Research Article

Study the Effect of Ciprofloxacin and Diclofenac Combination Treating Urinary Tract Infection by Resistance *E.coli* 0157:H7 in Female Rabbits

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Abstract | This study is designed to highlight some of the anti-bacterial effect for diclofenac aganist resist. *E .coli 0157: H7*, as well as the ability of the diclofenac represented by anti-inflammatory and ciprofloxacin combat the exposure of urinary tract infection. The virulence components responsible for colonization and penetration also have a role in the pathogenicity of the resistant *Escherichia coli 0157: H7* strain. Resistance *Escherichia coli 0157 H7* for antibiotics decreases the cure rate. Repurposing Food and Drug Administration (FDA) approved medications against the contributing factors is a novel method to overcome this scarcity. The analgesic medication diclofenac was reported to have anti-virulence action against resistance *Escherichia coli O157 :H7*. This study aimed to demonstrate the antimicrobial effect of diclofenac, Ciprofloxacin, and a combination (diclofenac +ciprofloxacin) against clinical resistance *E coli* o157 H7 isolates by using *Polymerase chain reaction* (PCR). The presented study, all rabbits, forty-eighth adult female rabbit aged (6-8 weeks) and weighing between (1500 and 2000) gm., was randomly divided into six equal groups(8/each) groups injection 0.1 ml in 1 x 10^9 CFU *Escherichia coli 0157: H7* by urinary catheterization route excepting in negative control and all groups resulted in a significant increase in urea creatinine, sodium, Potassium, chloride concentration in the blood after three days comparison with negative control and in all experimental period (14 days) which was no significant change ($P < 0.05$) between positive control (PC) and diclofenac treated groups but combination (ciprofloxacin 3.5 mg/kg+diclofenac1mg/kg) COM1 group and combination (ciprofloxacin 1.75 mg/kg+diclofenac1mg/kg) com2 significant decrease ($P< 0.05$) after (seven, fourteen) days comparison with positive control ,diclofenac group was used in combination with antibiotics as the anti-virulence agent which was enhance the ability of the immune system to eradicate infection. Altogether, we can conclude that the diclofenac has the required anti-boiflim effects to support ciprofloxacin effect and the body responses against urinary tract infection consequences of *E coli O157 :H7*

Keywords | Ciprofloxacin, Diclofenac, Multidrug resistance; *Escherichia coli 0157 H7*; Virulence, Rabbits.

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INTRODUCTION

In most cases, *Escherichia coli* are considered part of the non-pathogenic normal flora that lives in the digestive tracts of humans and other animals. Because this organism has the potential to acquire the genes that make it possible to become a pathogen, some strains of it may cause illnesses both within and outside the intestines (Cobo-Simón and Hart, 2023). Pathotypes, such as Shiga toxin or *E. coli O157:H7*, were used to classify the virulence components that the pathogenic strain-resistant *E. coli, 0157H7*, carries (Pokharel et al., 2023). These organisms are responsi-

ble for various epidemics in animals, including food-borne diarrhea, that is, bloody, and hemolytic uremic syndrome, among others. Food-borne diarrhea, bloody diarrhea, and hemolytic uremic syndrome each account for around 4% of all infection cases in children. (Al-Rudha, 2016). Urinary tract infections occurring by *E. coli O157:H7* are one of the most important illnesses in babies and children, and they are one of the leading causes of renal failure and mortality in patients (Al-Iraqi, 2017). Urinary tract infections (UTI) are important diseases in newborns. Cattle are reservoirs for *E. coli O157:H7*. Because these animals can carry *E. coli O157:H7* without exhibiting any signs and shed these organisms in their feces (Al-Zubaidy et al., 2018), it is believed that they are an important source of food-borne infections (Najim et al., 2017). Because no vaccination can protect against *E. coli O157:H7*, the infection must be diagnosed as quickly as possible so that treatment may be administered and kidney damage can be avoided. According to (Pokharel et al., 2023). *Polymerase chain reaction* (PCR) test is the most efficient diagnostic approach that is also highly specific and sensitive for these organs (Manzanas et al., 2023)

Because there is a lack of data on urinary tract infections caused by *E. coli O157 H7* in Iraq, as well as on the genotypic features of these organisms, In this study was done isolation and diagnosis the urinary tract infections caused by *E. coli O157:H7* in humans through the use of the PCR assay and the culturing method for enterohemorrhagic *E. coli* then infecting rabbits with the same isolate obtained from humans. The lining of blood arteries is the target of Shiga toxin, a multi-subunit protein with a 68-kilodalton molecular weight. After forming a bond with a component of the cell membrane, it then penetrates the cell and begins its work (Yousra et al., 2023). Once within the cell, it interacts with the ribosomes and causes them to become inactive. This stops the machinery responsible for protein synthesis, ultimately resulting in apoptosis and cell death (Al-Taii and Yousif, 2022). Shiga Toxin-Producing E. coli (STEC) strains may often be identified by their propensity to produce shiga toxin, which gives them the potential to form both adhering and effacing lesions. In spite of the fact that both of the Shiga toxins, *Stx1* and *Stx2*, are encoded on the same lambdoid bacteriophage, they are separate from one another. According to (Lee et al., 2007)'s research from 2007, *Stx2* has a genetic and amino acid similarity that is 55.6 percent identical to *Stx1*. In addition to *Stx, E. coli* generates enterohaemolysin, which is plasmid-encoded (Khudhir, 2022). This enterohaemolysin is encoded by *hlyA* gene. Strains that possess both the stx gene and the *hlyA* gene are thought to pose a greater threat to human health than strains that only have either the stx gene or the *hlyA* gene. It is interesting to note that bacteria generating only Shiga toxin type 2 (*Stx2*, encoded by *stx2*) seem to be more usually responsible for major problems such as Hemolytic-uremic syndrome (HUS) than strains producing Shiga toxin type 1 (Khudhir et al., 2017). More than 200 distinct serotypes of *E. coli* strains, including both O157 and non-O157: *Helicobacter pylori (H. pylori)*, have been identified to have genes for the Shiga toxin. The creation of biofilm is a typical process that enhances the survival of bacteria in various abiotic and biotic habitats. Biofilms may be found in a variety of environments. Biofilms are responsible for around 80 percent of chronic and recurring bacterial infections in people, some of which are linked with high rates of morbidity and death rate (Banerjee et al., 2020; Uruén et al., 2020).

Biofilms may be found in a variety of environments, including medical devices, food, and water. According to (Panxin et al., 2023) research, *E. coli o157 H7* may be killed by ciprofloxacin. However, genetic mutation or development of ciprofloxacin-resistant bacteria is possible. Biofilms are communities of sessile bacteria that adhere to a surface, interface, or other cells by producing proteins, polysaccharides, and external Deoxyribonucleic acid (DNA)(AL-Dujaily, and Mahmood, 2022). It acts as a natural physical barrier and Deoxyribonucleic acid (DNA) provides a constant flow of nutrients for microbial growth, conditions for interbacterial interaction, genetic material exchange, resistance to extreme potential of hydrogen (PH) and increased resistance to immune defenses and antimicrobial agents, making biofilm-related infections difficult to treat (Yin et al., 2019; Salgar-Chaparro et al., 2020).

Diclofenac has antibacterial and anti-biofilm properties against therapeutically important microorganisms. It is an analgesic, antipyretic, and anti-inflammatory non-steroidal anti-inflammatory medication. It reduces pain and inflammation in rheumatoid, gout, ankylosing spondilytis, osteoarthritis, dysmenorrhea, and spondylarthritis. It inhibits prostaglandin-producing cycloxygenase enzymes (Hasan et al., 2023). Blocking cyclooxygenase isoenzymes, (***COX***-1 and ***COX***-2) enzymes reduces prostaglandin synthesis and inflammation. Diclofenac's formula is C14H11Cl2NO2, and its molecular weight is 318.13. The hygroscopic, nearly white crystalline powder dissolves easily in methanol and butanol but sparingly in water. It melts at 288-290 C^0 (Ricciotti et al., 2011).

Aim of the study

Study the effect of the combination between ciprofloxacin and diclofenac and alone in treating urinary tract infection caused by *Escherichia coli (0157):H7*

MATERIALS AND METHODS

Identification Of *E.coli O157:H7*

This investigation isolated pure culture *E.coliO157:H7* using general and selective mediums (Mc Donough et al., 2000). UTI-afflicted females provided 200 urine samples from February to July 2022. Incubation of the urine samples was done at 37°C for (1-2) days on blood agar, MacConkey and detected by PCR assay using for DNAwas extracted using PrestoTM Mini g DNA Bacteria Kit Geneaid, U.S.A., and PCR master mix was made using AccuPower PCR premix Kit. PCR product electrophoresis (Fig.1). PCR testing verified that the bacterial isolates were *E.coli O157:H7*. Consecutive two fold dilutions, 50 µL of pathogenic *E. coli 0157 H7* suspension (107–109 CFU) is utilized to generate acute UTI by (Panda et al., 2010).The rabbit was given 0.1 ml of each dilution intraurethral and monitored for UTI symptoms. The rabbit's infectivity dose will remain the dilution that caused symptoms (Quinn et al., 2004). Each rabbit in the infected group will receive a canula (gauge 24G) with brain heart infusion broth dilution of 0.1 ml overnight at 37 Co. Aseptically insert urinary catheters. Forty-eight female rabbits will be divided into six groups (8 animals for each group) were purchased from local market New Zealand white rabbit (, during the experimental period, rabbits were individually in metal cages, at experimental animal house of Veterinary College / Baghdad University, they were kept at room temperature (23-28) C°, the rabbits were fed commercial pellet, food and water was supplied (Cam et al., 2008).

Institutional Animal Care And Use Committee Statement

Before performing any experiment, the experimental design and protocols used in current study were examined and approved in accordance with the animal welfare ethical measurements by the Scientific Committee of the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad and the Ethics Committee of the College of Veterinary Medicine, University of Baghdad, Baghdad – Iraq (IACUC#: P.G.-1354).

Animal Grouping

Group NC (Negative control): The uninfected group was gave only distilled water orally.

Group PC (Positive control): the animals were-infected with *E. coli o157:H7* and left without any treatment.

Group DC: Infected animals with *E. coli o157:H7* administrated diclofenac 1mg/kg B.W.

Group CIP: Infected animals with *E. coli o157:H7* and administrated with ciprofloxacin at 7mg/kg B.W

Group COM1 (combination): Infected animals with *E. coli 0157:H7* and treated with a variety of 3.5mg/kg B.W ciprofloxacin+ 1mg/kg B.W. Diclofenac.

Group COM2 were treated with 1.75mg/kg B.W ciprofloxacin + 1mg/kg B.W. All the treatments were administrated orally twice daily for 14 days.

Statistical Analysis

Data were analyzed statistically using the Microsoft Program (SPSS), the mean of variance was compared by T-test at ($P< 0.05$) as described by (Morgan et al. 2005).

RESULTS

Bacterial colonies on McConky medium at 37 degrees Celsius showed a rose pink hue 24-48 hours after the end of the incubation period, whereas colonies on E.M.B. media had a metallic green sheen color. In addition, the bacterial isolates displayed gram-negative motile bacilli, as determined by the assay, which is a speedier and more sensitive test for the detection of these microorganisms in materials that have been polluted. (Paton et al., 1998), Who indicated that the PCR assay was regarded to be the most sensitive approach to know if a sample includes *E.coli O157:H7* via detecting the genetic characteristic of these organisms supported these ideas. They said that the PCR assay was the sensitive way that employed.

The most recent result of the PCR assay identified the presence of the eae A, hlyA, Stx1and Stx2, flic, and rfbE genes in the local isolates of *E. coli* based on their microscopically, morphology, biochemical tests, It was determined that the bacterial isolates in question were of the *E.coli* kind. Colorless and smooth colonies appeared on the selective medium Cefixime Tellurite-Sorbitol MacConkey agar, but on Chrom agar O157, the colonies were a mauve hue. It was determined that the bacterial isolates were E. coli O157, and the PCR test verified the diagnosis that these strains were *E. coli* O157:H7 serotypes.

Detection Of *E.coli* O157: H7 Using Multiplex Pcr (Mpcr)

Amplification and melting conditions were optimized for the MPCR assay, using specific primers sequences targeted $fliC_{H7}$ (1522 bp), $rfbE_{O157}$ (259bp), *eaeA* (473 bp), stx_1 (180 bp) and stx_2 (780 bp) genes Figure (1). Genes used in the polymerase chain reaction (PCR) were similar to those used by (Hu et al., 1999), which developed MPCR assay that was able to identify these genes in animal urine samples. (Bai et al., 2010) have successfully managed the Optimization of multiplex PCR conditions to detect the presence of all five *E. coli* O157:H7 genes in water samples.

Serum Creatinine Concentration:

The present study increase serum creatinine in all groups after 3 days infected by resist. *E colli 0157 :H7* inducation

Table 1: Frequency of *rfb* *eaeA*, *stx* , *stx* and *fliC* genes in the samples:

Isolates source	rfb $_{O157}$ gene	fliC $_{H7}$ gene	eaeA gene	Stx $_{1}$ gene	Stx $_{2}$ gene
Human	+	+	+	+	+

Table 2: Creatinine concentrations (mg/dl) in infected groups by resistance *E coli* 0157 treated by Ciprofloxacin, diclofenac alone and combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups

Groups/ Serum creatinine concentration(mg/dl)	3 days	7 days	14 days
Control –ve(NC)	0.62±0.06 Ab	0.66±0.10 Ac	0.64±0.09 Ab
Control +ve(PC_	1.74±0.05 Ba	1.72±0.07 Ba	2.14±0.10 Aa
Diclofenac(DC) (1)mg/kg	1.62±0.10 Ba	B1.70±0.07a	A2.08±0.08a
Ciprofloxacin(CIP) (7)mg/kg	1.70±0.07 Aa	1.58±0.16 Aa	0.76±0.16 Bb
Diclofenac (1)mg/kg+Ciprofloxacin(3.5)mg/kg(COM1)	1.74±0.08 Aa	1.22±0.06 Bb	0.72±0.12 Cb
Diclofenac (1)mg/kg+Ciprofloxacin (1.75)mg/kg(COM2)	1.70±0.07 Aa	1.00±0.18 Bb	0.62±0.18 Cb

The small letter compares the values in the columns, and the capital letter compares the values in the row.

Table 3: Serum Sodium concentration mmol/l in infected groups by resistance *E coli* 0157H7 treated by Ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups

Groups/ Serum Sodium concentration mmol/l	Three days	Seven days	fourteen days
Control –ve(NC)	136.60±0.67 Ab	137.00±0.70 Ac	136.80±0.58 Ab
Control +ve(PC)	153.20±2.63 Ba	158.00±2.32 Ba	167.60±2.80 Aa
Diclofenac 1mg/kg(DC)	154.20±2.33 Ba	153.20±3.00 Ba	167.40±2.48 Aa
Ciprofloxacin 7mg/kg(CIP)	156.80±3.26 Aa	156.60±2.37 Aa	137.20±0.86 Bb
Diclofenac(1)mg/kg+Ciprofloxacin (3.5)mg/kg(COM1)	152.80±1.62 Aa	145.60±1.02 Bb	136.20±0.58 Cb
Diclofenac(1)mg/kg+Ciprofloxacin(1.75) mg/kg(COM2)	152.20±1.77 Aa	142.80±1.42 Bb	136.20±0.58 Cb

The small letter for comparison between the values in the columns, the capital letter for comparison between the values in the row.

Table 4: Potassium concentration mmol/l in infected groups by resistance E coli 0157H7 treated by Ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups

Groups/ Serum Potassium concentration(mmol/l)	Three days	Seven days	Fourteen days
Control –ve(NC)	3.84±0.10 Ac	3.76±0.07 Ac	3.64±0.05 Ab
Control +ve(PC)	6.30±0.19 Ba	6.30±0.13 Ba	6.96±0.06 Aa
Diclofenac (1)mg/kg(DC)	6.34±0.17 Aa	6.28±0.24 Aa	6.60±0.22 Aa
Ciprofloxacin(7)mg/kg(CIP)	6.12±0.18 Aa	6.38±0.21 Aa	3.80±0.12 Bb
Diclofenac+Ciprofloxacin (1+3.5)mg/kg COM1	5.96±0.12 Aa	5.26±0.08 Bb	3.70±0.07 Cb

Diclofenac+Ciprofloxacin (1+1.75)mg/kg COM2	A6.38±0.21 Aa	5.26±0.08 Bb	3.64±0.08 Cb

The small letter for comparison between the values in the columns, the capital letter for comparison between the values in the row.

Table 5: Serum chloride concentration mmol/l in infected groups by resistance *E coli* 0157 H7 treated by Ciprofloxacin, diclofenac alone, and combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups

Groups/ Serum Chloride concentration (mmol/l)	Three days	Seven days	fourteen days
Control –ve(NC)	99.00±0.44 Ab	103.20±1.82 Ab	102.60±2.24 Ab
Control +ve(PC)	118.20±1.15 Ba	118.80±0.86 Ba	A130.60±1.07 Aa
Diclofenac 1(mg/kg)(DC)	117.60±0.87 Ba	119.60±1.02 Ba	130.00±1.09 Aa
Ciprofloxacin 7 (mg/kg) (CIP)	118.20±0.86 Aa	118.00±0.70 Aa	103.60±2.44 Bb
Diclofenac 1 (mg/dl)+Ciprofloxacin3.5 (mg/kg) COM1	119.60±1.20 Aa	103.80±2.69 Bb	102.00±1.41 Bb
Diclofenac 1 (mg/dl)+Ciprofloxacin1.75 (mg/kg COM2	119.20±0.86 Aa	103.20±1.98 Bb	101.00±1.67 Bb

The small letter for comparison between the values in the columns, the capital letter for comparison between the values in the row.

Table 6: Serum blood urea concentration mg/dl in infected groups by resistance *E coli* 0157H7 treated by Ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups

Groups/ Blood Urea concentration (mg/dl)	Three days	Seven days	fourteen days
Control –ve(NC)	20.80±1.46 Ab	25.60±1.80 Ac	25.20±2.22 Ab
Control +ve(PC)	59.40±0.92 Ba	62.20±2.35 Aa	68.80±2.67 Aa
Diclofenac 1mg/kg(DC)	58.40±1.43 Ba	61.40±1.88 Aa	68.20±2.20 Aa
Ciprofloxacin 7mg/kg(CIP)	58.80±1.15 Aa	58.60±0.50 Aa	23.98±4.21 Bb
Diclofenac(1mg/kg)+Ciprofloxacin(3.5mg/kg) COM1	61.00±2.28 Aa	37.60±4.36 Bb	24.38±3.42 Cb
Diclofenac 1mg/kg+Ciprofloxacin(1.75mg/kg) COM2	60.40±2.08 Aa	37.20±4.05 Bb	23.90±3.12 Cb

The small letter for comparison between the values in the columns, the capital letter for comparison between the values in the row.

by urinary catheter excepted negative control this is evidence of infection the results of current study ciprofloxacin (CIP) group after 14 days no significant change (P< 0.05) compared with negative control (NC) however two groups treated by combination (ciprofloxacin+ diclofenac) and two doses (3.5+1 and 1.75+1) (mg/kg) after 7 and 14 days decrease significant (P< 0.05) this is evidence of the additive effect of diclofenac with ciprofloxacin, compared ciprofloxacin group and diclofenac group whereas diclofenac did not gave an effect on its own, even after 14 days of starting treatment compared with negative control (NC). Table (2) figure (7)

Serum Sodium

The presented study increase serum sodium in all groups after 3 days infected by resist. *E colli 0157 :H7* inducation by urinary catheter excepted negative control this is evidence of infection the results of current study ciprofloxacin (CIP) group after 14 days no a significant change (P< 0.05) compared with negative control (NC) however two groups treated by combination (ciprofloxacin+ diclofenac) and two doses(3.5+1 and 1.75+1)(mg/kg) after 7 and 14 days decrease significant (P< 0.05) this is evidence of the additive effect of diclofenac with ciprofloxacin, compared ciprofloxacin group and diclofenac group whereas diclofenac did not gave an effect on its own, even after 14 days of starting treatment compared with negative control

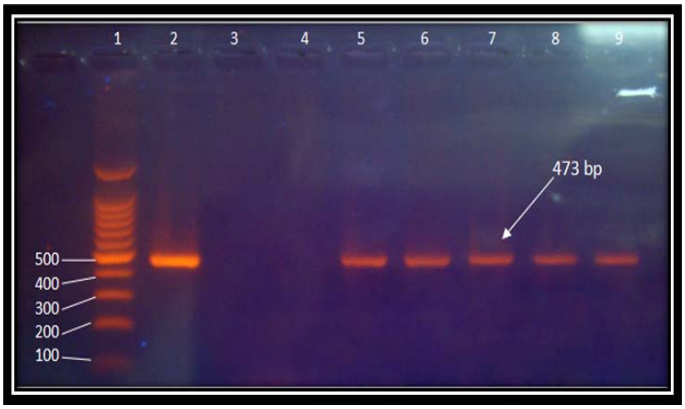

Figure 1: Shows gel electrophoresis, D.N.A. marker: 1 lane, negative control: 3-4 lane; amplification of eaeA gene: 5– 9 lane, stained with E.B. at 80 volts for 60 min, under U.V. light

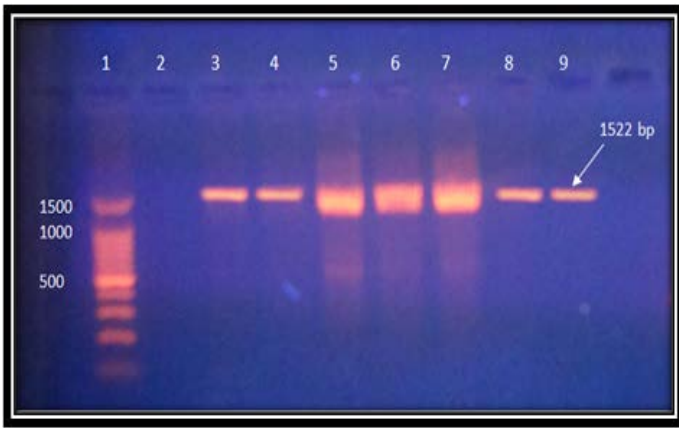

Figure 2: Shows gel electrophoresis, D.N.A. marker: 1 lane, the negative control: 2 lanes, PCR amplification of *flic* $_{H7}$ gene: 3-9 lanes, stained with E.B. at 80 volts for 60 minutes, under U.V. light.

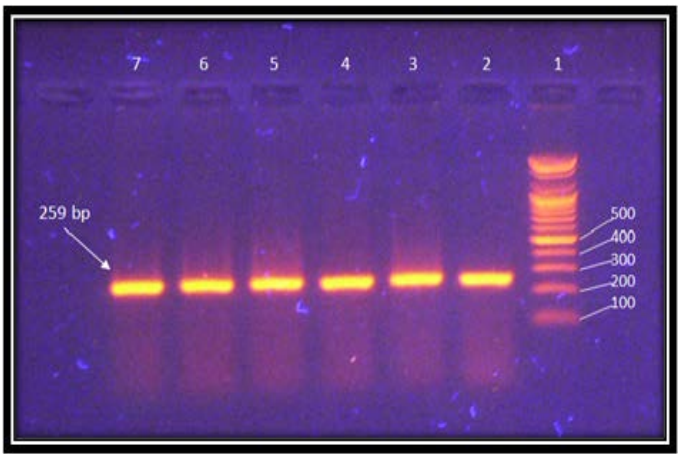

Figure 3: Shows gel electrophoresis, D.N.A. marker: lane 1; Amplification of *rfbE* gene: lanes 1-7, stained with E.B. at 80 volts for 60 minutes, under U.V. light.

(NC). Table (3) figure (8).

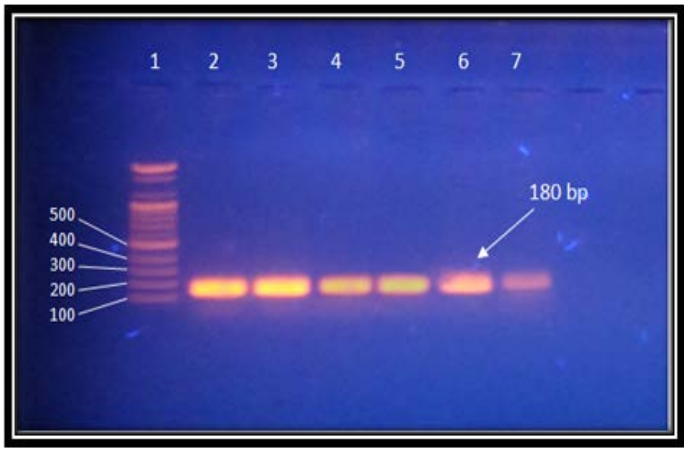

Figure 4: Shows gel electrophoresis, D.N.A. marker: 1 lane, amplification of *stx*$_1$ gene: lanes 2-7, stained with E.B. at 80 volts for 60 minutes, under U.V. light.

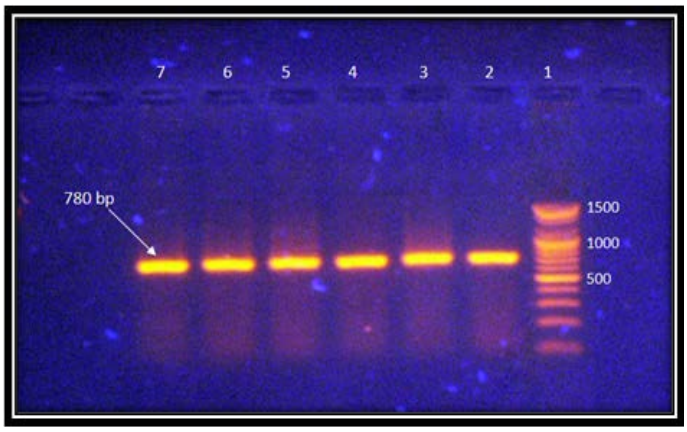

Figure 5: Shows gel electrophoresis, D.N.A. marker: lane 1; amplification of *stx*$_2$ gene: lanes 1-7, stained with E.B. at 80 volts 60 minutes, under U.V. light.

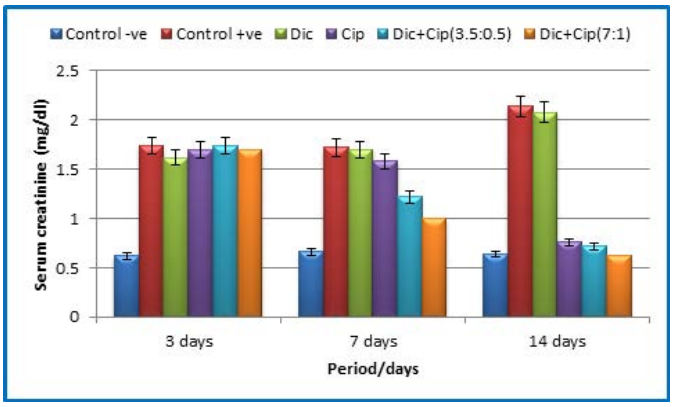

Figure 6: Creatinine concentrations (mg/dl) in infected groups by resistance *E coli* 0157 treated by ciprofloxacin, diclofenac alone, and a combination between (Ciprofloxacin + diclofenac) in two doses (mg/kg) and control groups
DC=diclofenac dosge 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg

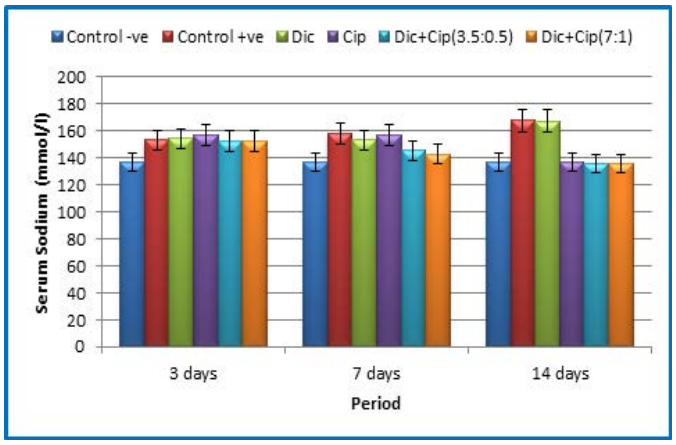

Figure 7: serum sodium mmol/l in infected groups by resistance *E coli* 0157H7 treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=diclofenac dosge 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

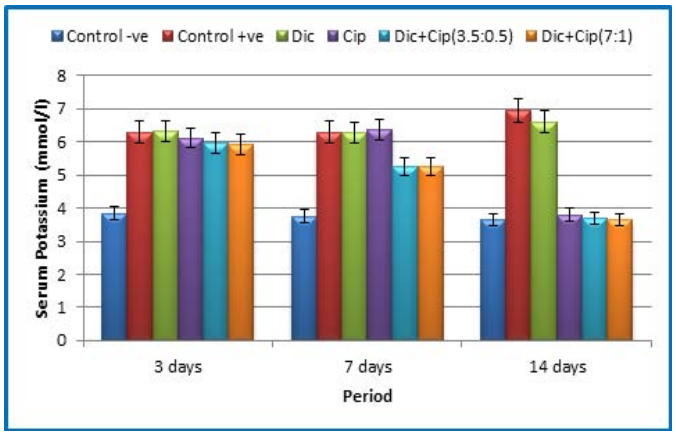

Figure 8: Serum Potassium concentration (mmol/l) in infected groups by resistance *E coli* 0157H7 treated by Ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups
DC=diclofenac dosge 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

Serum Potassium

the presented study increase serum potassium concentration in all groups after 3 days infected by resist. *E colli 0157: H7* inducation by urinary catheter excepted negative control this is evidence of infection after injection the presented study ciprofloxacin (CIP) group Serum potassium concentration after 14 days no a significant change ($P< 0.05$) compared with negative control (NC) however two groups treated by combination (ciprofloxacin+ diclofenac) in two doses(3.5+1 and 1.75+1)(mg/kg) after 7 and 14 days decrease significant ($P< 0.05$) this is evidence of the additive effect of diclofenac with ciprofloxacin, compared ciprofloxacin group and diclofenac groupwhereas diclofenac did not gave an effect on its own, even after 14 days of starting treatment compared with negative control (NC). Table (4) Fgure (9)

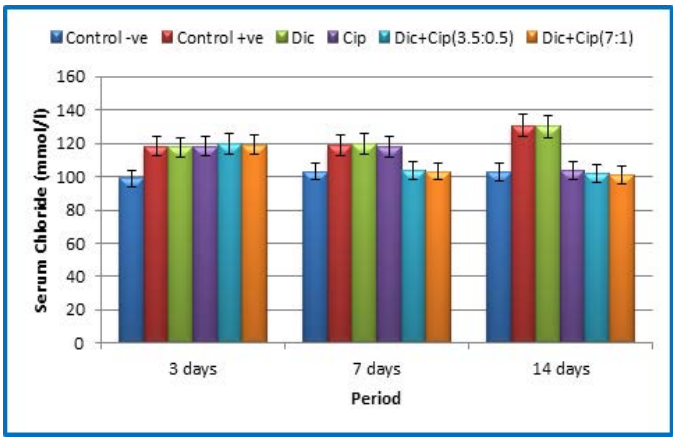

Figure 9: Serum chloride concentration (mmol/l) in infected groups by resistance *E coli* 0157H7 treated by Ciprofloxacin, diclofenac alone and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=daclofenac dosge 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1=((ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2=((ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

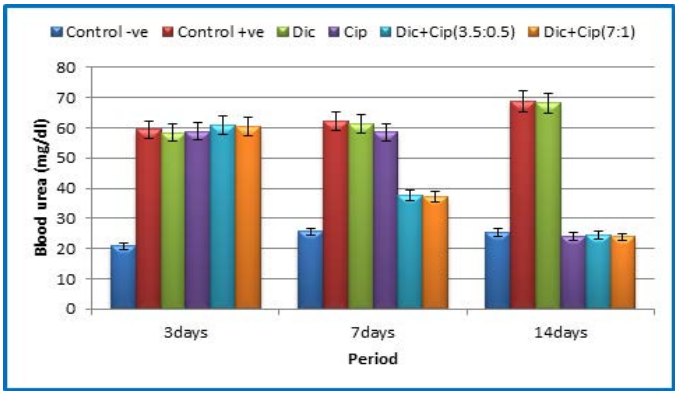

Figure 10: Serum blood urea mg/dl in infected groups by resistance *E coli* 0157H7 treated by Ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=daclofenac dosge 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1=((ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2=((ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

Serum Chloride

The present study increase serum chloride in all groups after 3 days infected by resist. *E colli 0157 H7* inducation by urinary catheter kidney dysfunction excepted negative control this is evidence of infection the results of presented study ciprofloxacin (CIP) group serum chloride after 14

days no a significant change (P< 0.05) compared with negative control (NC) but two groups treated by combination (ciprofloxacin+ diclofenac) and two doses, after 7 and 14 days significant decrease (P< 0.05) compared with (PC, DC and CIP) groups, and this is evidence of the additive effect of diclofenac with ciprofloxacin while diclofenac did not gave an effect on its own, even after 14 days of starting treatment compared with negative control (NC). Table (5) Figure (10)

Blood Urea

The presented study increase blood urea in all groups after 3 days infected by resist. *E colli* 0157: H7 induction by urinary catheter kidney dysfunction excepted negative control this is evidence of infection the results of current study ciprofloxacin (CIP) group after 14 days no a significant change (P< 0.05) compared with negative control(NC) however two groups treated by combination (ciprofloxacin+ diclofenac) and in two doses (3.5+1 and 1.75+1)(mg/kg), after 7 and 14 days the beginning of dosing significant decrease (P< 0.05) compared with (CIP,DC and PC) and this is evidence of the additive effect of diclofenac with ciprofloxacin, whereas diclofenac did not gave an effect on its own, even after 14 days of starting treatment compared with negative control (NC). Table (6) Figure (10)

DISCUSSION

All parameter above (creatinine, sodium, potissum, chloride and urea) decreased significantly after 14 days all groups (CIP, COM1 and COM2) Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class (Michałowska et al., 2023). It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein. Ciprofloxacin was first patented by Bayer (1983) and subsequently approved by the U.S. Food and Drug Administration (FDA) in 1987. Ciprofloxacin has 12 FDA-approved human uses and other veterinary uses, but it is often used for unapproved uses (off-label). Ciprofloxacin interacts with other drugs, herbal and natural supplements, and thyroid medications (Lukin et al., 2023). Ciprofloxacin is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA; thereby inhibiting cell division.This mechanism can also affect mammalian cell replication. In particular, some congeners of this drug family (for example those that contain the C-8 fluorine) (Imkamp et al., 2023). The duration of treatment with ciprofloxacin for UTI is typically 3 to 7 days moreover the presented study CIP group all parameter decreased significantly after 14 days because E coli o157H7 was resist according vitek2 however (COM1 and COM2) decreased significantly after 7 consequence, to additive effect between diclofenac and ciprofloxacin according checker board assay diclofenac anti- bacterial properties whilst combined with other medicines, it's also makes bacteria more susceptible to antibiotic agents (Chan et al., 2017). Consequently, a method for treating microbial diseases and reducing inflammation is to combine antibiotic drugs with diclofenac (Chan et al., 2017). diclofenac had an effect inactivating the E coli biofilm metabolism the authors observed a biofilm killing activity only when diclofenac was associated with kanamycin or tetracycline, ciprofloxacin (Leão et al., 2020). (Shah et al., 2016) The many traits of biofilms facilitate antimicrobial resistance (Park, 2023). In particular, creating an extracellular polymeric substance (EPS) matrix lessens the diffusion and penetration of antimicrobial drugs while shielding the bacteria from environmental stresses like dehydration and starvation (Espigares et al., 2023). Additionally, the EPS matrix hinders the biofilm from effectively absorbing oxygen and nutrients, causing certain cells, known as persisters, to enter a vegetative state and experience metabolic inactivity, making them inaccessible to antimicrobial drugs (Panxin et al., 2023) Resistance genes' expression, which results in the emergence of neutralizing enzymes, is another factor that influences antimicrobial resistance. diclofenac this drug are widely used in combination with antibiotics to treated infections since they are administered to reduce inflammation, pain, and fever (Rainsford, 2007). The mechanism of action of nonsteroidal anti-inflammatory drug (NSAIDs) such as diclofenac ibuprofen and acetylsalicylic acid (aspirin) depends on the inhibition of cyclooxygenase enzymes (Rainsford, 2007). antibacterial of diclofenac mechanism their anti-biofilm action (Abbas et al., 2020) may be related to their ability to affect the integrity of the cytoplasmic membrane of bacteria. In fact, cell permeation to propidium iodide, the release of intracellular K+ and changes in the physicochemical properties of the bacterial surface have been reported—suggesting cytoplasmic membrane damage (Laudy et al., 2016).

CONCLUSIONS

From the results of this study, the following observations are deduced:

Antibiotics susceptibility test was done for E. coli O157:H7 isolates that showed multiple antibiotic resistance, including Ciprofloxacin.

Shiga-like toxin 2 is the most important virulence marker of *E. coli O157:H7*.

Used diclofenac with ciprofloxacin in urinary tract infection (UTI)caused by *E. coli* O157:H7.for reduce the bacterial effect and inflammation

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CONFLICT OF INTEREST

There is no conflict of interest.

NOVELTY STATEMENT

Due to the absence of an approved vaccination for any resistance *E coli* 0157 H7 caused (U.T.I.) disease and the lack of easily accessible, secure, and effective medicines for some diseases resistant to synthetic treatments, it is imperative to seek alternate antibacterial sources. The study's novelty is that it focuses on antimicrobials for diclofenaca lone and combination with Ciprofloxacin) that could be used as antibacterial therapy.

AUTHORS CONTRIBUTION

All the mentioned authors are contributed in the current work.

REFERENCES

Abbas A, Atallah H, El-Sayed A, El-Ganiny M (2020). Diclofenac mitigates virulence of multidrug-resistant Staphylococcus aureus. Arch. Microbiol., 202(1):2751–2760. https:// doi:10.1007/s00203-020-01992-y

AL-Dujaily A, Mahmood, A (2022). Evaluation of Antibacterial and Antibiofilm Activity of Biogenic Silver Nanoparticles and Gentamicin Against Staphylococcus aureus Isolated from Caprine Mastitis. Iraqi J. Vet. Med., 46(1):10–16. https://doi.org/10.30539/ijvm.v46i1.1309.

Al-Iraqi O, Shareef Y, Dhahir H (2017). Urinary tract bacterial infection of local Iraqi buffaloes (Bubalus Bubalis) in Mosul city. Iraqi J. Vet. Med., 40(2):124–130. https://doi.org/10.30539/iraqijvm.v40i2.123.

Al-Rudha, A (2016). Distribution of E.coli O157:H7 in fecal and urine samples of cattle: E.coli O157:H7, Cattle, Fecal, Urine. Iraqi J. Vet. Med., 40(1): 79–82. https://doi.org/10.30539/iraqijvm.v40i1.142.

Al-Taii D, Yousif, A (2022). Effects of E.coli O157:H7 Experimental Infections on Rabbits. Iraqi J. Vet. Med., 43(1): 34–42. https://doi.org/10.30539/iraqijvm.v43i1.468.

Al-Zubaidy R, Shukr M, Ahmed M (2018). Contamination of the local produced broilers carcasses with Escherichia coli O157:H7 and its effect in public health in Diyala province. Iraqi J. Vet. Med., 41(2): 113–117. https://doi.org/10.30539/iraqijvm.v41i2.59.

Bai J, Shi X, Nagaraja T (2010). A multiplex PCR procedure for the detection of six major virulence genes in Escherichia coliO157:H7. J. Microbiolog. Methods, 82(1):85-89. https://doi:10.1016/j.mimet.2010.05.003.

Banerjee D, Shivapriya, P, Gautam, P, Misra, K, Sahoo A, Samanta S (2020). A review on basic biology of bacterial biofilm infections and their treatments by nanotechnology-based approaches. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci., 90: 243–259. https://doi.org/10.1007/s40011-018- 01065-7.

Bayer AS, Bolger AF, and Taubert KA et al. (1983). "Diagnosis and management of infective endocarditis and its complications". Circulation., 98 (25): 2936–48.

Cam Y, Atsever A, Eraslan G, Kibar M, Atalay O, Beyaz L, Inci A, and Liman C (2008). *Eimeria stiedae*: Experimental infection in rabbits and the effect of treatment with toltrazuril and ivermectin. Exp. Parasitol., 119 (1): 164-172.

Chan E, Yee Z, Raja I, Yap J (2017). Synergistic effect of non-steroidal anti-inflammatory drugs (NSAIDs) on antibacterial activity of cefuroxime and chloramphenicol against methicillin-resistant Staphylococcus aureus. J. Glob. Antimicrob. Resist.,10(1):70–74. https://doi.org/10.1016/j.jgar.2017.03.012.

Cobo-Simón M, Hart R , Ochman H *(2023). Escherichia Coli:* What Is and Which Are. Molecular Biol. Evol., (40) 1: msac273. https://doi.org/10.1093/molbev/msac273

Determination of diclofenac concentrations in human plasma using a sensitive gas chromatography mass spectrometry method. Chem. Cent. J., 10:52. doi: 10.1186/s13065-016-0199-3

Donough P (2000). Prevalence of Escherichia coli O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. J. Clin. Microbiol., 38(1): 318-322. DOI: https://doi.org/10.1128/jcm.38.1.318-322.2000

Espigares L F, Chico IH, Expósito-R M (2023). Antibiotic Resistance Changes in Gram-nagtive Bacteria from Urine Cultures: Development Analysis in a Health Area of South-EastSpain. Antibiotics , 12(7):1133.

Hasan Md Kamrul , Akhter S , Fatema K (2023). Selective modification of diclofenac to reduce the adverse effects; A computer-aided drug design approach. Informatics in Medicine Unlocked, 36(1):101159. https://doi.org/10.1016/j.imu.2023.101159

Hu Y, Zhang Q, and Meitzler C (1999). Rapid and specific detection of Escherichia coli O157:H7 in bovine faeces by a multiplex PCR. J. Appl. Microbiol., 87: 867–876.

Imkamp F, Bodendoerfer E , Mancini S (2023). QUIRMIA—A Phenotype-Based Algorithm for the Inference of Quinolone Resistance Mechanisms in *Escherichia coli*. Antibiotics., 12(7): 1119. https://doi.org/10.3390/antibiotics12071119

Khudhir Z (2022). Evaluation the Antibacterial Activity of the Brine, Nisin Solution, and Ozonated Water Against E. coli O157:H7 in the Experimentally Local Produced Soft Cheese. Iraqi J. Vet. Med., 45(1): 17–21. https://doi.org/10.30539/ijvm.v45i1.1035.

Khudhir Z, Hammad S (2017). The combined action of the Nisin and Lactoperoxidase system activation on the microbiological quality of raw milk with special emphasis against E.coli O157H7 in milk. J. Vet. Med., 40(2): 61–65. https://doi.org/10.30539/iraqijvm.v40i2.113.

Laudy E, Mrówka A, Krajewska J, Tyski S(2016). The influence of efflux pump inhibitors on the activity of non-antibiotic NSAIDS against gram-negative rods. PLoS ONE., 11(1):

e0147131.

Leão C, Borges A, and Simões M (2020). NSAIDs as a drug repurposing strategy for biofilm control. Antibiotics, 9:591. https://doi.org/10.3390/antibiotics9090591

Lee H, Popodi E, Tang H and Foster PL (2007). Rate and molecular spectrum of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing.National Academy of sciences of the United state of America, (109) 41.

Lukin A, Komarova K, Vinogradova L(2023). Synthesis and Antibacterial Evaluation of Ciprofloxacin Congeners with Spirocyclic Amine Periphery. Int. J. Mol. Sci. , *24*(2): 954. https://doi.org/10.3390/ijms24020954

Manzanas, C, Morrison E, KimYS et al (2023). Molecular testing devices for on-site detection of *E. coli* in water samples. Sci. Rep., 13 (4245): 2045-2322. https://doi.org/10.1038/s41598-023-31208-4

McDonald K, Smithee L (2011). Epidemiology of a large restaurantassociated outbreak of Shiga toxin-producing *Escherichia coli* O111,NM. Epidemiol. Infect., 140(9): 1644-1654. https://doi:10.1017/S0950268811002329

Mc Donough P (2000). Prevalence of *Escherichia coli* O157:H7 from cull dairy cows in New York state and comparison of culture methods used during preharvest food safety investigations. J. Clin. Microbiol., 38(1): 318-322.

Michałowska A , Kupczyk O , Czyrski A (2023). the Chemometric Evaluation of the Factors Influencing Cloud Point Extraction for Fluoroquinolones. Pharmaceutics . 15(6): 1774. https://doi.org/10.3390/pharmaceutics15061774

Morgan A, Leech A, Gloeciner W,Barrett C (2005). SPSS For Intermediate Statistic: Use and Interoretation, 2th ed, Laurence Erlbaum associates, Publishers Mahwah, NewJersey, London.

Najim N, Mustafa F (2017). The combined effect of bacteriocin-producing Lactobacillus rhamnosus GG-ATCC and Lactoperoxidase system activation on microbiological quality of raw milk with special emphasis against E.coli O157:H7 in milk. Iraqi J. Vet. Med., 40(2): 66–72. https://doi.org/10.30539/iraqijvm.v40i2.114.

Panxin Li, Rui Yin, Cheng J (2023). Bacterial Biofilm Formation on Biomaterials and Approaches to Its Treatment and Prevention. Int. J. Mol Sci., 24(14):11680. https://doi.org/10.3390/ijms241411680

Paes R, Silva R (2021). Antimicrobial Activity of Non-steroidal Anti-inflammatory Drugs on Biofilm: Current Evidence and Potential for Drug Repurposing Front Microbiol, 12(1); 707629. https://doi.org/10.3389/fmicb.2021.707629.

Panda, A.; Tatarov, I.; Melton-Celsa, A.R.; Kolappaswamy, K.; Kriel, E.H.; Petkov, D.; Coksaygan, T.; Livio, S.; McLeod, C.G. and Nataro, J.P.(2010). Escherichia. coli O157:H7 infection in Dutch belted and New Zealand white rabbits. Comp. Med, 60(1):31-37.

Park Inji, Jailani A, Lee JH (2023). The Antibiofilm Effects of Antimony Tin Oxide Nanoparticles against Polymicrobial Biofilms of Uropathogenic *Escherichia coli* and Staphylococcus aureus. Pharmaceutics, 15(6): 1679. https://doi.org/10.3390/pharmaceutics15061679

Paton A, Paton J (1998). Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbo111, and rfb O157. J. Clin. Microbiol., 36(2): 598-602 https://doi.org/10.1128/jcm.36.2.598-602.1998.

Pokharel P, Dhakal S,, Dozois CM (2023). The Diversity of *Escherichia coli* Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *11*(2): 344. https://doi.org/10.3390/microorganisms11020344

Quinn P, Carter M, Markey B, Carter G (2004). Clinical Veterinary Microbiology. 6th ed. Mosby an imp. Wolf, London, PP: 66 – 85.

Rainsford KD (2007). Anti-inflammatory drugs in the 21st century. Subcell. Biochem., 42: 3–27.

Ricciotti E, Fitz GA (2011). Prostaglandins and Inflammation. Arterioscle, Thromb, and Vasc Biol.,31(5): 986-1000. https://doi.org/10.1161/ATVBAHA.110.207449.

Salgar-Chaparro J, Lepkova K, Pojtanabuntoeng T, Darwin A, Machuca L (2020). Nutrient level determines biofilm characteristics and subsequent impact on microbial corrosion and biocide effectiveness. Appl. Environ. Microbiol.,86(7):e2885-19. https://doi.org/10.1128/AEM.02885–19.

Shah I, Barker J, Naughton DP, Barton S J, and Ashraf S S (2016).

Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime R, Arenas J (2020). Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics, 10(3). https://doi:10.3390/antibiotics10010003.

Yin W, Wang Y, Liu L, He J (2019). Biofilms the microbial protective clothing in extreme environments. Int. J. Mol. Sci., 20(14):3423. https://doi.org/10.3390/ijms20143423.

Yousra A. Abdlla,Raad A. Al-Sanjary(2023). The molecular identification of diarrheagenic Escherichia coli (DEC) isolated from meat and meat products. Iraqi J. Vet. Sci., (37) 1: 9-15. https://doi.org/10.33899/IJVS.2022.133244.2192