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Research Article

Antibacterial Effect of Co-Administration of Diclofenac and Ciprofloxacin against Infection Induced by Resistance *E coli* (O157-H7) in Female Rabbits

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Abstract | The aim of this project to study the effect of the combination between ciprofloxacin and diclofenac and alone in treating urinary tract infection caused by *Escherichia coli (0157):H7*. Samples were collected for the purpose of isolation and diagnosis resist.*E. coli 0157:H7* two hundred urine samples were collected from adult women patients suffering from UTIs during a period from February 2022 to July 2022 in Diwaniyah city Iraq . All urine samples were incubated aerobically at 37°C for 24-48 hrs on blood agar, MacConkey agar as well as biochemical tests identified special media and the isolates. The isolates were confirmed and diagnosed by Vitek assay. In this study, 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) as follow positive control(PC) , negative control(NC) diclofenac in 1mg/kg(DC), ciprofloxacin in 7 mg/kg(CIP), combination (ciprofloxacin 3.5 mg/kg+diclofenac1mg/kg) COM1 and combination(ciprofloxacin 1.75 mg/kg+diclofenac 1mg/kg) com2 groups all injection 0.1 ml in 1 x 10^9 CFU *E coli 0157 :H7* by urinary catheterization route excepting in negative control and all groups excepting negative groups resulted significant increase($P< 0.05$) in white blood cells, monocyte, and neutrophils in blood after three days comparison with negative control and in all experimental period (14 days) which was increase significant ($P< 0.05$) positive control (P.C.). However,(D-C,COM1,COM2) groups decrease significant ($P< 0.05$) in white blood cells, monocyte, and neutrophils values after (seven, fourteen) days comparison with the PC and CIP groups. diclofenac can be used in combination with antibiotics as an anti-virulence agent which will enhance the ability of the immune system to eradicate infection. In histopathological examination clear damage in kidney tissue specimens from positive group control (PC,DC) observed, with hyperplasia endothelial cell swelling, ulcer in the lining membrane, compared with(CIP,COM1and COM2). 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 | Diclofenac,Urinary tract infection, Antibacterial activity, Human pathogens, Rabbits, Ciprofloxacin, Multi-drug resistance and biochemical indices.

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INTRODUCTION

Urinary tract infections (UTI) are one of the most common medical complications during pregnancy (Kim and Lee, 2023). These infections are characterized by the presence of microorganisms in the genitourinary tract that cannot be explained by contamination, and have the potential to invade the tissues of the urinary tract and adjacent structures. Actually, the term UTI represents a wide variety of conditions, including asymptomatic bac-

teriuria, urethritis, cystitis, acute pyelonephritis and pyelonephritis associated with bacteremia or sepsis (Sanyaolu et al ., 2023). They usually named as a uropathogenic *E. coli* (UPEC) and have virulence properties that are associated with infection in the normal urinary tract including the expression of specific adhesions and toxins (Amábile, 2023).

Bacteria with Multidrug-resistant have rapidly increased in prevalence over the last several decades, resulting in persistent infections that are very difficult to treat with standard medicines and may last for long periods (Al-Iraqi et al., 2017). Multidrug-resistance (MDR) bacteria may also develop biofilms, which strengthens their resistance to both the human immune system and antibiotics (AL-Dujaily and Mahmood, 2022). The many traits of biofilms facilitate antimicrobial resistance (Park et al., 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. The capacity to overexpress efflux pumps allows antimicrobial compounds to be expelled from cells (Najim et al., 2017) including virulence and antibiotic resistance, is crucial for intercellular communication or quorum sensing (Kaur et al., 2023). *E. coli O157: H7* is a common pathogen linked to illnesses in the general public and healthcare facilities (Al-Zubaidy et al., 2018). *E. coli 015: H7* is a biofilm-producing bacterium linked to some of the most prevalent bacterial diseases globally (Al-Taii and Yousif, 2022). These bacteria become resistant to many antibiotics by acquiring gene clusters and plasmids(Kaur et al., 2023). Therefore, developing fresh approaches to managing bacterial infections is critica (Khudhir et al., 2017).

The lack of effective medications and the dangers involved in new plans seem to be solved by repurposing existing medications for novel therapeutic uses (Khudhir, 2022). Several recent studies have shown that nonsteroidal anti-inflammatory medicines (NSAIDs) diclofenac have antimicrobial properties foremother have analgesic, anti-inflammatory, and antipyretic effects (Oliviera et al., 2019). NSAIDs are medications that are often recommended together with antibiotics because they aid in lowering pain and fever brought on by bacterial infections (Hasan et al., 2023). The removal of generated biofilms by these NSAIDs in combination with antibiotics was thus examined to assess their potential to enhance the putative antibiofilm action of antibiotics (Chan et al., 2017). 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).

MATERIALS AND METHODS

Identification Of *E.coli* O157:H7

The media used for isolating resistance *E.coli* O157:H7 (Mc Donough et al., 2000) Two hundred humans urine samples were collected from females suffering from UTIs during the period from February 2022 to July 2022 incubation of the urine at 37°C for (1-2) days on the blood agar, MacConkey also, on specific media (Sorbitol MacConky, Chrom *E.coli* O157) and it were detected biochemically (Citrate utilization, Indole, Voges Proskeur, and Methyl Red) test the isolates were diagnostic by vitek2 assay Bacterial inoculum used to induce infection (acute UTI) will of 50 µL of pathogenic *E. coli 0157 H7* usually (107–109 CFU) of E.coli O157:H7 suspension, the inoculums preparation-standardize according to viable counting method-pour plate technique by using serial twofold dilutions (Quinn et al., 2004).. The dilution of 0.1 ml was injected intra urethral in the rabbit, then watched for clinical signs of UTI. The rabbit showed the signs depending on the dilution dose (Quinn et al., 2004). Culture overnight on brain heart broth of 0.1 ml is administrated by cannula to (infected groups). Insert urinary catheters using an aseptic technique.

Experimental Animals

Forty-eight female rabbits were split randomly into six groups, with eight rabbits in each group, and the serum was extracted from the coagulated blood samples by cen-

trifuge at 3000 rounds per minute for 15 minutes. The serum samples were then kept in a deep freezer at -20C until utilized for the serum biochemical test.

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.

GroupPC (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.

GroupCIP: 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 will be treated with 1.75mg/kg B.W ciprofloxacin + 1mg/kg B.W. All the treatments were administrated orally twice daily for 14 days.

Bacterial colonies on McConky medium at 37°C showed a rose pink hue 24 to 48 hours after incubation, whereas colonies on EMB had a metallic green shine. According to an assay, a quick and more accurate test for identifying these microorganisms in contaminated materials, the bacterial isolates also demonstrated gram-negative motile bacilli. The isolated bacteria were identified as E. coli. Smooth, colorless colonies may be seen on selective media such as Cefixime Tellurite-Sorbitol MacConkey and Chrom agarO157. The isolates were identified as *E. coli O157*, and this strain's diagnosis as *E. coli O157:H7* serotype was verified by the vitek2 test and serological detection of resistance. *O157:H7* serotyping test for *E. coli*

Blood Collection

Blood samples from the animals in each group were collected using the cardiac puncture technique and a 3 ml disposable syringe at zero time before infection and at 3, 7, and 14 days after infection induction. Blood samples were collected without any anticoagulant and allowed to stand and coagulate.

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

Dentification Of Resistance *E. Coli* O157:H7 By Vitek 2 System

Vitek2 system was used for the final identification, and GN-ID cards with 64 biochemical tests were used. The ID message confidence level for the isolate for resistant *E. coli O157:H7* was outstanding (the best likelihood percentage was 91%). This method is distinguished by quickly identifying Gram (-) from humans while reducing culture contamination and using little or no culture medium (Feng et al., 2020). In addition to enabling the interpretation of antimicrobial susceptibility tests (AST) for the proper treatment of patients, automated bacterial identification in the clinical laboratory offers a quick and reliable diagnosis for the majority of bacteria involved in the diseases with a high identification accuracy (Paim et al., 2014) discovered the value of the Vitek2 system for evaluating the biochemical properties of human *E. coli 0157: H7* isolates and the system's accurate identification of the species level. A sterile plastic stick applier used to take pure colonies from culture media and transfer a sufficient number of them to plastic test tubes. The age of colony was 24 hrs. Solutions content 3 ml of normal saline was prepared in especial plane tube of Vitek 2 system inoculated with a loop full of isolated colony. The test tube inserted into a dens check machine for standardization of colony to McFarland's standard solution (1.5×10^9 CFU/ml). This range for bacteria turbidity between 0.50 to 0.63. The standardization of inoculums placed into the cassette of Gram negative and a sample identification number entered into the computer software via barcode. Then, the cassette ID number was given. The cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader (incubator) module. The instrument controls the incubation temperature.

Serological Identification Of Resistance *E. Coli O157:H7* Serotyping Test (Wellcolex Resistance *E.coli* O157:H7, Remel)

Figure (1) shows the favorable and unfavorable outcomes based on the agglutination shown on the card test. The O157 and H7 antigens were identified in the colonies of non-sorbitol fermenting cultures using (SMA-CT). Because some of the resistant *E. coli* O157 strains were non-motile, isolates that had a positive response to the O157 antigen were cultured on blood agar overnight to detect the H7 flagellar antigen. When resistance *E. coli* O157:H7 was found using this test, the agglutination of the red color (positive result) for the O antigen compared

to the clear red color of the control, and the agglutination of the blue color indicated a positive result for the H antigen compared to the clear blue color of the control. In the current research, the latex agglutination test was regarded as a quick detection technique to shorten the process and eliminate additional pathogenic *E. coli* serotypes with biochemical character and culture. These findings concur with (March and Ratnam's, 1989) evaluation of the latex test as a quick and likely method of detecting *E. coli* O157:H7 based on laboratory tests.

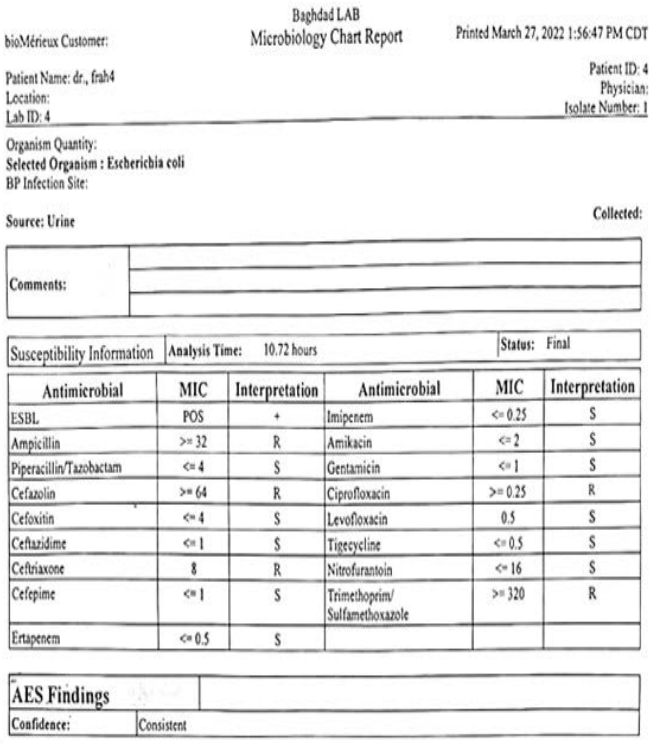
cited a related discovery when they said that the latex agglutination test was similarly effective for quickly identifying resistant *E. coli* O157 in urine. Vero toxins in culture filtrates of human *E. coli* may be detected and characterized using the latex agglutination technique, according to (Karmali et al., 2003). The Vero toxin (V.T.) test was quick, dependable, and simple. Its findings were also simple to understand, and it should enable more people to do V.T. testing

The values of white blood cell (WBC)

The values of white blood cell WBC x10^3/ul remains normal at the beginning of experiment in negative control group range during different times (3,7 and 14) days of the experiment which was (6.76±0.72,6.41±0.72,7.16±0.93) respectively however the infected group (PC,DC,CIP,COM2 and COM1) with resist. *E.coli O157: H7* showed a significant increase white blood cell (P< 0.05) which was (11.53±0.24, 11.60±0.24, 11.50±0.21, 11.37±0.22, 11.17±0.25) respectively as after 3 days compared with the N.C. group was (6.76±0.72) the values of white blood cell after 7 days showed a significant decrease (P< 0.05) in (COM1,COM2 and DC) groups which was(8.32±0.70, 8.33±0.72, 9.50±0.98) respectively compared with (PC and CIP) groups which was (12.27±0.51, 11.93±0.53) the values of white blood cell showed a significant increase (P< 0.05) in(COM2,COM1 and DC) groups which was(8.32±0.70, 8.33±0.72, 9.50±0.98) respectively compared with (NC) group was (6.41±0.72) after 7 days and the values of white blood cell after 14 days showed a significant decrease (P< 0.05) in (CIP, COM1, COM2 and DC) groups which was, (6.56±0.60, 5.21±0.54, 5.74±0.23) respectively as compared with (PC) groups was (14.36±0.36) respectively . The values of WBC count showed no significance change (P< 0.05) in (C.I.P, COM2, COM1, and DC) groups after14 days which was(6.56±0.60, 5.21±0.54, 5.74±0.23, 7.46±1.33) respectively, as compared with (NC) group was (7.16±0.93) the values of white blood cell significant increase white blood cell count (P< 0.05) in (PC) was (14.36±0.36) respectively compared with(NC) group was (7.16±0.93) after 14 days showed Table (2) and Figure (2).

The small letters are used for comparison between the columns. The capital letters are used to compare the rows.

Table 1: Antibiotic cards by Vitek2 compact used in the present study



Baghdad LAB
Microbiology Chart Report

bioMérieux Customer: Printed March 27, 2022 1:56:47 PM CDT

Patient Name: dr., frah4 Patient ID: 4
Location: Physician:
Lab ID: 4 Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
BP Infection Site:

Source: Urine Collected:

Comments:

Susceptibility Information | Analysis Time: 10.72 hours | Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	POS	+	Imipenem	<= 0.25	S
Ampicillin	>= 32	R	Amikacin	<= 2	S
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	S
Cefazolin	>= 64	R	Ciprofloxacin	>= 0.25	R
Cefoxitin	<= 4	S	Levofloxacin	0.5	S
Ceftazidime	<= 1	S	Tigecycline	<= 0.5	S
Ceftriaxone	8	R	Nitrofurantoin	<= 16	S
Cefepime	<= 1	S	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	<= 0.5	S			

AES Findings
Confidence: Consistent

Table 2: White blood cell counting 10^3/ul infected groups by resistance *E coli* 0157 H7 treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups

Groups/ WBCx 10^3/ul	3 days	7 days	14 days
Control –ve(NC)	6.76±0.72 A b	6.41±0.72 A d	A7.16±0.93c Ac
Control +ve(PC)	11.53±0.24 Ba	12.27±0.51 Ba	14.36±0.36 Aa
Diclofenac(DC) 1mg/kg	11.60±0.24 Aa	9.50±0.98 Bc	7.46±1.33 Cc
Ciprofloxacin (CIP) 7mg/kg	11.50±0.21 Aa	11.93±0.53 Ab	6.56±0.60 Bc

The Values Netrophilis

The values of netrophilis % remains normal at the beginning of the experiment in the negative control group range during different times (3,7 and 14) days of the experiment which was (44.25±1.03, 50.63±4.61, 44.25±1.03) respectively, however the infected groups (PC, DC, CIP, COM1and COM2) with resist. *E.coli O157: H7* showed a significant increase (P< 0.05) which was (72.35±3.82, 76.49±0.72, 76.04±0.87, 76.09±0.85, 76.04±0.88) respectively after three days compared with the NC group was (44.25±1.03) the values of netrophilis after seven days

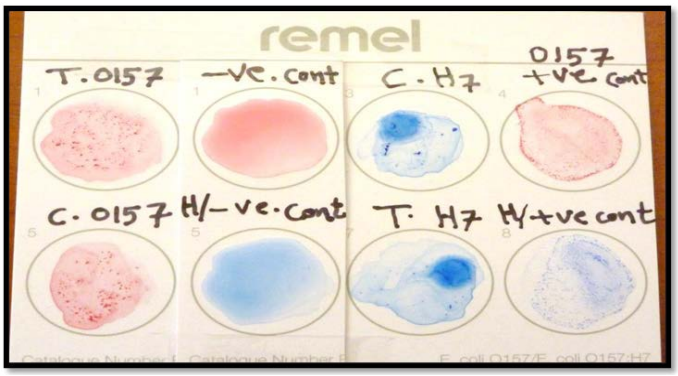


Figure 1: Serotyping of isolates by Wella colex color *resistance E. coli* O157:H7test.

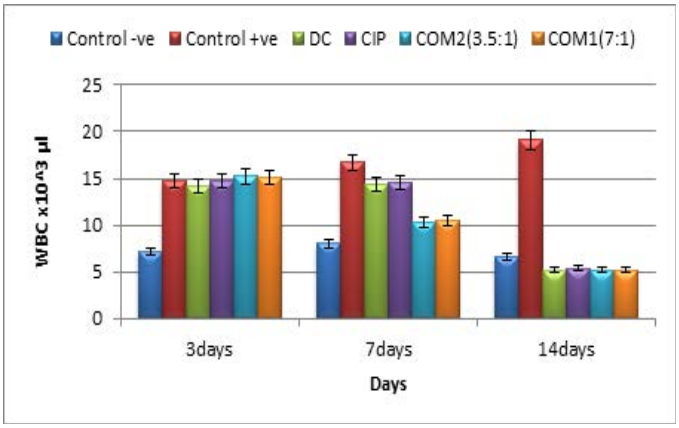


Figure 2:White blood cell counting x 10^3/ul in infected groups by resistance *E coli* 0157 H7 treated by ciprofloxacin ,diclofenac alone and combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=diclofenac dosage 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

showed a significant decrease (P< 0.05) (DC, COM1 and COM2) groups which was (42.95±2.39, 55.19±1.61, 55.44±1.37) respectively compared (CIP and PC) which was (74.18±2.44, 81.92±3.29), the values of netrophilis after 14 days showed a significant decrease (P< 0.05) (DC, CIP and COM2, COM1) groups which was (39.70±1.55, 41.49±0.35 44.61±1.3, 41.86±0.81) respectively as compared with (PC) group was (92.72±1.37) The values of neutrophils showed no significance change (P< 0.05) in (DC, CIP, COM2 and COM1) groups after14 days which was (39.70±1.55, 41.49±0.35, 44.61±1.3, 41.86±0.81) respectively, as compared with (NC) groups was (44.25±1.03) the values of neutrophils showed increase significant (P< 0.05) (P.C.) was (92.72±1.37) respectively compared with(NC) group was (44.25±1.03) Table (3) Figure (3).

The Values Of Monocyte

The values of monocyte x10^3/ul remains normal at the beginning of experiment in negative control group range

Table 3: Neutrophilis% infected groups by resistance *E coli* 0157 H7 treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups

Groups/ Neutrophilis%	Three days	Seven days	Fourteen days
Control –ve (NC)	44.25±1.03 Bb	50.63±4.61 Ac	44.25±1.03 Bb
Control +ve (PC)	72.35±3.82 Ca	81.92±3.29 Ba	92.72±1.37 Aa
Diclofenac (DC) 1mg/kg	76.49±0.72 Aa	42.95±2.39 Bd	39.70±1.55 Cb
Ciprofloxacin (CIP) 7mg/kg	76.04±0.87 Aa	74.18±2.44 Aa	41.49±0.35 Bb
Diclofenac (DC) 1mg/kg+ Ciprofloxacin (3.5 mg/kg) COM2	76.09±0.85 Aa	55.19±1.61 Ba	44.61±1.37 Cb
Diclofenac 1mg/kg+ Ciprofloxacin (1.75mg/kg) COM2	76.04±0.88 Aa	55.44±1.37 Ba	41.86±0.81 Cb

*The small letters are used for comparison between the columns. The capital letters are used to compare the rows.

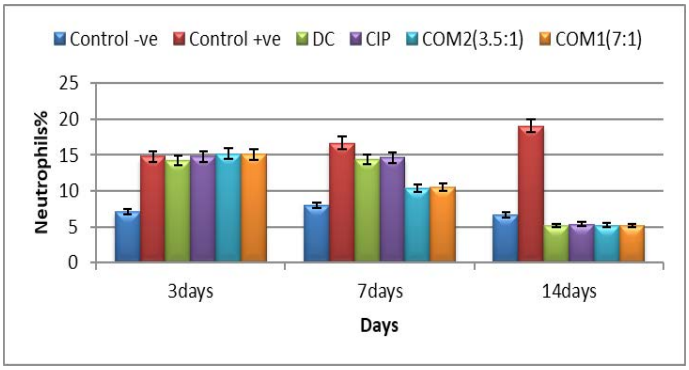


Figure 3: Neutrophils% in infected groups by resist. *E coli 0157 :H7* treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=diclofenac dosage 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)
capital letters are used to compare the rows.

during different times (3,7 and 14) days of the experiment which was (7.10±0.86, 8.02±0.68, 6.66±0.50) respectively however the infected groups (PC, DC, CIP, COM2 and COM1) with resist. *E.coli O157: H7* showed a significant increase (P< 0.05) which was(14.82±0.83, 14.24±0.96, 14.77±0.86, 15.20±1.05, 15.12±1.40) respectively as after 3 days compared with the NC group

was (7.10±0.86) the values of monocyte after seven days significant decrease(P< 0.05) (COM2,COM1 and DC) groups which was (10.33±0.60, 10.49±0.57, 14.41±0.89) respectively compared (PC,CIP) which was (16.70±0.77, 14.62±1.03) the values of monocyte significant increase (P< 0.05) (COM2, COM1 and DC) groups which was (10.33±0.60, 10.49±0.57, 14.41±0.89) respectively compared with (NC) group was (8.02±0.68) after 7 days the values of monocyte after 14 days significant decrease (P< 0.05) (C.I.P, COM2, COM1and DC) groups which was, (5.16±0.37, 5.38±0.20, 5.20±0.20, 5.19±0.19) respectively as compared with (PC) groups was (6.66±0.50) respectively. The values of monocyte showed no significance change (P< 0.05) (C.I.P, COM2, COM1and DC) groups after14 days which was (5.38±0.20, 5.20±0.20, 5.19±0.19) respectively, as compared with(NC) groups was (6.66±0.50) the monocyte significant increase (PC) was (19.08±0.98) respectively compared with (NC) group was(6.66±0.50) after 14 days Table (4) and Figure (4).

Table 4: Monocyte% 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/ Monocyte%	Three days	Seven days	Fourteen days
Control –ve (NC)	7.10±0.86 Ab	8.02±0.68 Ad	6.66±0.50 Ab
Control +ve (PC)	14.82±0.83 Ba	16.70±0.77 Ba	19.08±0.98 Aa
Diclofenac 1mg/kg (DC)	A14.24±0.96 Aa	14.41±0.89 Bb	5.16±0.37 Cb
Ciprofloxacin 7 mg/kg (CIP)	14.77±0.86 Aa	14.62±1.03 Aa	5.38±0.20 Bb
Diclofenac 1mg/kg+Ciprofloxacin (3.5mg/kg) (COM1)	15.20±1.05 Aa	10.33±0.60 Bb	5.20±0.20 Cb
Diclofenac 1mg/kg+Ciprofloxacin 1.75 mg /kg (COM2)	15.12±1.40 Aa	10.49±0.57 Bb	5.19±0.19 Cb

*The small letters are used for comparison between the columns. The capital letters are used to compare the rows.

Histopathological Change In Organs Of Rabbits

Histopathological Examination After 14 Days Post-Infection With Resistance *E.coli* O157:H7

Kidney and urinary bladder: The negative control group revealed the normal architecture of the kidney from the glomeruli in the cortex and renal tubules and the normal collecting tubules of the medulla. The bladder appeared

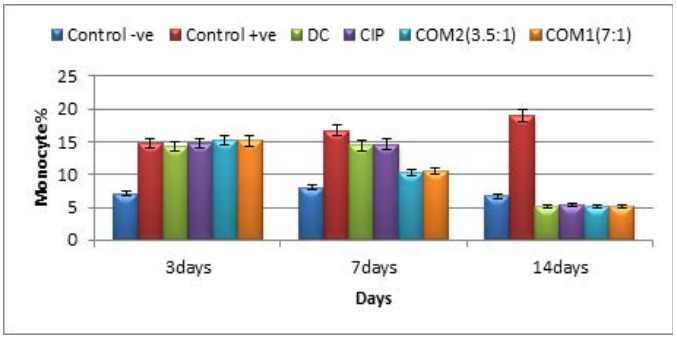


Figure 4: Monocyte % in infected groups by resistance *E coli* 0157: H7 treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups
DC=diclofenac dosage 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2=(ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

with normal mucosa of transitional epithelium, submucosa, and muscular layer Figure (5,6)

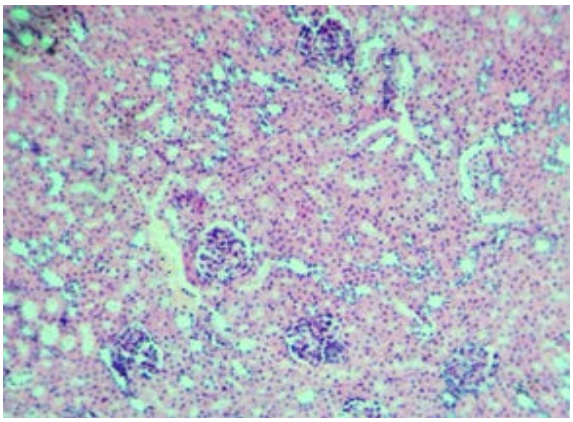


Figure 5: Histological section in the kidney from negative control shows: normal renal cortex from glomeruli and renal tubules. (H&E stain, 100X).

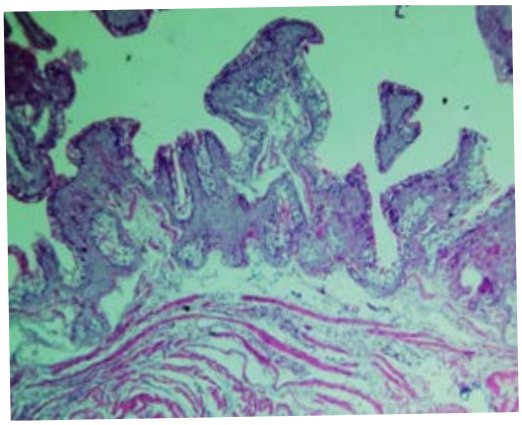


Figure 6: Histological section in the urinary bladder from negative control shows: normal transitional mucosal epithelium, submucosa, and muscle coat. (H&E stain, 100X).

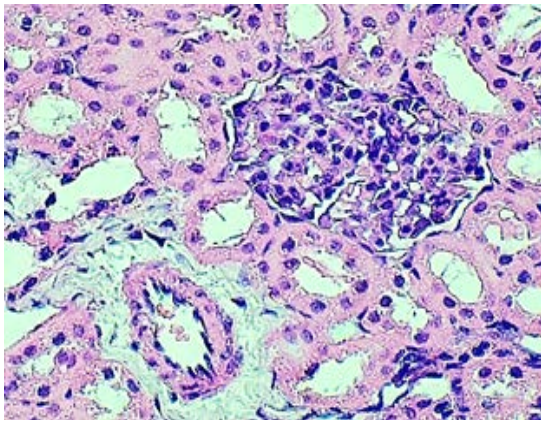


Figure 7: Histopathological show for renal from ciprofloxacin group female rabbit (CIP) shows: enlarged-congested glomerulus glomerular hypercellularity and perivascular edema . (H&E stain, 400X).

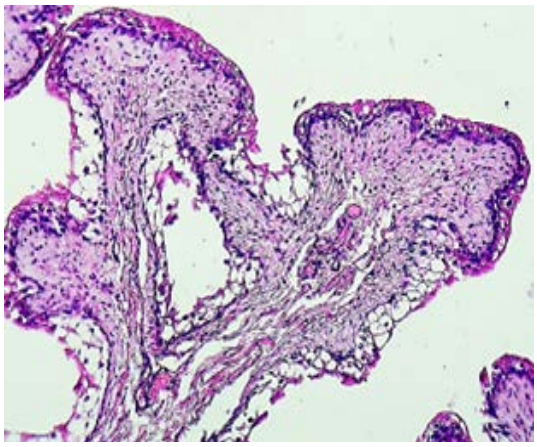


Figure 8: Histopathological show for urinary bladder from positive control (P.C.) female rabbit shows: vacuolar degeneration of transitional epithelium and eosinophilia (arrow). (H&E stain, 200X).

Figure (8) vacuolar degeneration of transitional epithelium and eosinophilia The kidney tissue sections in rabbits treated with CIP showed enlargement of glomeruli due to congestion of their capillaries and moderate to mild cellular degeneration of the lining epithelium of renal tubules. The urinary bladder in rabbits treated with CIP appeared thick with increased eosinophilia in superficial epithelial cells, and vacuolar degeneration is seen (Figures 7). In a group of rabbits treated with DC, the renal tissues revealed shrinkage and atrophy of glomerular tufts. Bowmans' distention contained eosinophilic material, which showed marked vacuolar degeneration of the transitional epithelial cells (Figures 9,10). atrophic- folding of transitional epithelium urinary bladder with com1 are shown in (Figure 11). In the com 2 group ,kidney thickening of transitional epithelium with vacuolation and mild atrophy of inner muscular layer (Figure 12).

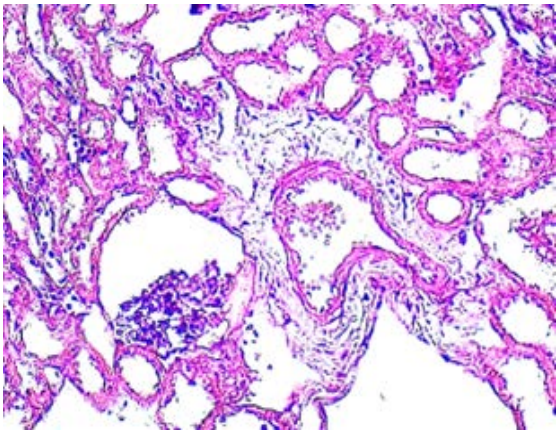


Figure 9: Histopathological show for renal from diclofenac group(D.C.) female rabbit shows: atrophic glomerular tuft, distended Bowman's space containing slight edema. interstitial perivascular edema and segmental necrosis (H&E stain, 200X).

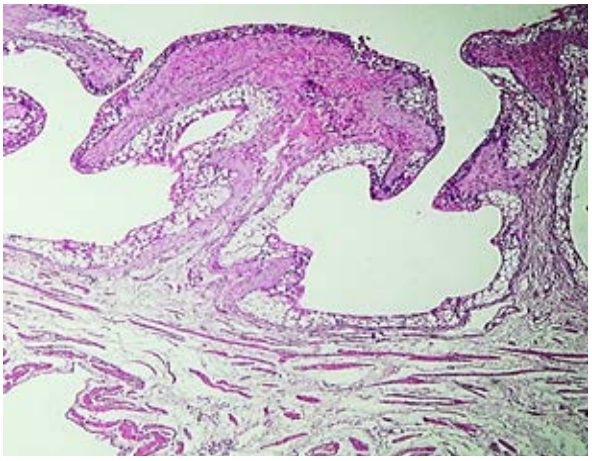


Figure 10: Histopathological show for renal from diclofenac group (D.C.) female rabbit shows: necrotic-eosinophilic transitional epithelium with vacuolation and mild atrophy of inner muscular layer. (H&E stain, 200X).

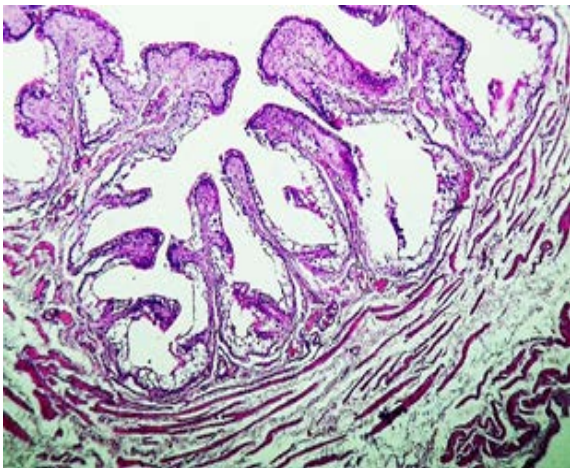


Figure 11: Histopathological show for urinary bladder from combination COM1)female rabbit shows: atrophic-folding of transitional epithelium. (H&E stain, 40X).

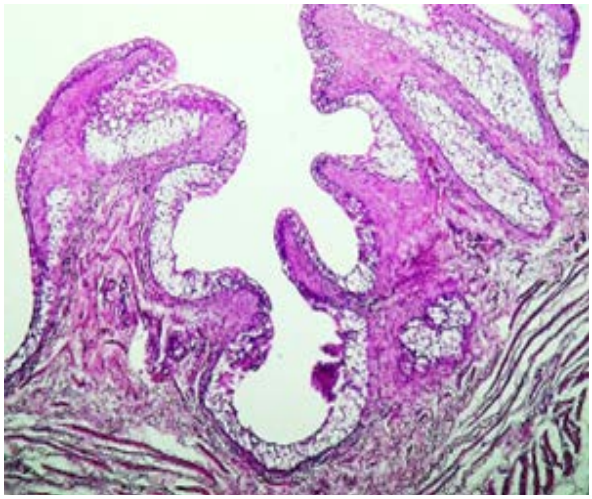


Figure 12: Histopathological show for renal from combination group (COM2) female rabbit shows: thickening of transitional epithelium with vacuolation and mild atrophy of inner muscular layer. (H&E stain, 200X).

DISCUSSION

The presented study showed increasing the monocyte, neutrophils and white blood cells in all groups after three days infected by resist. *E colli 0157: H7* indication by urinary catheter kidney dysfunction excepted negative control. This is evidence of infection. After inoculation, rabbits in groups PC, DC CIP,COM1,COM2were monitored daily for development of clinical signs, showed lethargy and weight loss compared with the pre-inoculation weight, and lack of movement around the cage when manually stimulated (toe or ear touch or pinch), include cloudy or milky urine.

After twenty four hours of infection showed a signs, all animals were suffering: poor appetite, frequent urination, dyspnea, emaciation occurred in 5 infected rabbits after 3 day post infection; Morbidity (infection) rate was 100% in infected groups along the period of experiment.

The presented study, ciprofloxacin (CIP) group the value monocyte, neutrophils and white blood cells after 14 days did no significant change compared with (NC) however three groups treated by diclofenac (DC), combination (ciprofloxacin+ diclofenac) and in two doses (3.5+1 and 1.75+1) (mg/kg), after 7and 14 days the significant decrease (P< 0.05) compared with (CIP, PC) and this is evidence of the additive effect of diclofenac with ciprofloxacin, while diclofenac gave effect, after seven days of starting treatment . diclofenac is a nonsteroidal anti-inflammatory drug that is used to relieve pain, fever, and inflammation by inhibiting the prostaglandins.

Prostaglandins are produced in response to injury or certain diseases and promote inflammation, pain and fever. They are produced by an enzyme called cyclooxygenase (COX). There are two forms, COX-1 enzyme and COX-2 enzyme. Diclofenac inhibits COX-1 and COX-2, leading to decreased prostaglandins, pain, fever, and inflammation. it inhibits COX-2 more than COX-, which indicates its potential effect in decreasing inflammation (Attri et al., 2015) the results presented study after seven days of the therapy, it reduces WBC, neutrophils, and monocytes. it can remove the clinical signs of inflammatory diseases. Diclofenac was a Competitive antagonist p2x. According to (Klein et al., 2022),

P2X receptors are contributed to pain transmission in CNS. It modulates P2X receptor-mediated by decreasing the ATP. it affects P2X receptor activation and pain transmission. P2X receptors have a role inflammation and immune responses. P2X promotes the release of pro-inflammatory. (Mansoor, 2022).

Diclofenac reducing inflammation and suppressing the production of inflammatory mediators may indirectly affect P2X receptor-mediated processes. It's important to note that the effects of diclofenac on P2X receptors may vary depending on the specific context and cell types involved. Diclofenac's modulation of P2X receptor function is considered secondary to its primary mechanism of action through COX inhibition and reduction in prostaglandin production (Neese et al., 2020) P2X receptors have been identified in rabbits. P2X receptors are a widely distributed family of ion channels found in various species, including humans, rodents (such as mice and rats), rabbits, and other mammals. (Mcgarvey et al., 2022) In rabbits, P2X receptors have been investigated in various physiological systems, such as the nervous, cardiovascular, gastrointestinal, and immune systems. They are involved in diverse processes, including neurotransmission, regulation of blood pressure, smooth muscle contraction, sensory perception, and immune responses. The specific functions and roles of P2X receptors in rabbits may vary depending on the tissue or organ system being studied. The additive effect between diclofenac and Ciprofloxacin against *resist. Escherichia coli 0157: H7* was found to decrease significant (white blood cells, monocyte, and neutrophils in blood) % (p<0.05), compared diclofenac, ciprofloxacin groups alone this study agreement with some studies that found antibacterial effects of NSAIDs against *K. pneumoniae, E. coli, P. aeruginosa, and S. aureus* (Chan et al., 2017). NSAIDs (diclofenac) have antibacterial effects with antibiotics, increasing bacteria susceptibility to antibiotic agents (Chan et al., 2017). Using antibiotics and NSAIDs could treat microbial infections and prevent inflammation; the effect of the combination (diclofenac + ciprofloxacin) dosage (3.5+1 and 1.75+1) mg/kg decreased the (white blood cells, monocyte, and neutrophils in blood) after 7 days,14 days

of the duration of the experiment compared with Ciprofloxacin alone.

The histopathological study clarifies the role of ciprofloxacin and combination (diclofenac + ciprofloxacin and groups (CIP, COM 1 and C0M2) where no effective histological disorders appear as normal tissue section observed in all dissecting organ. However, some sections showed mild disorders that did not affect organ function. The study demonstrated that rabbit experimentally infected positive control group (PC) with resistance *E coli 0157H7* developed obvious damage in tissue and loss of organ function. the mechanism led to this damage, as observed in the kidney section, which showed necrosis in tissue with necrotic foci and inflammatory cells indicating inflammation. Necrosis is the premature death of cells in living tissue by autolysis leading to unregulated digestion of cell components. Kidney section showed the most severe disorder occurred in a body organ, diagnosed as ulcers in the lining membrane, glomerular and tubular necrosis (loss nuclei, intense eosinophilic homogenous cytoplasm but preserved shape), without evidence of glomerulus thrombi, It was reported that the glomerular damage is the conscience of cytotoxic effect of Stx on capillary endothelial cells. As mentioned, Shiga toxin have an effect against only small blood vessels of the digestive tract, urinary bladder, and kidney. Although the kidney of the positive group control (PC and DC) in this study model showed swelling of epithelial cells and mononuclear cell infiltration, STEC-mediated HUS's pathogens were not demonstrated.

Resistance *E coli 0157 H7*infection and kidney necrosis indicated the necrosis cells fall into the tubular lumen obliterating it and determining acute renal failure, with the intact basement membrane, so the regeneration of the tubular epithelium is possible as the glomeruli are not severely infected (Willysson et al., 2020). Kidney section showed the most severe disorder occurred in the body organ, diagnosed as ulcers in the lining membrane, glomerular and tubular necrosis (loss of nuclei, intense eosinophilic homogenous cytoplasm but preserved shape), without glomerulus thrombi; Although the kidney of the positive control group (PC) rabbit in this study model showed swelling of epithelial cells and mononuclear cell infiltration, the pathogens of resistance *E coli 0157 H7*mediated HUS were not demonstrated.

(Lingwood, 2020) also, acute tubular necrosis without glomerular thrombi was observed in ciprofloxacin and combination (ciprofloxacin +diclofenac) treated rabbits. However,h the existence of the functional Stx-receptors Gb3 rabbit survived with E coli 0157 H7 infection and kidney necrosis, indicating the necrosis cells fall into the tubular lumen obliterating it, and determining acute renal failure, with the intact basement membrane. Hence,o the regeneration of the tubular epithelium is possible as the glomeruli are not severely infected (Willysson et al., 2020).

The Shiga-like toxin causes damage in the renal microvasculature, resulting in edema, localized tissue ischemia, and an influx of inflammatory cells into the renal mucosa despite intestinal hemorrhage having been detected by gross examination of the evidence. This is because polymorphonuclear leucocyte extravasation or direct release into the kidney compartment was a prominent feature in the purulent destruction of the renal mucosa. Polymorphonuclear leucocytes - PMN may contribute to developing systemic complications during *E. coli* O157:H7 infection by allowing the Shiga-like toxin to reach circulation and carrying the toxin to target organs, including CNS. Transmigration of human PMN into the intestinal lumen in response to Shiga-like toxin-induced inflammatory signals facilitates the movement of Shiga-like toxin into the bloodstream; according to (Legros et al., 2017), Shiga-like toxin bound to circulating PMN can be detected in blood from human patients with HUS, human PMN do not express the Shiga-like toxin receptor Gb3, but appear to bind it via a lower affinity receptor. The ability of PMN to transfer bound shiga-like toxin 2 to sensitive cells expressing Gb3 as well as to other PMN *in vitro* provides evidence that PMN may play a role in transporting shiga-like toxin 2 to the intestine and central nervous system – CNS (Tam et al., 2008). Shiga-like toxin 2 binds to isolated human PMN and may delay the onset of apoptosis in these cells. A delay in PMN apoptosis may prolong the functional lifespan of these cells and the amount of time they are in circulation and triggers the PMN oxidative burst that may result in tissue damage. According to research by (Legros et al., 2017), Shiga-like toxins, the putative target of the toxin in the development of HUS, may interact preferentially with rabbit renal microvascular endothelial cells in human E. coli O157:H7 UTI infections, leading to life-threatening vascular complications such as acute renal disease or HUS.

CONCLUSIONS AND RECCOMENDATIONS

From the results of this study, the following observations are deduced:
E. coli O157:H7 in the female urine, identified by *E. coli O157:H7* chromogenic agar medium, biochemical, serological, and vitek2 assays.
An antibiotics susceptibility test was done for *E. coli O157:H7* isolates that showed 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 infec-

tion (UTI)caused by *E. coli O157:H7*.for reduce the bacterial effect and inflammation

Study the antibacterial activity of the diclofenac on other G+ve and G–ve bacteria and Fungus.
2-Using the diclofenac with antibiotic (ciprofloxacin) to treat human and animals infected with urinary tract infection by *resist . E coli 0157 :H7* disease to overcome the bacteria resistance to antibiotics and reduce the bacterial effect and inflammation
3- study the antibacterial activity for other NSAIDs types.
4- Investigate the specific mechanisms and underlying interactions between diclofenac and other antibiotics and its wider effectiveness against resistant bacterial pathogens.
5- Combinations may be more useful than individual drugs in the treatment of *resist . E coli 0157 H7* infection.

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CONFLICT OF INTEREST

Authors declare that there is no commercial or any other conflict of interests related to this manuscripts or authors

NOVELTY STATEMENT

Due to the lack of an approved vaccine for any resistance E coli 0157 H7 caused (UTI) disease and the lack of easily accessible, secure, and effective medications for some diseases resistant to synthetic treatments, it is essential to look into alternative sources of antibacterial medication. The study's novelty focuses on (a diclofenac + ciprofloxacin) combination that can be used as novel antibacterial pharmaceuticals.

AUTHORS CONTRIBUTION

All the mentioned authors are contributed in the current work achievement.

DATA AVAILABILITY

Authors will provide all data at the reasonable request.

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