exhibits antibacterial properties (El Atki *et al.* 2019).

In vitro studies in this work revealed that the essential oil from cinnamon completely suppressed the growth of all examined *E. coli* isolates (100%) at concentration of 500 ppm, meanwhile at concentrations of 250

ppm, 125 ppm and 63 showed no antibacterial effect on all tested isolates. Regarding clove EO, inhibited the growth completely of all examined *E. coli* isolates (100%) at concentration of 1000 ppm, whereas at 500 ppm inhibited the growth of 26.6% of examined *E. coli* isolates, whereas at concentrations of 250 ppm, 125 ppm and 63 ppm showed no antibacterial effect on all tested isolates. Concerning oregano EO, the growth of all tested *E. coli* isolates completely inhibited (100%) at concentration of 250 ppm while at concentrations of 125 ppm and 63 ppm showed no antibacterial effect on all tested isolates. Meanwhile, eucalyptus EO, showed no antibacterial effect on all examined *E. coli* isolates at concentrations of 1500 ppm, 1250 ppm, 1000 ppm, 500 ppm, 250 ppm, 125 ppm and 63 ppm. Also, blue green algae showed no antibacterial effect on all tested *E. coli* isolates at concentrations of 1%, 3%, 5%, 7% and 10%.

Several studies reported the antibacterial activities of cinnamon, clove and oregano EOs either with lower or higher concentrations than this study against *E. coli*. The antimicrobial effect of cinnamon and oregano EOs had been reported in previous studies by many authors (Radwan *et al.*, 2016; See and Jenwitheesuk, 2018 and Pouyan *et al.*, 2021) who revealed that cinnamon and oregano EOs were effective against *E. coli*. Moreover, the antimicrobial effect of clove EO against *E. coli* had been stated in previous studies by de Almeida *et al.* (2023) and Zych *et al.* (2024).

Additionally, the spread of MDR bacteria in the veterinary and medical sectors is ascribed to the transferring of mobile genetic elements including extra chromosomal DNA (plasmids), transposons or integrons. Together, these elements are crucial in promoting horizontal genetic transfer which in turn facilitates acquiring and dissemination of genes of resistance (Partridge *et al.*, 2018). Plasmids are the vital vectors that disseminate resistance genes across the bacteria (Radwan *et al.*, 2016). *E. coli* frequently harbored antimicrobial resistance genes, among them those responsible for resistance against β -lactams (*bla*_{TEM}), (Parvez *et al.*, 2016) and sulfonamides (*sul1*), (Parvin *et al.*, 2020). The *qnrS* gene possesses a public health risk because *qnr*-plasmids are often linked to integrons and include resistance factors, contributing resistance to many antimicrobial classes, including aminoglycosides and lactams (Li, 2005).

In the present investigation, PCR was conducted on 5 MDR *E. coli* isolates to determine 3 resistance genes: β -lactamase (*bla*_{TEM}), sulfonamides (*sul1*) and fluoroquinolones (*qnrS*). The results indicated that all examined isolates (100%) carried *bla*_{TEM} and *sul1* genes while *qnrS* was detected in 3 isolates (60%). These results are agreed with Enany *et al.* (2023) and Radwan *et al.* (2016) as they demonstrated *bla*_{TEM} gene in 100% of tested isolates. Nearly similar results were conducted by Abd Elaziz *et al.* (2022) and Abed *et al.* (2020) who determined the presence of *bla*_{TEM} with percentages of 95.7% and 90%, respectively. As well as Radwan *et al.* (2016) reported the presence of *sul1* with a percentage of 92.9%. Meanwhile, a lower prevalence of *bla*_{TEM} is demonstrated by Kagane *et al.* (2021); Ibrahim *et al.* (2019); Messaili *et al.* (2019) and Eraky & Abd EL-Ghany (2024) with percentages of 80%, 72.9%, 70% and 33.33%, respectively. Also, Eraky & Abd EL-Ghany (2024) found *sul1* with a percentage of 33.33%. Moreover, *sul1* and *qnrS* is recorded with a lower prevalence by Messaili *et al.* (2019) and Kim *et al.* (2020) with percentages of 14% and 2.5%, respectively.

APEC have probable virulence factors associated with haemagglutinin temperature sensitive, increased serum survival and complement resistance (Mainil, 2013). The type I fimbrial adhesion gene (*FimH*) involved extraintestinal *E. coli* invasion via the intestinal mucosa (Poole *et al.*, 2017). Moreover, *FimH* gene reliable for encoding the synthesis of type 1 fimbriae (Ionica *et al.*, 2012). The *iutA* gene encodes for siderophores that is characteristic for invasive *E. coli* and facilitates the growth of *E. coli* on media with a low concentration of iron (Garénaux *et al.*, 2011). Furthermore, the *iss* gene (increased serum survival) is connected to serum resistance and it is more prominent in pathogenic *E. coli* than commensals (Vandekerchove, 2004), and its presence in conjugative ColV plasmid may explain the relationship between *E. coli* pathogenicity and the *iss* factor (Johnson *et al.*, 2008).

In the present investigation, PCR was conducted on 5 MDR *E. coli* isolates and tested for harboring *fimH*, *iss* and *iutA* virulence genes and the results revealed that all examine isolates (100%) carried all tested genes. Such results are agreed with the results of Salam *et al.* (2024) who reported the presence of *fimH* and *iss* with a percentage of 100%, Hassan *et al.* (2020); *iss* and *iutA* (100%), Sedeek *et al.* (2020) and Azim *et al.* (2021); *iutA* and *fimH* (100%). Nearly similar results recorded by Awawdeh *et al.* (2024) as they identified *iutA* and *iss* in 93% of tested isolates, Halfaoui *et al.* (2024) and Messaili *et al.* (2019); *fimH* (91%), Ali *et al.* (2019); *iutA* and *fimH* (90% and 84%, respectively). High prevalence of *iss* detected by Varga *et al.* (2018), Lounis *et al.* (2020) and Mahmoud *et al.* (2023) with percentages of 88%, 82.3% and 84.6%, respectively. Moreover, Mbanga & Nyararai (2015) and Varga *et al.* (2018) detected *iutA* with percentages of 80% and 79%, respectively. While a low prevalence of *iutA* and *iss* is detected by Habak *et al.* (2020) and Messaili *et al.* (2019) with percentages of 9.53% and 20%, respectively.

Conclusion

This study demonstrated high incidence of MDR *E. coli* associated with colibacillosis in broilers and the existence of resistance and virulence genes. Clove, cinnamon and oregano oils have a remarkable antibacterial action against the growth of MDR *E. coli* isolates.

Conflict of interest

The authors have no conflict of interest to declare.

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