Study the Antibacterial Mechanism of Diclofenac and its Activity Alone or Combined with Ciprofloxacin in Treating Urinary Tract Infection

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Abstract: This study aimed to compare the effects of diclofenac, ciprofloxacin and their combination groups on urine tract infection (U.T.I.s) caused by resistance E *coli 0157 H7* in *vitro* and in *vivo*. Two hundred urine samples were collected from adult women patients suffering from U.T.I.s from February 2022 to July 2022. The urine specimen was cultured on the blood agar and then incubated at 37°C for (1-2) days; MacConkey agar is the media used for determining the biochemistry results, and identification by Rapidchek test which was performed on typical colonies from the selective plate. The M.I.C effect of Diclofenac began at (25600µg/ml) against resistance *E coli 0157H7* while M.I.C of ciprofloxacin it was at (0.96mg/ml), but M.I.C of a combination between (diclofenacn+ciprofloxacin) it was at (400+0.48) mg/ml. Resistance induction assay,with the ciprofloxacin has increased four times, while with the diclofenac has doubled, whilst the combination between (diclofenacn+ciprofloxacin) has no changed after 21 days, according to M.I.C.in the time-kill assay, it was proved that the ciprofloxacin and the combination between (diclofenacn+ciprofloxacin) works after 6 hours, compared to the diclofenac, which started to reduce bacterial counting after 40. After that, the examination was carried out by scanning electronic microscope (S.E.M.) to exam the mechanics of diclofenac,work which was found that it was works as anti-biofilm In this study, forty-eighth (48) rabbits were used and divided into six groups as follow positive control (P.C.), negative control (N.C.), diclofenac (D.C.) in 1mg/kg group, ciprofloxacin (C.I.P.)in7mg/kg group, combination (ciprofloxacin 3.5 mg/kg+diclofenac1mg/kg)COM1 group and combination (ciprofloxacin 1.75 mg/kg+diclofenac1mg/kg) com2 All animals (injected with 0.1 ml in 1×10^8 CFU E *coli 0157 H7* by urinary catheterization route excepting negative control all group excepting negative control resulted in a significant increase in pus cell, epithelium cell concentration in the urine after three days *E coli 0157 H7* infection comparison with negative control while in all experimental period after (14 days) showed no significant ($P < 0.05$) between positive control (P.C.) and diclofenac treated groups but combination COM1 group and com2 decrease significantly ($P< 0.05$) after (seven and fourteen) days comparison with positive control. diclofenac can be combined with antibiotics as an anti-virulence agent, enhancing the immune system's ability to eradicate infection.

Keywords: Diclofenac; urinary tract infection; antibacterial activity, human pathogens.

INTRODUCTION

Pathogens that are regarded the focus of infection, including outpatients and hospitalized patients, are becoming more resistant to antibiotics on a global scale. According to (Al-Zubaidy *et al*., 2018) these resistances might develop via mutation or by the appropriation of resistance genes from different species. Antimicrobial usage in animals and humans and the transfer of resistant strains among them are the major causes of rising antibiotic resistance. Antibiotic resistance is connected to the release of pharmaceutical industry effluents that have not been properly handled, particularly in nations where bulk medicines are produced (Najim *et al*., 2017).

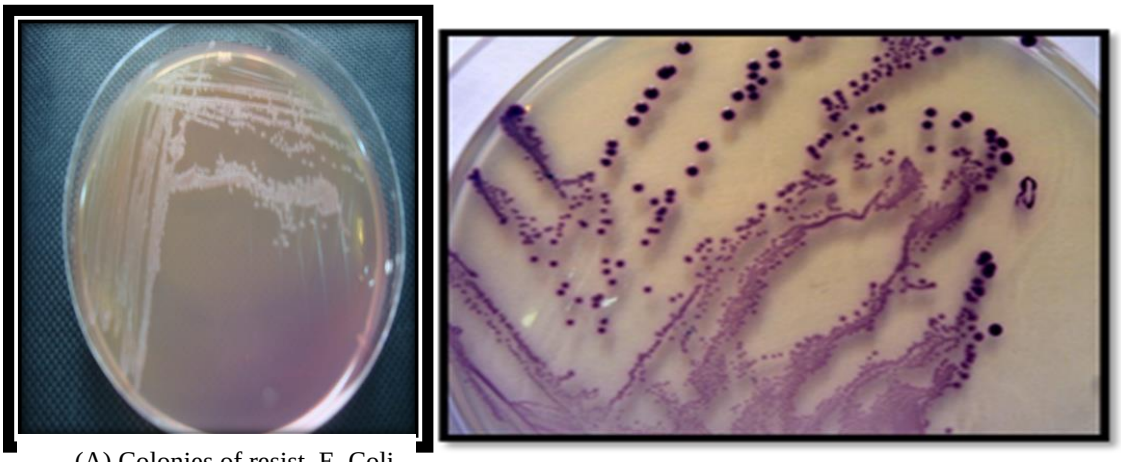
The overall result fraction of resistant bacteria that continue to proliferate rises as a result of antibiotics' increased selection pressure on bacterial populations, which kills more susceptible germs. Even at relatively low antibiotic concentrations, resistant bacteria may proliferate more quickly than susceptible germs. Alternative therapies are more necessary as antibiotic resistance spreads. Urinary tract infections (U.T.I.s) are among the most common bacterial infections seen in primary healthcare, and they are among the diseases with increasing antibiotic resistance (Al-Iraqi, 2017). Calls for new antibiotic therapies have been made, but new drug development is becoming rarer (Kapoor *et al*., 2017). With up to 35% of nosocomial diseases occurring in hospitals and being the second leading cause of bacteremia in patients in hospitals, these diseases have also emerged as the most prevalent hospital-acquired illnesses. Therefore they affecting patients of all ages and sexes but in Females account for 87.5% in compared to males (71.3%), affecting patients of all ages and sexes (AL-Dujaily and Mahmood, 2022).

This is related to women's small urethral tubes and the anus's near closeness to the urethral entrance, which makes it easier for germs to enter the urethra. According to estimates by(Al-Taii and Yousif ,2022), 50% of women repeated cases of acute cystitis throughout their adult lives. Although it is disputed and controversial, combination antibiotic treatment is routinely used to treat severe infections by gram negative. In comparison to monotherapy, combinations may be more effective because they have a wider antibacterial range, synergistic effects, and a lower likelihood of resistance developing during treatment. Combinations are being used more often to boost the antibacterial activity of existing medications against multidrug-resistant pathogens in the lack of evidence-based therapeutic choices. However, excessive usage of combinations must be avoided as it may lead to greater expenses, superinfections, resistant strain selection, and increased toxicity risks (Malema *et al*., 2018),. In addition to infecting adults,as well as these organisms are also responsible for various outbreaks in animals, poultry, and humans around the world. These organisms cause food-borne diarrhea, urinary tract infection, bloody diarrhea, and hemolytic uremic syndrome, which occur with an average of 4% of infections in women and children (Khudhir *et al*., 2022).One of the most serious illnesses in babies and children which induce renal failure or result in patient death is urinary tract infection brought on by *E. coli O157:H7* (Al-Rudha, 2016).

MATERIALS AND RESULTS

Identification of *E.coli O157:H7*

The culture of bacteria on Sorbitol MacConkey showed as amber or colorless, negative to sorbitol fermentation, while positive sorbitol of *E. coli* showed pink color Fig(1).



(A) Colonies of resist. E. Coli O157:H7 on C-SMAC.

(B) resist. E .coli O157:H7 culture on Chrom agar

Figer(1) Culturing *E. coli O157:H7*on Chromogenic agar base medium with cefixime tellurite the culture of *E. coli O157:H7* on Chromogenic medium with cefixime tellurite isolates belong to serotype resistance *E. coli O157:H7*; colonies showed pink to red in the color.

Specific biochemical tests for resistance *E. coli O157:H7*

The biochemical examination of the resistance *E. coli O157:H7* cannot ferment cellobiose sugar (cellobiose fermentation -ve), cannot grow in the presence of potassium cyanide (K.C.N. -ve), and it can produce enterohemolysin on blood agar (enterohemolysin production +ve),table(1),fig(3and4).

Table (1): biochemical examination of *E. coli O157:H7*

Biochemical tests	Results
fermentation of the Cellobiose	-ve
K.C.N.	-ve
production of the Enterohemolysin	+ve

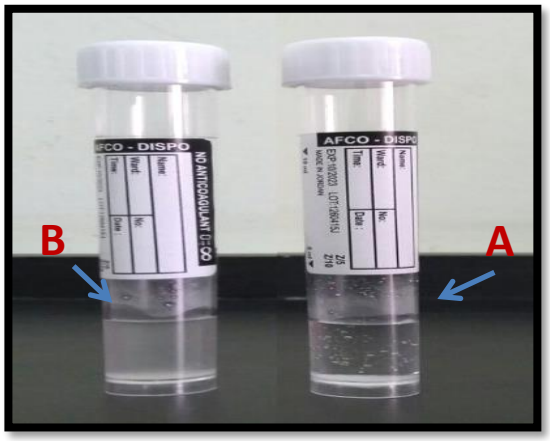


Figure (2): Cellobiose fermentation test.
A:Tube with turbid solution (negative result).
B: tube with clear solution (positive result).

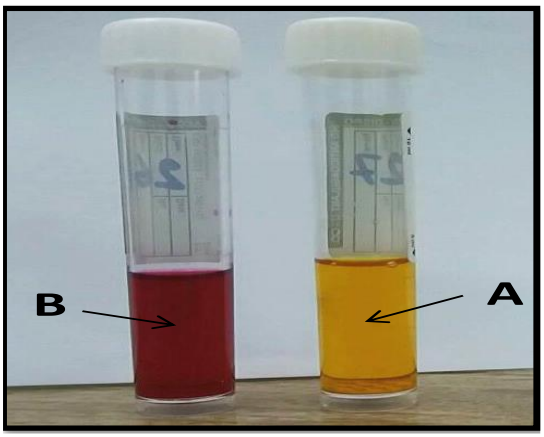


Figure (3): K.C.N. test.
A: tube with yellow color (cellobiose fermentation)
B: Tube with red color (no cellobiose fermentation)

Resistance E. *coli O157:H7* isolates lack the ability to grow in the presence of potassium cyanide Culturing of resist. *E. coli O157:H7* in potassium cyanide broth showed a clear solution after a period of incubation because it was unable to grow in the presence of potassium cyanide *while E. hermanii* is able to grow in potassium cyanide broth (B.A.M., 2001). *E. hermanii* is biochemically similar to resistance *E. coli O157:H7*. Therefore the main biochemical tests required to distinguish between them was their ability to ferment Cellobiose and grow in the presence of potassium cyanide (K.C.N.) (Al-Dawmy and Yousif, 2013).

The third confirmatory test involved the production of enterohemolysin. when *E. coli O157:H7* isolates grew on washing sheep blood agar, they demonstrated their capacity to produce enterohemolysin; -hemolysis manifested as small, turbid, unclear areas around the colonies. these findings were compatible with (Gould et al., 2009). One of the serum risk indicators for HUS. is IL-1, which is produced as a result of the pore-forming toxin enterohemolysin (Taneike, 2002).

Identification of *E.coli O157:H7* by Rapidchek test:

The two lines of the positive test of the sample broth is transferred to a test tube after the appropriate enrichment, and a test strip was then placed inside the tube. No heat treatment is necessary. Ten minutes will pass before the outcome.when two lines had appeared indicate a positive sample, whereas one line indicates a negative sample. The lateral flow strip includes a built-in control line, letting you know if the test was successful (Guerini et al., 2006).fig.(4)

Figure (4) RapidChek resist. *E. coli O157: H7* Negative results in the right, positive in the middle.

Estimation of the minimum inhibitory concentration (M.I.C.) value for resist. *E coli 0157 H7* against ciprofloxacin, diclofenac and combination of (ciprofloxacin + diclofenac)

The M.I.C. of selected antibiotics was obtained for resist. *E. coli O157: H7* isolate used. Serial dilutions of ciprofloxacin, Diclofenac, and a combination of (ciprofloxacin + Diclofenac) were made in broth, and a standard inoculum of *E. coli O157: H7* of the tested bacteria was added and incubated for 24 hours. Then determination of the highest dilution showed no turbidity. A control tube was prepared by adding culture to broth without antibiotic in addition to blank (no bacteria and no antibiotic). After overnight incubation at 37°C, tubes were inspected for growth which was indicated by turbidity. Tubes which the antibiotic was present in sufficient concentrations to inhibit bacterial growth remained clear. The last "clear" tube in the M.I.C. results showed that bacterial isolates were more affected by combination at low concentrations (0.48 µg/ml ciprofloxacin + 400 µg/ml Diclofenac) against resistance *E.coli o157 H7*, whilst ciprofloxacin showed a mild activity against resistance *E.coli 0157 H7* (0.96 µg/ml), the result is considered resistant compared to CLSL (2020) which it was ≤ 0.25. The clinical *E. coli* detected in the hospitals showed that Diclofenac has an antibacterial effect against different bacteria at (5-50) µg/ml and could treat U.T.I.s. (Mazumdar at el., 2009;Salem-Milani et al., 2013). The results have depicted significant antibacterial activity of Diclofenac and Ibuprofen(I.B.P.) at 50µg/ml diclofenac decreased the M.I.C. for ciprofloxacin against resistance *E coli 0157 H7*. One or more of the clinical strains utilized in the experiment showed a high ciprofloxacin M.I.C. without diclofenac (M.I.C. = 0.96 µg/ml) but a considerable decrease in M.I.C. with diclofenac at two different doses (M.I.C. = 0.48 µg/ml). This outcome is consistent with the conclusions achieved by (Abbas et al., 2020).. In addition to its antibacterial properties when combined with other medicines,

diclofenac 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. Ibuprofen, diclofenac, aspirin, and celecoxib have been demonstrated to have antibacterial properties against a variety of bacteria, including *K. pneumoniae, E. coli, P. aeruginosa, and S. aureus* in many investigations (Chan *et al*., 2017; Paes Leme *et al*., 2021). NSAIDs, diclofenac (D.C.), as previously reported by (Abbas *et al*., 2020),which have inhibitory actions against the formation of S.T.X. and were able to reduce the virulence of *E. coli*. diclofenac may prevent the growth of a number of microorganisms, including *S. aureus, E. coli, C. albicans, M. tuberculosis, and L. monocytogenes*, according to investigations by (Mazumdar *et al*., 2009). whereas, in disagreement with (Leo *et al*., 2020), who said that diclofenac showed *in vitro* activity against *E coli* isolate with a high M.I.C. (2,000 mg/mL) and no measurable minimum bactericidal levels >2,000 mg/m. According to several researches, NSAIDs may decrease bacterial pathogenicity and antibiotic sensitivity through a variety of methods. According to different research,Ibuprofen (I.B.P.)and diclofenac may bind to D.N.A. gyrase and prevent the development of bacteria by acting as antibiotics. As stated by a recent research, diclofenac has structural similarities with the quinolones and fluoroquinolone families of antimicrobials (Kahlous *et al*., 2017). The anti- virulence effects of NSAIDs, such as the reduction of hemolysis and staphyloxanthin formation in these bacteria, provide indications that they are active against the pathogenicity of *S. aureus* in addition to their anti-biofilm action (Abbas *et al*., 2020).shown table (2)

Table (2): Minimum inhibitory concentration (M.I.C.) (µg/ml) **(ciprofloxacin, diclofenac, combination (ciprofloxacin +diclofenac)**

DRUGs	**concentration (µg/ml)**									**M.I.C. (µg/ml)**
Ciprofloxacin (C.I.P.)	0.06		0.12		0.24			0.48		0.96
Diclofenac(DC)	50	100	200	400	800	1600	3200	6400	12800	25600
COM(ciprofloxacin +diclofenac)	0.03+25			0.06+50			0.12+100		0.24+200	0.48+400

Resistance Induction Assay

The results that depended on M.I.C. were shown for each of the ciprofloxacin, diclofenac, and combination (ciprofloxacin +diclofenac)after zero and four days was M.I.C. for ciprofloxacin, Diclofenac and combination (ciprofloxacin +diclofenac) was (o.96,25600, (0.48+12800)) mg/ml respectively but after 8,14 and 21 days combination (ciprofloxacin +diclofenac) remained stationary this agrees with (Abbas *et al*.,2020) whom used diclofenac as an inhibitor on virulence *E. coli 0157 H7*, including biofilm formation increased antibiotic resistance (Verma *et al*., 2018). Diclofenac decreased the biofilm formation while ciprofloxacin change appeared, yet in M.I.C. ciprofloxacin after 8,14 and 21 days was (1.92 , 3.84and 7.68) µg/ml, respectively these finding were consistent with studies that have *E. coli 0157 H7 i*ncreased resistance to (ampicillin, fluoroquinolones, tetracycline, cephalosporins, and chloramphenicol) (Belfield *et al*., 2017; Pereira *et al*., 2018; Abidi *et al*., 2019; Dai *et al*., 2019). After 21 days increased to four times M.I.C. while diclofenac change appeared after 14and 21 days and was (51200, 51200) µg/ml respectively but it did not fourfold These finding were consistent with other studies shown by (Abidi *et al*., 2019). diclofenac cannot induce the antibiotic resistance in combination (ciprofloxacin +diclofenac) in the experimental studies, values which was same in all period experimental as shown in the table (3).

Table (3) Resistance induction assay in infected groups treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac)

Days	M.I.C. after				
	0	4	8	14	21
Ciprofloxacin µg/ml	0.96	0.96	1.92	3.84	7.68
diclofenac µg/ml	25600	25600	25600	51200	51200
ciprofloxacin+diclofenac µg/ml	0.48+400	0.48+400	0.48+400	0.48+400	0.48+400

Time Kill Assay

In the time-kill assay, the results presented in terms of the changes in the log10 cfu/mL of viable colonies indicated that the diclofenac exhibited little bactericidal activity.

The results showed that, after zero time, there was no significant ($P<0.05$) change in all groups of control and groups treated with ciprofloxacin 0.96 ,diclofenac 25600 and combination (ciprofloxacin 0.48 + diclofenac 400) which were 2.28±0.06, 2.17±0.01, 2.25±0.01and 2.15±0.04 respectively while after 2 hours it increased significantly ($P<0.05$) in control and diclofenac groups compared with ciprofloxacin ,combination (diclofenac and ciprofloxacin)groups which was (3.94±0.18, 3.42±0.10) (2.55±0.13, 2.22±0.05) respectively after 4 hours it increased significantly ($P<0.05$) control and diclofenac groups compared ciprofloxacin and combination (diclofenac and ciprofloxacin) groups which was (5.09±0.28,

4.47±0.12)(1.63±0.07,.14±0.01) respectively after 6 hour it increased a significantly (P<0.05) in groups of control and diclofenac compared ciprofloxacin, combination (diclofenac and ciprofloxacin)groups which was (5.15±0.21, 4.70±0.11),(0.00±0.00, 0.00±0.00) respectively which was decrease a significant(P<0.05) compared diclofenac it was (5.15±0.21), (4.70±0.11) respectively in same time whilst in 8,24,30,36 hours there was significant increase (P<0.05) in control and diclofenac comparison with ciprofloxacin, and combination (diclofenac and ciprofloxacin) groups it was (5.25±0.06, 5.92±0.15, 5.94±0.18, 5.87±0.16 , 5.57±0.20), (4.22±0.26, 4.16±0.28, 3.41±0.16, 2.45±0.14,) (0.00±0.00, 0.00±0.00, 0.00±0.00 ,0.00±0.00, 0.00±0.00), (0.00±0.00, 0.00±0.00, 0.00±0.00, 0.00±0.00, 0.00±0.00) respectively As well as results showed significant increase a (P<0.05) in control compared to diclofenac which was (5.25±0.06, 5.92±0.15, 5.94±0.18, 5.87±0.16, 5.57±0.20), (4.22±0.26, 4.16±0.28, 3.41±0.16, 2.45±0.14, 0.00±0.00) respectively After 40 hours resuts in table (4) the results after 4 hours ciprofloxacin in concentration 0.96 µg/ml and combination (diclofenac and ciprofloxacin) in concentration (400+0.48) µg/ml no growth bacteria resistance *E coli 0157 H7* but diclofenac alone killing bacteria after 40 hour and in concentration 25600 µg/ml ciprofloxacin concentration in combination with diclofenac was half 0.48 µg/ml compared with alone which was 0.96 µg/ml and diclofenac it was 400 µg/ml compared alone was 400µg/ml this result agree with (Leão *et al*., 2020). A high level of diclofenac has an effect on the biofilm metabolism of inactivating *E coli*; the biofilm showed a killing effect only with tetracycline or kanamycin, ciprofloxacin (Leão *et al*., 2020).

Table (4) Killing time(log) in infected groups by resist *E coli 0157* treated by ciprofloxacin, diclofenac alone, and combination between (ciprofloxacin +diclofenac) and control groups.

Groups	Zero time	2hr	4hr	6hr	8hr	24hr	30hr	36hr	40hr
Control	2.28 ±0.06 Da	3.94 ±0.18 Ca	5.09 ±0.28 Ba	5.15 ±0.21 Ba	5.25 ±0.06 Ba	5.92 ±0.15 Aa	5.94 ±0.18 Aa	5.87 ±0.16 Aa	5.57 ±0.20 Aa
Ciprofloxacin (0.96) µg/ml	2.17 ±0.01 Ba	2.55 ±0.13 Ab	1.63 ±0.07 Cb	0.00 ±0.00 Dc	0.00 ±0.00 Dc	0.00 ±0.00 Dc	0.00 ±0.00 Dc	0.00 ±0.00 Dc	0.00 ±0.00 Dc
Diclofenac (25600) µg/ml	2.25 ±0.01 Da	3.42 ±0.10 Ca	4.47 ±0.12 Aa	4.70 ±0.11 Ab	4.22 ±0.26 Bb	4.16 ±0.28 Bb	3.41 ±0.16 Cb	2.45 ±0.14 Db	0.00 ±0.00 Eb
Ciprofloxac0.48in+diclofenac 400 µg/ml	2.15 ±0.04 Aa	2.22 ±0.05 Ab	1.14 ±0.01 Bc	0.00 ±0.00 Cc	0.00 ±0.00 Cc	0.00 ±0.00 Cc	C0.00 ±0.00 Cc	C0.00 ±0.00 Cc	C0.00 ±0.00 Cc

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

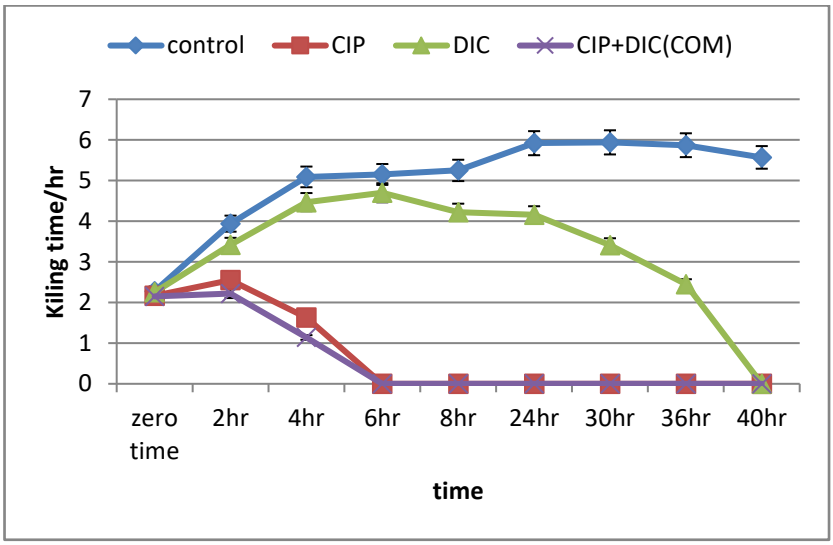


Figure (6) killing time(log) in infected groups by resist. *E coli 0157* treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) and control groups
DC=diclofenac dosge 25600 µg/ml
CIP=ciprofloxacin dosage 0.98 µg/ml
COM= (ciprofloxacin0.48 µg/ml +diclofenac400 µg/ml)

Scanning Electron Microscope (S.E.M.).

Electron microscopy results showed Regarding the mechanics of diclofenac work, destroyed the formed biological membranes, which increased its resistance *E coli 0157 H7* ciprofloxacin. The results were consistent with (Leão *et al.*, 2020) where mentioned published an interesting study evaluating the *in vitro* activity of NSAIDs against resistance *E. coli 0157 H7and S. aureus* biofilms. *In vitro*, experiments showed that aspirin (A.S.A.)diclofenac and ibuprofen have anti-biofilm activity in concentrations similar to those found in human pharmacokinetic studies (plasma concentrations). S.E.M. images of Diclofenac (1× M.I.C.) treated and untreated resistance *E coli 0157 H7* cells. Normal morphology of untreated diclofenac resistance Smooth cell surfaces with no apparent ultrastructural alterations are shown in *E. coli 0157 H7 fig* (7,a); diclofenac-treated cells exhibit deformities and core holes during the exposure period fig (8)(b, c, and d); A large amount of cell surface damage is seen in the S.E.M. picture as shown by spurting of cellular contents, loss of form (bending), and the central hollowing of numerous biconcave cells with a central depression and cavity indicating concurrent leaking of cytoplasmic contents. A few deformed cells may be seen at the field's perimeter, and colonies with scratches, surface blebs, and plicated surfaces have also been noted. Numerous cells had the appearance of being pierced and spewing cytoplasmic material. Black solid arrows have denoted major structural modifications.

In fact, cell permeation to propidium iodide and 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)

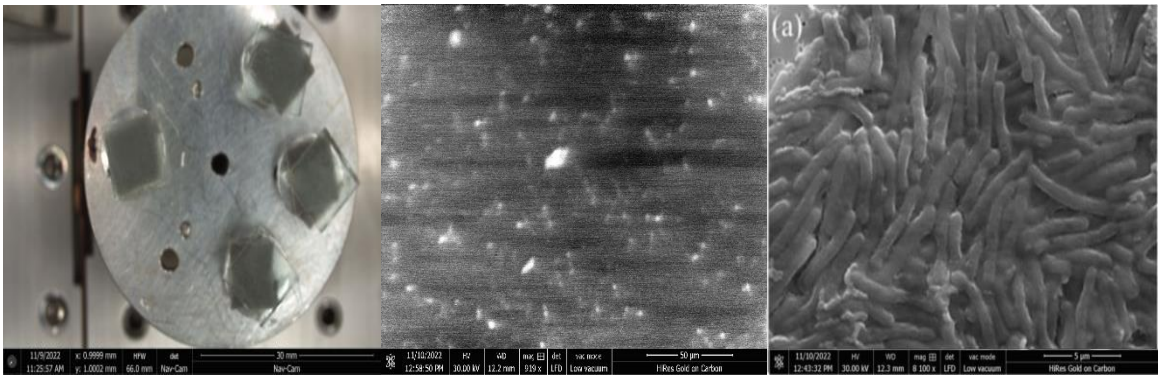


Figure (7): S.E.M. images of resistance *E. coli 0157 H7cells*: (a) untreated

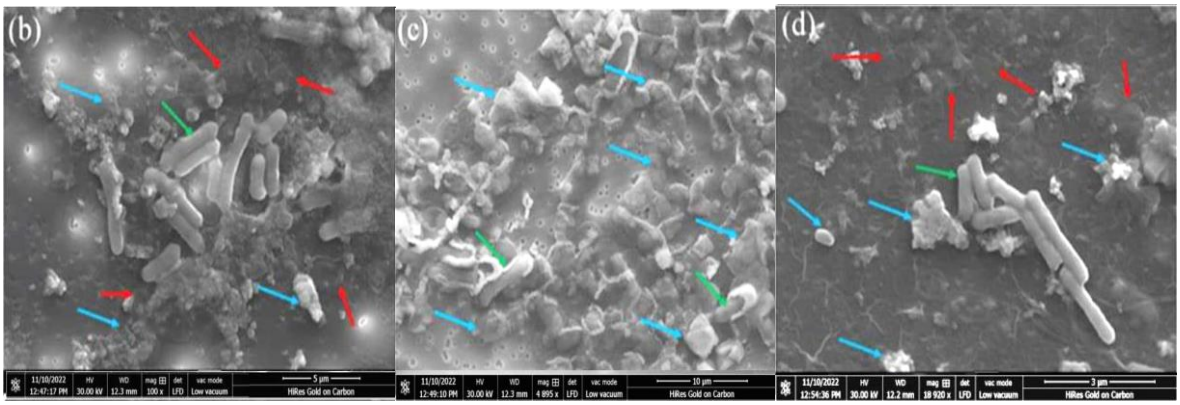


Figure (8) S.E.M. images of resist, *E. coli 0157 H7*cells: (b, c, d). treated diclofenac 25600 (mg/kg) intact cells (green arrows), cellular debris (blue arrows), and ghost cells (red arrows)

Urine Culture

All urine samples were collected from infected rabbits with urinary tract infection (in sterile containers, midstream urine, after cleaning the genitals and centrifuged 2000rpm for 2min) from infected rabbits with urinary tract infections. Immediately, the sediment was incubated with brain heart infusion broth at 37oC overnight and streaked (by sterile swab) on blood and MacConkey agar surface and incubated aerobically at 37oC for 24h (Collee *et al.*, 1996).

Forty-eight female rabbits were divided randomly into six groups (8 rabbits in each group).

1. Group NC (Negative control): The uninfected group were only distilled water orally.
2. GroupPC (Positive control): the animals will-infected with *E. coli o157-H7* and left without any treatment.
3. Group D.I.C.: Infected animals with *E. coli o157-H7* treated with diclofenac 1mg/kg B.W.
4. GroupCIP: Infected animals with *E. coli o157-H7* and treated with ciprofloxacin at 7mg/kg B.W

5. Group COM1(combination): Infected animals with *E. coli o157 H7* and treated with a combination of 3.5mg/kg b.w ciprofloxacin.+ 1mg/kg B.W. diclofenac.
6. Group COM2 were treated with 1.75mg/kg B.W ciprofloxacin.+ 1mg/kg B.W. All the treatments will be administrated orally twice daily for 14 days.

Pus cell scor in urine

The values of pus cell score were normal at the starting point of the experiment in negative control group range during different times (3,7and14) days of the experiment it was (2.00±0.00,2.00±0.00 and 2.00±0.00) respectively; but the infected group (P.C., DC, C.I.P., COM1 and COM2) with resist. *E.coli O157: H7* showed a significant increase pus cell concentration in urine (P< 0.05) was (9.00±0.70, 9.40±0.50, 9.20±0.70, 9.30±0.70, 9.50±0.70) respectively as after 3 days compared with the N.C. group which was (2.00±0.00) in same time. The values of pus cell concentration after 7 days showed a significant decrease concentration in urine (P< 0.05) in (COM1and COM2) groups which was (4.70±0.25, 4.80±0.22) respectively compared to (C.I.P., P.C.and DC) which was (18.60±2.50, 18.20±2.67,18.60±2.50) The values of pus cell levels demonstrated increase in (COM1and COM2) groups which was (4.70±0.25, 4.80±0.22) respectively compared with(N.C.) group which was (2.00±0.00) after 7 days The values of pus cell after 14 days showed a significant decreased (P< 0.05) in (C.I.P., COM1and COM2) groups, (1.90±0.24, 2.40±0.29, 2.50±0.20) respectively as compared with (P.C.and DC) groups it was (25.30±3.26, 24.30±2.84) respectively. Whereas no significance difference (P< 0.05) in (C.I.P., COM1and COM2) groups after14 days was (1.90±0.24, 2.40±0.29, 2.50±0.20) respectively, in compared with (N.C.) groups (2.00±0.00) in same time The values of pus cell concentration in urine increase a significant pus cell concentration in urine (P< 0.05) in (P.C., DC) (25.30±3.26 and 24.30±2.84) respectively in compared with(N.C.) group (2.00±0.00) after 14 days table (5).

Table (5) pus cell score at high power field (HPF) in urine-infected groups by resist. *E coli 0157* treated by ciprofloxacin, diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses (mg/kg) and control groups.

Groups/ Pus cells concentration in urine HPF	Three days	Seven days	Fourteen days
Control –ve(NC)	2.00±0.00 Ab	2.00±0.00 Ac	2.00±0.00 Ab
Control +ve(PC)	9.00±0.70 Ca	18.60±2.50 Ba	25.30±3.26 Aa
Diclofenac 1mg/kg(DC)	9.40±0.50 Ca	18.20±2.67 Ba	24.30±2.84 Aa

Ciprofloxacin 7 mg/kg(CIP)	9.20±0.70 Aa	18.60±2.50 Aa	1.90±0.24 Bb
Diclofenac 1mg/kg +Ciprofloxacin (3.5mg/kg)COM1	9.30±0.70 Aa	4.70±0.25 Bb	2.40±0.29 Cb
Diclofenac 1mg/kg +Ciprofloxacin (1.75mg/kg)COM2	9.50±0.70 Aa	4.80±0.22 Bb	2.50±0.20 Cb

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

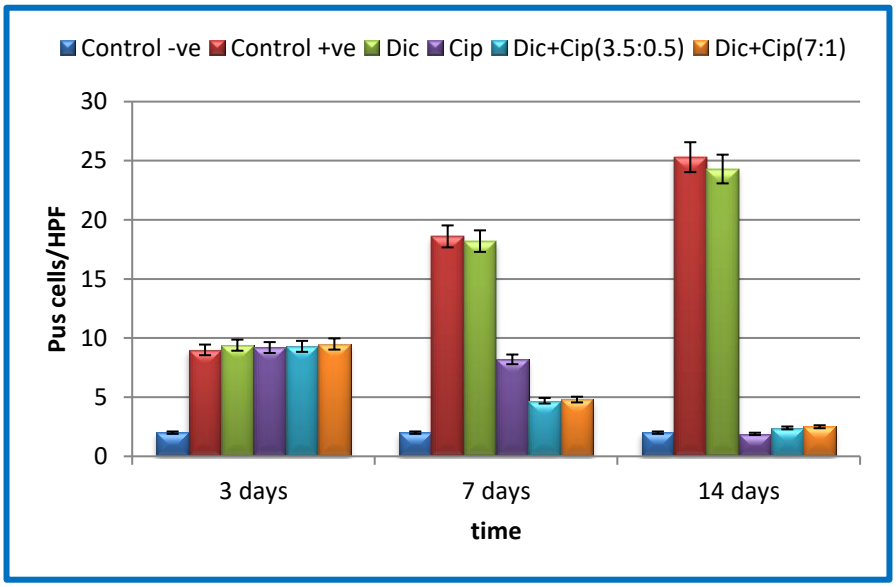


Figure (9) pus cell score in urine at high power field (HPF) in infected groups by resistance *E coli 0157* treated by ciprofloxacin, Diclofenac alone, and combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups

DC=diclofenac dosage 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)

Pus cells, also known as leukocytes, are white blood cells that are present in the urine. The presence of pus cells in urine may indicate an infection or inflammation in the urinary tract (Akoachere et al.,2012).

Common causes of pus cell concentration in urine include urinary tract infections (U.T.I.s), such as bladder or kidney infections. U.T.I.s can occur when bacteria enter the urinary tract and multiply, leading to symptoms such as frequent urination, pain or burning during urination, cloudy or foul-smelling urine, and a feeling of urgency.

In addition to U.T.I.s, other conditions that can cause pus cells in urine include kidney stones, interstitial cystitis (a chronic bladder condition), prostatitis (inflammation of the prostate gland in men), and sexually transmitted infections (S.T.I.s) such as gonorrhea or chlamydia. (Muvunyi *et al* .,2011) In this study, increased pus cell concentration in the urine of all groups after three days of infected by resistance *E colli 0157 H7* which indication by urinary catheter kidney dysfunction excepted negative control. This is evidence of infection after the injection resistance *E coli 0157 H7* in this study after 14 days no ciprofloxacin (C.I.P.) group showed no significant difference ($P< 0.05$) compared with negative control (N.C.). However, two groups treated by combination (ciprofloxacin + Diclofenac) and in two doses 3.5+1(mg/kg) and 1.75+1(mg/kg), after 7,14 days from the beginning resulted showed a significant decrease ($P< 0.05$) compared with (C.I.P., DC, P.C.) and this is evidence of the additive effect of diclofenac with ciprofloxacin, while diclofenac did not give an effect on its own, even after 14 days of starting treatment compared with negative control (N.C.).

Epithelium Cells score in urine

The values of epithelium cells score in urine were normal at the starting point of the experiment in negative control group range during different times(3,7and14) days of the experiment (8.70±0.83,8.80±0.66, 8.70±0.70) respectively; but However the infected group (P.C., DC, C.I.P., COM1, COM2) with resistance *E.coli O157: H7* showed a significant increase epithelium cells concentration in urine ($P< 0.05$) (18.00±0.63,17.80±0.37, 17.80±0.37, 17.20±0.25, 17.40±0.18)respectively as after three days compared with the N.C. group it was (8.70±0.83) in the same time the values after seven days demonstrated decrease the epithelium cells numbers in the urine ($P< 0.05$) in (COM1, COM2) groups (9.20±1.11, 9.20±1.11) respectively compared (P.C., D.I.C., C.I.P.) was (20.30±0.80, 18.80±0.80, 17.40±0.18),in contrast the values of epithelium cells showed a significant increase ($P< 0.05$) in (COM1andCOM2) groups which was (9.20±1.11 and 9.20±1.11) respectively compared with (N.C.) group,was (8.80±0.66) after seven days, and the values of epithelium cells after 14 days showed a significant decrease ($P< 0.05$) in (C.I.P., COM1, COM2) groups which was, (4.50±1.00, 4.40±0.84, 4.60±1.00) respectively as compared with(P.C and DC) groups which was (37.90±2.21, 37.60±2.48) respectively moreover. no significance difference ($P< 0.05$) in (C.I.P., COM1, COM2) groups after 14 days in the values of epithelium cells was(4.50±1.00, 4.40±0.84, 4.60±1.00) respectively, as compared with (N.C.) groups, was(8.70±0.70) the values of epithelium cells increased significantly epithelium cells concentration in urine ($P< 0.05$) in(P.Cand DC)groups (37.90±2.21, 37.60±2.48) respectively compared with (N.C.) group (8.70±0.70) after 14 days table(5).

Table (5) epithelium cells score 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

Groups/ epithelium cells concentration in urine(HPF)	Three days	Seven days	fourteen days
Control –ve(NC)	8.70±0.83 Ab	8.80±0.66 Ab	4.40±0.84 Cc
Control +ve(PC)	18.00±0.63 Ba	20.30±0.80 Ba	37.90±2.21 Aa
Diclofenac 1mg/kg(DC)	17.80±0.37 Ba	18.80±0.80 Ba	37.60±2.48 Aa
Ciprofloxacin 7mg/kg(CIP)	17.80±0.37 Aa	17.40±0.18 Aa	4.50±1.00 Bc
Diclofenac 1mg/kg+Ciprofloxacin (3.5mg/kg)(COM1	17.20±0.25 Aa	9.20±1.11 Bb	4.40±0.84 Cc
Diclofenac 1 mg/kg+Ciprofloxacin (1.75mg/kg)COM2	17.40±0.18 Aa	9.20±1.11 Bb	4.60±1.00 Cc

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

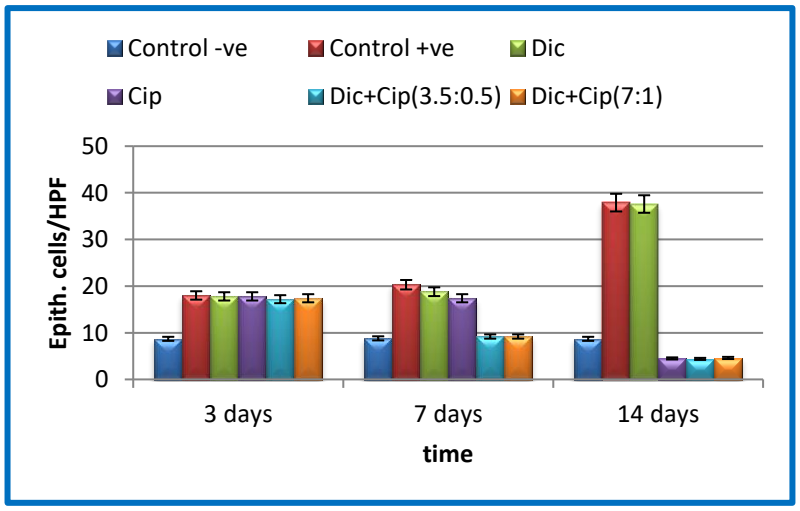


Figure (10)epithelium cells score in urine in infected groups by resistance *E coli 0157* treated by ciprofloxacin, Diclofenac alone, and a combination between (ciprofloxacin +diclofenac) in two doses(mg/kg) and control groups

DC=diclofenac dosage 1mg/kg
CIP=ciprofloxacin dosage 7 mg/kg
COM1= (ciprofloxacin3.5mg/kg +diclofenac1mg/kg)
COM2= (ciprofloxacin1.75mg/kg +diclofenac1mg/kg)
Epithelial cells in urine are originate from various parts of the urinary tract, including the kidneys, bladder, ureters, and urethra. The presence of epithelial cells in urine can be an indication of certain conditions or diseases affecting the urinary system. (Mundt *et al*., 2011) types of epithelial cells commonly found in urine include squamous epithelial cells, which have a flat and scale-like appearance They are typically derived from the urethra and can be considered normal if present in small numbers. However, increased squamous epithelial cells in urine may indicate contamination during sample collection.

Transitional epithelial cells: also known as urothelial cells are found in the lining of the urinary tract. These cells have a rounded or irregular shape and may be indicative of inflammation or infection in the urinary system, such as a urinary tract infection (U.T.I.). these cells: these cells are derived from the renal tubules in the kidneys The presence of renal tubular epithelial cells in urine may suggest kidney damage or diseases, such as acute tubular necrosis or other kidney disorders Columnar epithelial cells: These cells are typically found in the lining of the urinary tract and can be seen in cases of inflammation or infection. (De la Salle *et al* .,2017) In this study, increased epithelial cells concentration in urine all groups after three days infected by resistance E coli 0157 H7 inducation by urinary catheter kidney dysfunction excepted negative control this is evidence of infection in this study ciprofloxacin (C.I.P.) group showed no significant ($P< 0.05$) changed in the epithelial cells concentration in urine after 14 days no a compared with negative control (N.C.) whilst the two groups treated by combination (ciprofloxacin+ diclofenac) and in two doses 3.5+1(mg/kg),1.75+1(mg/kg), after 7and 14 days presented asignficant decreased t ($P< 0.05$) compared with (C.I.P., DCand P.C.) and this is evidence of the additive effect of diclofenac with ciprofloxacin, while diclofenac did not give an effect on its own, even after 14 days of starting treatment compared with negative control (N.C.).

The results of current study agreement with (Bartlett ET AL, 2010), ciprofloxacin (C.I.P.) group pus cell, epithelium cell in urine after 14 days in compared with negative control (N.C.).

The additive effect between diclofenac and ciprofloxacin against resistance *Escherichia coli 0157* was found to decrease pus cell and epithelium cell significantly ($p<0.05$) compared diclofenac, ciprofloxacin groups alone in addition the current study agreement with many reports which demonstrated that ibuprofen, diclofenac, celecoxib, and aspirin could use against *K. pneumoniae, E. coli, S. aureus, and P. aeruginosa* (Chan *et al*.,2017; Paes Leme *et al.*,2021). NSAIDs (Diclofenac), the antimicrobial activity of the antibiotics combination, increase the bacteria's susceptibility to the antibiotics (Chan *et al.,* 2017). administration of antibiotics with NSAIDs can treat bacterial and inflammatory diseases the presented study,

the effect of both groups combination (diclofenac+ciprofloxacin) in dosage (3.5+1and1+75) mg/kg decreased significantly ($P< 0.05$) the pus, epithelium cell concentration after 7, 14 days of the duration of the experiment compared with diclofenac and ciprofloxacin alone. diclofenac is used together with antibiotics to treat diseases, decrease inflammation, fever, and pain. The mechanism of action of diclofenac depends on cyclooxygenase enzyme inhibition (Rainsford, 2007). Concentration after 14 days was no significant changed ($P< 0.05$) compared with negative control (N.C.).

Conclusions:

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

1. *E. coli O157:H7* cultured from females' urine at the hospital, identified by chromogenic agar medium, biochemical and molecular assays.
2. An antibiotics susceptibility test was done for *E. coli O157:H7* isolates that showed multiple antibiotic resistance, including ciprofloxacin.
3. Shiga-like toxin 2 is the most important virulence marker *of E. coli O157:H7*.
4. Used Diclofenac with ciprofloxacin in urinary tract infection (U.T.I.)caused by.for reduce the bacterial effect and inflammation

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 Diclofenac and ciprofloxacin) that could be used as antibacterial therapy.

AUTHORS CONTRIBUTION:

All the mentioned authors contributed to the current work.

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