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Combined Effect of Plantaricin and Ciprofloxacin Against UTIs Induced by E. coli O157: H7 in Female Rats

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Abstract

The purpose of the present study was to determine how well plantaricin and ciprofloxacin worked to prevent urinary tract infections in female rats caused by E. coli O157H7, which consisted of two steps; the first included isolation and identification *of E.coli* O157 H7 by using biochemical tests and VITEK II system while the second step was studying the therapeutic activity of plantaricin (which isolate from Lactobacillus plantarum and identified genetically by PCR) and ciprofloxacin against the UTIs that induced experimentally by *E.coli* O157:H7 in 40 female rats (which were divided into five equal groups), and comparison of these effects with ciprofloxacin. The results of this research showed that combination of planaricin and ciprofloxacin has a clear therapeutic effect in the treatment of UTIs through its effect on body weight and improved clinical markers as well as urine bacterial count and level of creatinine in serum of female rats that have been infected with *E. coli* O 157;H7 in compartion to animals that have been infected with *E.coli* O157:H7 and not been treated or with animals that treated with plantaricin or ciprofloxacin alone .This antibacterial activity that characterizes the combined of plantaricin may be due to safe antimicrobial effects against *E.coli* O157:H7

Key words: Combination, Plantaricin, Utis, *E.Coli* O157:H7, Rats, Creatinine

Introduction

In clinical practice across the world, UTIs, or infections of the urinary tract, are the most common medical conditions. Despite several attempts, UTIs continue to be serious health problems that afflict millions of people annually around the world, resulting in significant incidence and huge healthcare costs **(1).** It is also a common and complicated infection among human or animals. Global estimates revealed that both females and male experienced no less than one UTI episode cases **(2) (3).** Typically, there are two classifications of UTIs. Acute cystitis, categorized as the first, affects the lower urinary tract. The second disorder is acute pyelonephritis, which impacts the upper urinary system. Another problem that has to be watched out for is asymptomatic bacteriuria, which refers to the presence of specific bacteria levels in the urine but no obvious signs (**4) (5**) , and high bladder bacterial burdens (**6).** Escherichia coli, Proteus, and Staphylococcus organisms account for 90% of the initial clinical UTIs and 70% of recurrent infections that are caused by infections caused by bacteria, which are also the most prevalent source of UTI (**7).** Gram negative Escherichia coli bacteria are the cause of both the frequency and

severity of illnesses (8, 9). Hemolytic uremic syndrome (HUS) is one of the most common causes of acute renal failure, even though infections with Shiga toxin-producing Escherichia coli (STEC) account for the majority of cases. *E.coli* O157 is still the most common STEC genotype **(10).** The progressing with uses of ciprofloxacin lead to side effects, as well as hyper or hypoglycemia, photosensitivity, and tendinitis, are among the significant adverse effects of ciprofloxacin **. (11).** Probiotics are types of microbes that are alive that, when given in sufficient doses, have positive effects on the well-being of the recipient **(12).** Lactobacillus plantarum produces the new bacteriocin known as plantaricin which usually included in both class I and II. Class I includes bacteriocins which named plantaricin **(13).** In the future, plantricin may replace conventional antibiotics as an efficient drug for the avoidance and management of infectious infections **(14).** The research aims to assess the efficaciousness of ciprofloxacin and plantaricin in treating urinary tract infections caused by E. coli O157:H7.

Materials and Methods

Source of *E.coli* O 157:H7

An E. Coli O157:H7 bacterial strain was acquired from AL-Karama hospital in Wasit Governorate, from female suffering from acute UTIs, this bacterium identified by Vetik IIsystem and biochemical characteristics according to **(15**).

Extraction of plantaricin from Lactobacillus plantarum

Plantaricin was produced from lactobacillus plantarum that isolate from local sample (sourdough sample) by using a growth medium called MRS broth according to (**16**). The plantaricin gene identify by PCR according to (**17)**.

Animals

Forty (40) female Wister albino rats that were between three and four months old and weighed between 176 and 250 g. have been kept in plastic containers measuring 20 x 50 x 75 cm and allocated to a special housing section at the University of Baghdad College of Veterinary Medicine for a fortnight to allow for adjustment. There was plenty of tap water and commercial feed pellets, the standard rat diet, accessible. Air-conditioned accommodations with 20–25 Co. housing units were maintained. Ventilation vacuums were used on a regular basis to replenish the air in the rooms. Every day, the containers' litter was replaced**.**

Ethics and Animals of Experiments:

In compliance with the moral recommendations on the handling and utilization of animals in research (PG/1417) of animal welfare, all laboratory animals used in this study were authorized by The Scientific Committee of the College of Veterinary Medicine, University of Baghdad (2023/7/6).

Inducing of Infection (UTI)

The bacteria utilized *E. coli* O157:H7 suspension (2.6 x 10^6) CFU/ml is the source of the illness (acute UTI). The inoculations are prepared and standardized using a pour plate approach and repeated tenth dilutions. The rats will receive 0.1 ml of each dilution intra urethrally (**figure 1**), and the animals are to be monitored for signs of UTI. The solution that caused the rat's infection, as shown by the symptoms, will be used as the rats' infectivity dose during the whole illness (**15**). 24-hour culture over night at 37°C in 0.1-ml dilutions of brain-heart infusion broth give with a canula (gauge 24G) to each rat of (infected groups).

Figure (1): intra-urethral orifice injection of E. coli O157:H7

Experimental Design:

40 female and Five groups of eight rats each were randomly assigned to the animals.**1. Group A (Negative control):** 8 normal female rats not infected with *E.coli* O157 :H7 , given only distilled water orally.

2. Group B (Positive control): 8 female rats having an E. Coli O157:H7 infection and not treated.

3. Group C: 8 female rats infected with *E. coli* O157 H7 were given ciprofloxacin orally twice daily at a dose 14.28 mg/kg (**18)**.

4. Group D: 8 female rats infected with *E.coli* and given orally plantaricin 0.5g/kg ,twice daily **(19)**

5. Group E : 8 female rats infection with *E.coli* O157:H7 treated concurrent orally with plantaricin and ciprofloxacin (half doses) twice daily , for 14 day

Body Weight Changes

Animals' weights were recorded before an infection was induced, throughout the first week of treatment, and after seven and 14 days of therapy.

Clinical Signs: Clinical signs, urine color, unusual frequency in urination, cloudy urine or foul smelling, changes in behavior, activity, food and water conception and death rate in animal groups were continuously recorded during the period of the experiment.

Blood Serum Samples

drawn samples of blood prior to infection, seven days after infection, and fourteen days following treatment. All female rats were anesthetized with chloroform. Direct cardiac puncture of rats was used to obtain blood samples, which were then placed in dry, clean, and sterile tubes (gel tubes), allowed to clot for a short period of time 15 minutes of centrifuging at 4000 rpm at room temperature to separate the clear sera, and this were then placed in Eppendorf tubes by micropipette and kept in a deep freezer at (-8^{o}C) till performing the biochemical analysis **(20).**

Determination of serum creatinine concentration (mg/dl)

This test was made by using creatinine kit (Biosystem company,/Spain) to determine the serum creatinine concentration following 7-day therapy period and 14 -day infection-inducing period.

Urine bacterial count

Urine sample were collected within a sterile glass tube and Following a week of infection induction and 14 days of therapy, there was an increase in the amount of E. coli O157:H7 found in urine samples. 0.1 ml of urine sample was suspended to 0.9 ml of diluents that containing (0.1%) of peptone water that's according to **(21, 22).** The bacterial counting (CFU/ ml) was calculated by using the following formula **(15).**

$$\textbf{Number of bacteria /ml} = \frac{\textbf{No.of colonies (CFU)}}{\textbf{dilution factor X amount plated}}$$

Statistical analysis

A tool called the Statistical Assessment System- SAS (2018) (23) was employed , a significant comparison of means was made using the difference that was least significant (LSD) test ((ANOVA).

Results and Discussion

Biochemical identification of *E. coli* O157:H7

The result of biochemical tests is explained in the table (1) and this results were in agreement with **(24)**

7:H7

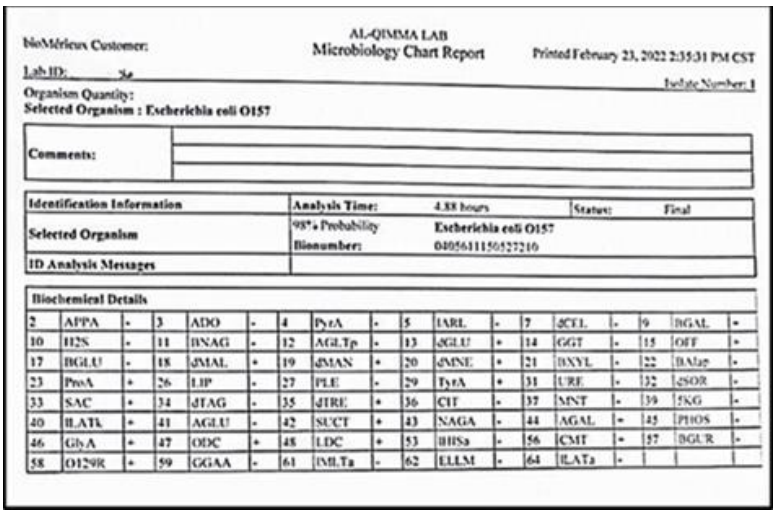
Table (1): "Biochemical tests for identification of E. coli O157:H7"

No.	**Biochemical test**	
1	Catalase.	**+**
2	Oxides.	**-**
3	Indole.	**+**
4	Methyl red**.**	**+**
5	Voges-Proskauer.	**-**
6	Citrate utilization.	**-**
7	KIA**.**	**A/A**
8	Ureas**.**	**-**
9	Motility.	**+**

(+) positive result, (-) negative result, (KIA) Kliger Iron Agar test, (A/A) Acid slant/ Acid bottom

Vitek II System

Based on the manufacturer's technical datasheet, considering a likelihood of 98%, the isolated bacteria have attained an excellent identification level. This was done with the automated Vitek II system by using GN-ID cards which include many biochemical tests (Figure: 2). This method is distinguished through the rapid identification of bacteria minus requiring for several mediums for culture and the decreased pollution of populations (**25).**



bioMérieux Customer: AL-QIMMA LAB
Microbiology Chart Report
Printed February 23, 2022 2:35:31 PM CST

Lab ID: 56 Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli O157

Comments:

Identification Information	Analysis Time: 4.88 hours	Status: Final
Selected Organism	98% Probability: Escherichia coli O157	
	Bionumber: 0405611150527210	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAla	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	+	53	BHSa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Figure (2): Identification of E. coli O157:H7 by Vitek II system

Extraction of plantaricin from *Lactobacillus plantarum*

37 ◦C was found to be between 30 and 37 degrees Celsius is the ideal temperature for development. were better for plantaricin production. Which was in agreement with (16) , (17) and the identification of plantaricin was found to be as following :

FASTA Sequence for plantaricin gene

AGTGCTTAAACTTGATGGCTTGAACTATCCGTGGATGAATCCTCGGACAGCGCTAATGAC 60 Subject 474 533

Query61CCAATCGGCAGGCCCAACAGCACTTTTATAATTATTCCGAGCGCCACGCGCGCTATAGGC 120 Subject 534 .....A..... 593. Query 121 ATGGAAAACGCCACCTGAAATAGCATTTAATTCACGGTCACGCAAAACTAGAAAATTTTT 180 Subject 594 .....T..... 653 Query 181CATAATTGTTGATCTCCCCCAAGAAAATTAACGAATACTTTTCAAAATACCACGAATGCC **.**

Induction of Urinary tract infection

Urinary tract infections were observed in rats after 24 hrs. after inoculation with the pathogenic *E coli* O157:H7.

Clinical Signs:

Prior to the infection's development, all healthy female Wistar rats displayed normal urination and light to moderately yellow urine that was normal in color. After two days of infection urine had become dark yellow, all the animals exhibited clinical signs of illness characterized by fever, dehydration, crowding, dullness, and frequent urination**.**

Body Weight Changes

Differences in body mass (grams) demonstrated a relationship among sickness, kind of therapy as shown in (table: 2). After 7 days of infection induction, in comparison to group (-ve control), Body weight decreased in groups B (+ control), C (plantaricin treated), and D (ciprofloxacin treated) although not significantly (P>0.05). After receiving treatment for 14 days, the animals in group C gained weight more slowly than the animals in the two treated groups (C and D), which gained weight more slightly than the animals in group A (-ve control). The identical trend showed up after 14 days of therapy, when group B (+ control) continued to show a substantial decline in comparison to control group A (-ve control), as well as with comparing group C was present. Although groups D and E continued to gain weight normally during the course of the trial, group C showed a substantial change from its weight at the end of the days of therapy.

Table (2): Rat body weight change (grams) in several groups after infection and ciprofloxacin and plantaricin treatment during the course of experiment

Mean ± SE of Body weight (gm)

Groups	**Before infection**	**After day 7 of infection**	**After day 14 of infection**	**LSD value**
Negative Control (A)	179.37 ±2.07 A a	180.25 ±1.48 A a	13.28 NS	3.28 NS
Positive Control (B)	178.87 ±1.88 A a	175.50 ±0.88 B ab	172.62 ±0.65 B b	5.16 *
Ciprofloxacin (C)	178.87 ±1.98 A a	177.25 ±1.48 AB a	181.12 ±2.01 A a	4.74 NS
Plantaricin (D)	180.12 ±1.68 A a	178.37 ±1.72 AB a	181.00 ±1.56 A a	3.78 NS
Combination (E)	180.87 ±1.65 A a	179.12 ±2.08 AB a	182.00 ±2.01 A a	3.94 NS
LSD value	5.353 NS	4.538 *	4.688 *	----------

This shows that little characters in the same row and various big letters in the same column are noticeably distinct. (P≤0.05).

Urine Bacterial Count

E. coli O157:H7 colony forming unit/ml (cfu/ml) estimates for each of the four groups are displayed in (table: 3) The bacteria in urine were counted using the pour plate technique, investigated a number of it was demonstrated that the spread plate approach and this method on CCA agar were both effective ways to count urine *E. coli* were simpler to use, easier to carry out, less costly, and would produce results in only one day. Colony forming units per milliliter (CFU/ml) were used to express the amount of *E. coli* found in urine samples from the four groups. In all infected groups, a substantial rise in the *E. coli* viable count (P >0.05) was discovered. The result of bacterial count were agreement with **(37)**. Also, that was agreement with **(33**) and **(35)** they demonstrated that, within 7 days of causing urinary tract infection in female rats by injecting pathogenic *E. coli* O157:H7 into the ureter, When the rats were vaccinated with (2.6 106) CFU/ml, E. coli O157H:7 was successfully colonized **(33 , 53).**

Table(3): Effect of of plantaricin , ciprofloxacin and concurrent use of plantaricin/ ciprofloxacin on urine bacterial count x10^7

Mean ± SE of Body weight (gm)			
Groups	**After day 7 of infection**	**After day 14 of infection**	LSD value
Negative Control (A)	0.00 ±0.00 B a	0.00 ±0.00 B a	0.00 NS
Positive Control (B)	10735.00 ± 3144.00 A b	39462.5 ± 21825.03 A a	17602.47 *
Ciprofloxacin (C)	1986.25 ± 1295.37 B a	136.12 ± 73.53 B b	1348.92 *
Plantaricin (D)	4247.50 ± 2130.0 B a	342.50 ± 127.54 B b	1726.76 *
Combination (E)	25.50 ± 10.29 B	14.42 ± 8.09 B	19.02 NS
LSD value	4526.93 *	7705.64 *	---
This shows that little characters in the same row and various big letters in the same column are noticeably distinct. (P≤0.05).			

Serm creatinine concentration (mg/dl)

The variations in mean serum creatinine concentrations among all affected female rats after being treated with scheduled treatment for 7 and 14 days as well as control group are listed in (**Table 4**). It can be seen that serum creatinine concentration was within the normal values in all groups (p>0.05) before the infection. After 7 days of infection, the serum creatinine concentration increased significantly (P<0.05) in all infected groups except the negative control group. The impact of plantaricin on the kidneys affected by bacterial infection was assessed using a creatinine serum analysis.

Table (4): Serum Creatinine values of rats infected with *E. coli* O 157:H7 and treated with Plantaricin, Ciprofloxacin, and concurrent use of combination (Plantaricin / Ciprofloxacin).

Mean ± SE of Body weight (gm)			
Groups	**After 7 days of infection**	**After 14 days of infection**	LSD value
Negative Control (A)	0.650 ±0.08 C a	0.650 ±0.08 B a	0.055 NS
Positive Control (B)	1.317 ±0.11 A a	1.148 ±0.12 A a	0.194 NS
Ciprofloxacin (C)	1.067 ±0.09 AB a	0.737 ±0.07 B b	0.206 *
Plantaricin (D)	0.937 ±0.06 B a	0.712 ±0.07 B b	0.197 *
Combination (E)	0.800 ±0.12 BC a	0.500 ±0.10 B b	0.206 *
LSD value	**0.277 ***	**0.259 ***	---

This shows that little characters in the same row and various big letters in the same column are noticeably distinct. (P≤0.05).

Discussion

For most types of pathogens responsible for infectious diseases, automated bacterial identification in a clinical laboratory provides a rapid, precise diagnosis with a highly desirable level of recognition precision (**26**).On the other hand, the VITEK II system is beneficial for comparing the biochemical characteristics of *E. coli* O157:H7 **(27).**

Based on molecular weight, the discovered protein was anticipated to be plantaricin that agrees with (**30).**

The main clinical signs of urinary tract infection represented acute infected female rats with *E. coli* O157: H7 before treatment female rats suffering from urinary disturbance **(31 , 32)**.

That clarified the very effective antibacterial components in plantaricin . Weight loss and anorexia brought on by infection were shared by all affected groups **(31, 33, 34, 35, 52).** Although the treatment groups' weights reduced, the control group's body weight marginally rose. The body's weight differences between treatment groups and the control group were not appreciably different. The weight of body was also not significantly different from control which that was agreement with **(36, 29).** The results showed that the concurrent treatment of plantaricin / ciprofloxacin led to the lowering of urine bacterial count to no bacterial growth before ending the 14 day of therapy, hence, there was no discernible decline from the seven-day treatment period. Also, animals treated with plantaricin or ciprofloxacin Urine bacterial count did not significantly decrease (P≤0.0.05) after seven days of therapy, After seven days of treatment, treated group showed the bacterial count returned to normal, exactly as it had been Combined of plantaricin/ciprofloxacin could inhibit the growth of *E.coli* **(29) ,**before the infection is induced, whereas group (B) bacterial count slightly decreased (P≤0.05) when compared with seven days of treatment.

This resulting in bacterial cell death and slowing the emergence of resistance **(39, 40).** While plantaricin dramatically decreased the viable cell count of pathogenic urine bacteria cfu/ml following treatment .According to **(41)**, pointed upon how numerous studies have focused on plantracin with ciprofloxacin remarkable capacity to inhibit pathogen growth through its bactericidal activity and enable the body's immune system to combat a getting ill lacking the need of antimicrobials (**42**). Its been demonstrated to be a spontaneous, secure antibacterial agent that is extremely efficient versus a variety of harmful bacteria. As a result, it is advised that it be employed as a replacement to other chemicals' stabilizers **(43 ,44)** noted that the initial defense

against cationic bacteriocins is the anionic cell membranes of bacteria. Plantaricin is thus frequently employed to destroy specific bacteria by permeabilizing the cell membrane **(45).** As compared to ciprofloxacin, plantaricin's ability to permeabilize membranes *against E. coli* O157 H7 was discovered in this investigation **(47).** Plantaricin dissipated power, through electrolyte outflow and subsequent membrane permeation led to the suppression of intracellular ATP, which brought in cell death. The findings imply that plantaricin's constituents have some anti-*E. coli* action **(47)**

Mixture treatment, which inhibits numerous cellular targets, has been proposed as a promising approach that may postpone the emergence of tolerance despite lowering dose and associated adverse reactions **(48, 49).**Plantaricin has been shown to improve the bactericidal and inhibitory effects of conventional antibiotics at doses below the MIC which could decrease the danger of cytotoxic side effects and the emergence of antibiotic resistance. Using precise therapies in mixed forms is a beneficial and essential strategy that has the ability to increase the arsenal of available antibiotics versus infections and repurpose presently off-patent drugs that agree with **(50).** Traditional antibiotics were significantly improved in clinical settings when synthetic peptides were present because they decreased the size of abscesses and improved bacterial clearance . Enhanced membrane permeability and improved antibiotic penetration are among the proposed underlying mechanisms **(51)**. The research being conducted makes the case for the ongoing development of plantaricin as possible treatments for *the E. coli* O157:H7 bacterium strain.

Conclusion

Concurrent use of plantaricin and ciprofloxacin showed more efficiency than the treatment with ciprofloxacin alone, also the highest rate of synergism for plantaricin and ciprofloxacin were active in combination against acute urinary tract infection by *E. coli* O175: H7.

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Novelty Statement

The work is unusual in that it focuses on the combined effects of gene sequences and plantaricin, which is made from L. plantarm, to treat UTIs brought on by E. Coli O157:H7 as a substitute form of antibacterial drugs.

Authors Contribution

Everyone who wrote made an equal contribution. Conflict of interest: There isn't one that the writers have disclosed.

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