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Herbal Oils and Probiotic Efficacy in Rabbits Challenged with Multidrug-Resistant *Escherichia coli*

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Abstract

Colibacillosis is a common infectious bacterial disease that can cause enteritis and high mortality in young rabbits, threatening the breeding industry. This work was performed to express some virulence-determining genes and antimicrobial resistance patterns of isolated *E. coli* and to study the efficacy of herbal oils and/or probiotic on reducing *E. coli* infection in rabbits. Vaginal swabs and semen samples were collected from rabbits with reproductive problems (farm 1) while anal swabs were collected from rabbits with diarrhea (farm 2) in Ismailia Governorate, Egypt. Samples were subjected for bacterial identification, antibiogram-testing and molecular monitoring of *iss* and *eaeA* virulence genes. An experiment was performed in which 54 weaned California rabbits were divided into 6 equal groups, negative control group (G1), non-infected treated with probiotic and both fennel and moringa oils (G2), infected and treated with probiotic, fennel and moringa oils (G3), treated with probiotic only (G4), treated with colistin sulfate (G5) and positive control (G6). Infected groups (G3, G4, G5 and G6) were challenged orally with *E. coli* ($1x10^7$ CFU). The detection of *E. coli* was 15.3% (2/13) and 62.5% (10/16) in farm (1) and (2) respectively. The isolated *E. coli* was multidrug-resistant (MDR), carrying virulence genes *iss* and *eaeA*, with prevalence of 100% (12/12) and 50% (6 /12) respectively. Clinical symptoms were prominent in the experiment *E. coli* infected untreated group (6), with the least evidence in group (5). The *E. coli* colony counts were significantly higher in group (6) while rabbits in group (3) had significantly higher body weights compared to infected groups. Thus, it could be concluded that the use of two herbal oils and probiotics were able to reduce mortality rates, clinical signs, and the total *E. coli* count in experimentally infected rabbits.

KEYWORDS
E. coli, Herbal oils, Probiotics, Rabbits, Virulence gene

INTRODUCTION

Rabbits are an important source of animal protein for many countries in the world (El-Deep *et al*., 2021). Rabbits' meat has a healthy source of protein with low fat and cholesterol especially for young and old people (Imam *et al*., 2020). Therefore, in order to maintain the growth and development of rabbits, optimal growth conditions should be maintained (El-Deep *et al*., 2020a).

Young rabbits are particularly susceptible to various diseases such as gastrointestinal disorder especial during the weaning period, and the main pathogenic bacteria is *Escherichia coli* (*E. coli*) which cause high economic loss in rabbits farming, It can be transmitted to rabbits through ingestion of contaminated food and water, causing loss of appetite, weakness, diarrhea, abdominal distention and death (Camer *et al*., 2012).

Virulence-determining genes influence the pathogenicity of bacteria inside the host and allow them to produce enteric or extraintestinal disease, the *eae* gene is considered the main genetic marker used to describe enteropathogenic *E. coli* strains (Milon *et al*., 1999), which enable bacteria to adhere to the epithelial cells in intestinal lumen (Badagliacca *et al*., 2018). Episomal increased serum survival protein (*iss*) is a good indicator of the pathogenicity of bacteria and is more common in infective strains (Pfaff-McDonough *et al*., 2000).

Antibiotics are often used by rabbit breeders to prevent or treat such problems, but continued use of these antibiotics may compromise the animal's natural immunity, and accumulate in meat, which could be transmitted to human and weaken the natural immunity against infection (El-Deep *et al*., 2020b). Prohibition of antibiotics and discovery of new alternative biological additives forced scientists to study some non-antibiotic compounds such as prebiotics, probiotics, herbal extracts, and organic acids (Oso *et al*., 2013; Olorunsola *et al*., 2016).

Probiotics play many roles in rabbits farming, like decreasing the incidence of gastrointestinal infection and increase immune response which may help in decreasing the use of antibiotics, enhancing rabbits growth through increasing the feed conversion rates which lead to increase the quality of the final product (Mancini and Paci, 2021). Combination of medicinal herbs and their extracts primarily used as natural immune boosters, growth promotion and antioxidant supply to promote animal health and productivity (Al-Sagan *et al*., 2020; Dawood, 2021).

As one of the most promising alternative feedstuffs, *Moringa oleifera* (moringa) has antioxidant properties and strong antimi-

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crobial activity, and can be used as a natural food antibacterial agent in food systems (Sharma *et al*., 2020). As well as fennel (*Foeniculum vulgare*), which has antimicrobial properties and may be used to reduce the number of undesirable gut microbes and enhance digestion (Elgayyar *et al*., 2001).

The current study aimed to monitor the antimicrobial resistance patterns and the presence of certain virulence-determining genes (*iss* and *eaeA*) in *Escherichia coli* isolated from rabbits. As well as investigating the efficacy of fennel, moringa oil, and/or probiotics as alternatives to antibiotics in reducing *E. coli* infections and promoting growth in rabbits.

MATERIALS AND METHODS

Ethical approval

The materials and protocols applied in this study were accepted by the Scientific Research Ethics Committee of the Faculty of Veterinary Medicine, University of Suez, Ismailia, Egypt, Approval No (2023012).

Sampling and clinical examination

Twenty-nine samples; vaginal swabs (n=9), semen samples (n=4), anal swabs (n=12) and liver specimens (n=4) were collected under sterile conditions from two rabbit farms in Ismailia province, Egypt. Vaginal swabs were gathered from rabbit's suffering from reproductive problems (infertility, abortion, dystocia, and stillbirth) while semen samples were collected from animals with history of sperm abnormalities (Farm 1). Anal swabs and liver specimens were collected from clinically diarrhetic rabbits (Farm 2) with dirty fur around anal opening, and combined with weight loss, lethargy, anorexia and mortalities. The obtained samples were immediately inoculated on peptone water.

E. coli isolation and identification

Loops of peptone water were inoculated aseptically in nutrient broth (Oxoid, UK) and incubated aerobically at 37°C for 24-48 h. Eosin methylene blue (EMB) and MacConkey agar (Oxoid, UK) were streaked using loopful of broth-culture. Identification of the isolated colonies was done based on their colonial characters, microscopical examination, and biochemical reactions (indole, oxidase, Voges-Proskauer, methyl-red, lactose fermentation, H_2S, citrate-utilization, and urease tests) as mentioned by Quinn *et al*. (2011).

Antimicrobial susceptibility testing

Escherichia coli isolates were monitored for susceptibility to antimicrobial agents using the disk diffusion method. Using these antimicrobials; ampicillin (AMP 10 µg), tetracycline (T 30 µg), norfloxacin (NOR 10 µg), streptomycin (S 10 µg), fosfomycin (Fo 200 µg), colistin (CL 10 µg), penicillin (P 10u), erythromycin (E 15 µg) and lincospectin (LS 100 µg) (Oxoid, UK). The sensitivity degree was interpreted after incubating the plates at 37°C for 18 h according to (CLSI, 2017).

Molecular typing of E. coli virulence-determining genes

Monitoring of *E. coli* virulence-determinant genes (*iss* and *eaeA*) was performed. These genes were selected based on their important roles in disease induction as mentioned in previous studies (Hassan and Al-Azeem, 2009; Eldin and Reda, 2016). DNA extracted from the examined samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications as for the manufacturer's instruction. Reaction volume was 25-µl and contained 1 µl of each primer with concentration of 20 pmol, 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 5 µl of DNA template, and 5.5 µl of water. Performing the reaction was in an Applied biosystems 2720 thermal cycler. Primers used were obtained from Metabion (Germany) and are listed in Table 1. The separation of PCR products was applied by electrophoresis on 1.5% agarose gel stained with 0.5µg/ml ethidium bromide (Applichem, Germany, GmbH). Gel documentation system (Alpha Innotech, Biometra) was used to photograph the gels, and the data were evaluated by computer software.

Experimental design

This experiment was designed to evaluate *in vitro* and/or *in vivo* antimicrobial action of herbal oils and probiotic against *E. coli* field isolate. The experiment was done in experimental Research Center, Animal Health Research Institute, Dokki, Giza, Egypt

Bacterial culture

E. coli strain used in this experiment was obtained from field isolates in this study after bacteriologically, serologically, and molecularly identification of virulence gene. The *E. coli* strain was inoculated in peptone water and incubated for 24 h at 37°C. Then prepare an inoculum of 10^7 and 10^9 colony forming units (CFU)/ml based on Mc-Farland criteria.

Drug used in this experiment

Colistin Sulfate (Royal link Pharma): on 3rd day post-infection (PI) a dose of 20 mg/kg bwt was administered orally for 5 days.

ProBax probiotic (Key Vet Cooperation KVC): contains (*Lactobacillus casei* $1x10^{11}$ CFU and *Bacillus subtillus* $1x10^{11}$ CFU). administered in drinking water at dose of 1 g/L throughout the experiment.

Fennel and moringa oils were optioned from National Research Center, Egypt. Giving in drinking water at dose of 6 ml/L throughout the experiment period.

Table 1. Target genes, oligonucleotides sequences, amplified segment size, and PCR cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35cycles)				Final Extension	References
			Primary Denaturation	Secondary denaturation	Annealing	Extension		
iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266	94˚C 5min	94˚C 30sec	54˚C 30sec	72˚C 30sec	72˚C 7 min.	Yaguchi *et al*. (2007)
eaeA	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248	94˚C 5min	94˚C 30sec	51˚C 30sec	72˚C 30sec	72˚C 7min.	Bisi-Johnson *et al*. (2011)

Antimicrobial activity of herbal oil (in vitro test)

The *in vitro* antibacterial effects of fennel and moringa oils were assessed and appropriate levels of these oils against challenged *E. coli* strain was determined. Antibacterial evaluation was performed according to the MIC assay method (Kowalska-Krochmal and Dudek-Wicher, 2021). Mueller Hinton Broth (100 µl) was poured into wells of a microtiter plate, then 100 µl of herbal oil was added to the first well, followed by a double-fold serial dilution. Next, 100 µl of *E. coli* field isolate suspension (10^9 cfu/ml), estimated at 0.5 Mc-Farland, was inoculated into each well and incubated at 37°C for 24 h. Bacterial growth was indicated by the existence of turbidity. MIC is the smallest concentration of herbal oil that can inhibit bacterial growth after incubation for 24-48 h.

In vivo evaluation of the effectiveness of probiotics and herbal oils in rabbits experimentally infected with E. coli

Fifty-eight weaned California rabbits (30 days old) of both sexes were purchased from a private rabbit's farm in Cairo, Egypt. On arrival, four rabbits were sacrificed, and their livers cultured to confirm that they were free of *E. coli*. During the experiment, rabbits were kept in metal cages with good sanitary conditions and fed commercial balanced ration ad libitum. Rabbits were randomly divided into 6 equal groups (9 rabbits each), G1: Negative control, none infected, and none treated. G2: None infected and treated with probiotic (at dose rate of 1 ml/L in drinking water) and both fennel and moringa oils (at dosage rate of 6 ml/L in drinking water) throughout the experiment period. G3: Infected and treated with probiotic (at dose rate of 1 ml/L in drinking water) and both fennel and moringa oils (at dosage rate of 6 ml/L in drinking water) throughout the experiment period. G4: Infected and treated with probiotic only (at dose rate of 1 ml/L in drinking water). G5: Infected and treated with colistin sulfate orally at the 3rd day post-infection (at dosage rate of 20 mg/kg bwt. for 5 consecutive days). G6: Positive control: infected non treated. Infected groups were orally administered 0.1 ml of *E. coli*, with infective dose ($1x10^7$ CFU). Mortality rate, clinical signs, post-mortem examination, and weekly mean body weight were recorded during the experiment period (1 month).

Total bacterial counts

Total *E. coli* counts of the infected groups at the 7th, 15th, and 21st days PI was evaluated. One gram of liver tissue was added into 9 ml sterile nutrient broth and homogenized well, later the suspension was vortexed. Serial 10- fold dilutions were made. One ml of each dilution was inoculated onto double MacConkey agar plates and incubated aerobically at 37°C for 24-48 h and the number of CFU per gram of liver was determined.

Statistical analysis

Statistical analysis and graphs were performed with R (A language and environment for statistical computing) (R Core Team, 2020). Results were described as mean ± SD, comparison of treatments for each group was evaluated by analysis of variance (ANOVA), then for multiple comparisons the Tukey's post-hoc test was applied, and calculation of p-value.

RESULTS

Detection of E. coli in tested samples

The identification of isolated *E. coli* was done, based on their morphology and biochemical reaction. *E. coli* grown on MacConkey and EMB agar to produce characteristically growth colonies, pink and metallic sheen colonies, respectively, which appear microscopically as medium-sized Gram-negative bacilli. Biochemically, *E. coli* isolates tested positive for indole, methyl red, and lactose fermentation. Additionally, they tested negative for urease, Voges-Proskauer, H_2S production and citrate utilization. Detection of *E. coli* was 22.2% (2/9), 0% (0/4), 66.6% (8/12), and 50% (2/4) from vaginal swabs, semen samples, anal swabs, and liver samples respectively, for an overall occurrence of 15.3% (2/13) and 62.5% (10/16) in farms (1) and (2) respectively.

Antimicrobial resistance pattern of isolated E. coli

Antimicrobial susceptibility tests revealed that *E. coli* isolates from both farms were only sensitive to three antibiotics, norfloxacin, fosfomycin and colistin, while *E. coli* isolated from farm (2) was intermediate sensitive to ampicillin and streptomycin. As well as the examined isolates displayed high resistance to erythromycin, lincospectin, penicillin, and oxytetracycline besides, streptomycin in farm (1) as shown in Table 2.

Detection of virulence-determining genes of E. coli isolates

Molecular detection of *E. coli* virulence-determining genes showed that the corrected size of the *iss* positive DNA band was 266 bp (Figure 1a), and the corrected size of the *eaeA* gene was 248 bp (Figure 1b). The *iss* and *eaeA* virulence genes were present in 100% (12/12) and 50% (6/12) of the tested isolates, respectively as shown in Figure 1.

Table 2. Characterization of antimicrobial resistance pattern of *E. coli* isolates

Tested antibiotic	Inhibition zone (mm) of isolated *E. coli*		Interpretive criteria of zone diameter (mm) according (CLSI, 2017)		
	Farm (1)	Farm (2)	Sensitive	Intermediate	Resistance
Ampicillin	15	16	≥17	14–16	≤13
Tetracycline	0	0	≥15	12–14	≤11
Norfloxacin	26	28	≥17	13–16	≤12
Streptomycin	11	14	≥15	12–14	≤11
Fosfomycin	26	28	≥16	13–15	≤12
Colistin	13	14	≥11	-	≤10
Penicillin	15	15	≥29	-	≤28
erythromycin	0	8-9	≥23	14–22	≤13
Lincospectin	0	0	≥20	-	≤16

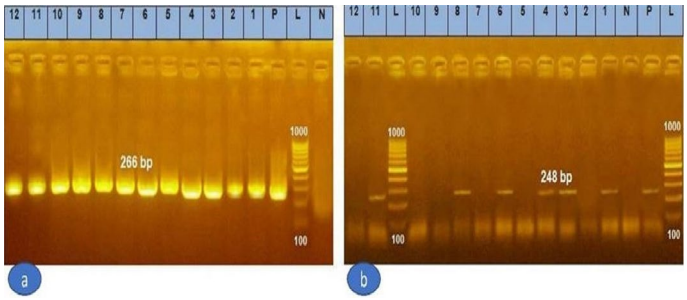

Fig. 1. Detection of *E. coli iss* (a) and *eaeA* (b) virulence gene by PCR agarose gel electrophoresis pattern. Lane L: 100bp DNA ladder, Lane 1:12: tested samples, Lane P: positive control, Lane N: negative control.

In vitro assessment of the antimicrobial activity of herbal oil against E. coli isolate

Antimicrobial activity of fennel and moringa oils against isolated *E. coli* was evaluated by determination of MIC. From the results obtained, it was noted that *E. coli* was sensitive to both oils. These oils had MIC value of 1:16 and were able to completely inhibit the growth of bacteria on MacConkey agar after incubation for 24 h at 37°C.

In vivo evaluation of the effectiveness of probiotics and herbal oils against E. coli infection in rabbits

Clinical symptoms, mortality rate and post-mortem examination

Symptoms appeared on the 3rd day PI, infected rabbits suffered from anorexia, general depression, dullness, abdominal distention, perineal staining with watery, brown diarrhea. These findings were prominent in the control-infected untreated group (6), followed by the probiotic treated- infected group (4), with the least evidence in the colistin sulfate-treated group (5). Control positive (G6) recorded the highest mortality rate during the experiment, which was 33.3% (3/9), while that of the other infected groups was 11.1% (1/9). Dead rabbits showed congestion in all internal organs, pericarditis, enteritis, ballooning of the intestine with petechial hemorrhage on intestinal wall.

Effects of probiotics, herbal oils and colistin on the total colony count of E. coli in experimentally infected rabbits

E. coli experimentally infected and untreated rabbits (G6) showed significantly higher total colony counts compared to the other infected-treated groups (3, 4, and 5) during experiment period. Whereas groups (3, 4 and 5) recorded nonexistence of *E. coli* counts at the 21st PI, as shown in Figure 2.

Effects of probiotics, herbal oils and colistin on body weight of experimentally infected rabbits

The effect of commercial probiotic and two herbal oils and colistin antibiotic on body weight of healthy and *E. coli* experimentally infected rabbits at the 7th ,15th and 21st days PI was illustrated in Table 3. At the 7th day, experimentally infected rabbits with *E. coli* and probiotic treated (G4) and colistin treated (G5) showed body weights significantly decreased as compared to other groups. At the 15th and 21st days, PI experimentally infected rabbits with *E. coli* and treated with probiotic and both fennel and moringa oils (G3) showed body weights significantly increased as compared to other groups. In non-infected groups rabbits treated with both fennel and moringa oils and probiotic (G2) showed body weights significantly increased at the 7th, 15th and 21st days of the experiment in comparison to non-treated group 1.

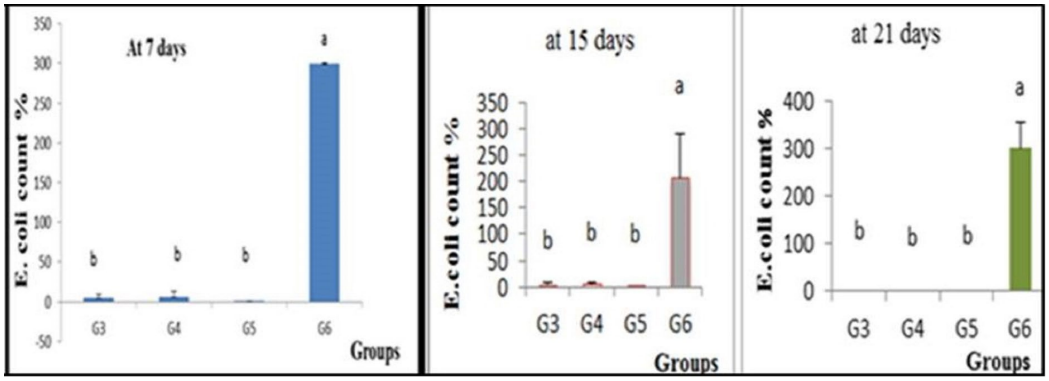

Fig. 2. Effects of probiotics, herbal oil and colistin on the total colony count of *E. coli* in experimentally infected rabbits.
G3: Infected and treated with probiotic and herbal oils. G4: Infected and treated with probiotic only. G5: Infected and treated with colistin sulfate. G6: infected non treated (positive control). Bars carry different small letters sre significantly $p < 0.01$ different. *P- value < 0.01

Table 3. The effect of probiotics and herbal oils (fennel and moringa) on body weight of healthy and *E. coli* experimentally infected rabbits at 7th, 15th and 21st days PI. Mean ± SD.

Groups	Days post *E coli* infection		
	7 days	15 days	21 days
G1	921.11±116.05[a]	987.78±96.92[b]	1124.44±121.46[b]
G2	921.11±116.05[a]	1147.50±205.27[a]	1400.0±180.28[a]
G3	936.11±145.53[a]	1116.67±103.28[a]	1350.0±50.0[a]
G4	741.5±208.73[b]	856.67±120.94[b]	1210.0±52.92[b]
G5	702.22±121.35[b]	902.22±121.35[b]	1150.0±50.0[b]
G6	912.22±70.89[a]	980.22±70.89[b]	1100.0±50.0[b]

Means within the same column and carry different small letters are significantly $p < 0.05$ different.
G1: non infected non treated (negative control). G2: non infected and treated with probiotic and herbal oils. G3: Infected and treated with probiotic and herbal oils. G4: Infected and treated with probiotic only. G5: Infected and treated with colistin sulfate. G6: infected non treated (positive control).

DISCUSSION

Colibacillosis, is a common and major infectious disease of rabbits that endangers the rabbit industry (Zhao *et al*., 2018). The virulence factors associated with the pathogenicity of *E. coli* infection are several and display a broad range of activities, including toxins, iron acquisition, and adhesion factors (Sarowska *et al*., 2019). The aim of this study was to monitor the presence of two important virulence-determining genes (*iss* and *eaeA*) and to study the pattern of antimicrobial resistance in *Escherichia coli* isolated from rabbits as well as studying the efficacy of herbal oils and/or probiotic as an antibiotic alternative compared to colistin sulfate for reducing *E. coli* infection and promoting growth in rabbits.

The bacteriological examination revealed that the detection of *E. coli* was 15.3% (2/13) and 62.5% (10/16) in farm 1 (suffering from reproductive problems) and farm 2 (clinically diarrhetic rabbits) respectively. *Escherichia coli* is a commensal or pathogenic bacteria that can cause intestinal or extraintestinal infection in rabbits (Okerman, 1994). There was a variation in the detection of *E. coli* among the inspected two farms, but it is difficult to generalize this result due to the small number of samples. Our results are consistent with a previous report that *E. coli* was isolated from 70.12% of diarrheal rabbits (Sakr *et al*., 2019) and 16.66% of aborted live does (EL-Sayed and Abd EL-latif, 2006).

Combination of virulence-determining genes and antibiotic resistance genes may lead to emergence of unexpected new bacterial strains with high mortality and morbidity. In this study, the *iss* and *eaeA* virulence genes were present in 100% (12/12) and 50% (6/12) of *E. coli* isolates detected using PCR, a finding consistent with other studies (Eid *et al*., 2017; Roshdy *et al*., 2021). With regard to antimicrobial susceptibility assessment, recovered *E. coli* isolates exhibited significant resistance to tetracycline, erythromycin, lincospectin and penicillin, which indicate E.coli isolated in this study are multidrug resistant (MDR) isolates (Moawad *et al*., 2017). Widespread use of antibiotics in an uncontrolled manner in the veterinary sector leads to the development of such resistant strains (Algammal *et al*., 2020b). This result is in line with Aboelhadid *et al*. (2022) who mentioned that *E. coli* isolates displayed high resistance to almost all antimicrobials tested.

The efficacy of drugs in the treatment of bacterial diseases requires not only the active search for new therapeutic strategies, but also the careful selection of antibiotics based on various parameters, including antimicrobial susceptibility testing (Kowalska-Krochmal and Dudek-Wicher, 2021), Isolated *E. coli* showed susceptibility to norfloxacin, fosfomycin, and colistin sulfate. Based on antibiotic susceptibility testing and previous reports (Algammal *et al*., 2020a), which inform that colistin sulfate has good activity against MDR *E. coli*. A trial was performed to investigate the effectiveness of herbal oils and/or probiotics compared with colistin sulfate in reducing *E. coli* infections.

In this experiment, rabbits infected with *E. coli* developed symptoms such as anorexia, dullness, abdominal distension, and brown watery diarrhea on the 3rd day PI. Necropsy of dead rabbits revealed congestion in all visceral organs, pericarditis, enteritis, ballooning of the intestine with petechial hemorrhage on the wall, these results are similar to previous reports (Prescott, 1978; Licois, 2004; Ismail *et al*., 2017). The symptoms were prominent in infected untreated group (6), followed by probiotic treated-infected group 4, with the least evidence in the colistin sulfate-treated group 5. The choice of treatment should be based on susceptibility results, colistin may be a good option against multidrug resistant (Gram-negative) bacteria (Li *et al*., 2005).

In terms of mortality, the infected untreated group 6 had the highest mortality rate during the experiment, 33.3% (3/9), while the mortality rate of other infected groups was 11.1% (1/9). The use of probiotics, fennel and moringa oils, and colistin sulfate was able to reduce mortality in this experiment, as probiotic supplementation overcame the severity of clinical symptoms and reduced mortality in experimentally infected rabbits (Abdelhady and El-Abasy, 2015). More recently moringa is commonly used in several medicinal applications to control various digestive disorders (Gupta *et al*., 2018) as well as there is evidence that the fennel Foeniculum constituents had antimicrobial properties (Mohammed and Abbas, 2009).

The total colony count of *E. coli* was significantly higher in the experimentally infected and untreated rabbits (G6) compared to the other groups (3, 4, and 5) during the experiment. However, groups 3, 4, and 5 had no *E. coli* counts recorded at the 21st day PI, this can be attributed to the dominance of gut beneficial bacteria over pathogenic bacteria or inhibitory effect of probiotics on *E. coli* in the gut (Mattar *et al*., 2001; Panda *et al*., 2010). In addition, rabbits in the probiotic and herbal oil treatment group 3 gained significantly more body weight compared to the other infection groups. Infections may directly reduce performance and feed efficiency by disrupting gastrointestinal absorption capacity or indirectly by reducing normal gut microflora and metabolic processes (Abou-Kassem *et al*., 2021). But supplementation with fennel oil improves feed conversion and body weight by increasing length of intestinal villi. (Imbabi *et al*., 2021). Fennel is useful for degassing and reducing gas from dietary fermentation in the gastrointestinal tract, and also contains anethole, which eliminates pathogenic bacteria and improves body weight and feed conversion (Mohammed and Abbas, 2009). Synergy of herbal oils and probiotics enhances body weight due to the dynamic effect of probiotics on competing intestinal pathogenic microorganisms and/or enhancing immunity, fighting infectious agents, promoting digestion, absorption, promoting intestinal development (Chen *et al*., 2018)

CONCLUSION

Escherichia coli isolates are multidrug resistant (MDR) to erythromycin, lincospectin, penicillin, and oxytetracycline, and carry the virulence genes *iss* and *eaeA* with a high prevalence. Herbal oils and probiotics synergistically enhance body weight, compete with *Escherichia coli* infection, reduce mortality and clinical symptoms in experimentally infected rabbits.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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