A STUDY ON THE PATHOGENICITY OF ESCHERICHIA COLI PRODUCING EXTENDED-SPECTRUM B ISOLATED FROM UROLOGICAL PATIENTS AND ITS ASSOCIATION WITH SOME IMMUNOLOGICAL BIOMARKERS FROM HOSPITALIZED ADULT PATIENTS

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Abstract

Objective: The objective of this research is to discover Escherichia coli bacteria that produce the broad-spectrum beta-lactamase enzyme and assess their resistance to antibiotics, as well as their toxicity in animals. A total of 40 clinical samples were obtained from inpatient individuals diagnosed with urinary tract infections. These samples were collected from both serum and urine sources, and the collection process was overseen by a specialist doctor from Balad General Hospital, Al-Dujail Surgical Hospital, as well as several well-known clinics. The data collection period spanned from January 2, 2023, to March 1, 2023.

Methodology: The identification of the isolates involved a two-step process. Initially, selective media were employed to detect the bacteria responsible for urinary tract infections and differentiate them from other bacterial species. This was achieved by cultivating the isolates on UTI ChromoSelect Agar medium. Subsequently, the isolates were cultured on ESBL Chrome Agar medium, which specifically targets bacteria that produce the broad-spectrum beta-lactamase enzyme. The distinctive characteristics of the resulting colonies were utilised for further identification. In terms of morphology, the analysis revealed that 10 out of the total isolates, accounting for 25%, were identified as Escherichia coli, a bacterium known for its production of the broad-spectrum beta-lactamase enzyme.

Results: All bacterial isolates exhibited resistance to multiple drug-resistant (MDR) antibiotics. Specifically, all isolates shown resistance to Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, and Pipracillin. Additionally, 30% of the isolates displayed resistance to Ciprofloxacin, while 40% of the isolates were resistant to Gentamycin and Nitrofurantoin. All specimens exhibited sensitivity to Imipenem.

The results obtained from the enzyme-linked immunosorbent assay revealed that all samples that secreted broad-spectrum beta-lactamase exhibited elevated serum concentrations of interleukin IL-17. Notably, the minimum serum concentration (QTA = 135.2 pg/ml) surpassed the median serum concentration standard $\bar{X}$ = 59.158 pg/ml by a factor greater than two. A statistically significant difference was observed with a 99% confidence level ($p = 0.000 < 0.01$) in the serum concentrations of interleukin between samples exhibiting urinary system infection without detectable growth of ESBL E.Coli on the appropriate culture media, and samples exhibiting urinary system infection with visible growth. The enzyme-linked immunosorbent test (ELISA) revealed a statistically significant distinction, with a 99% confidence level ($p = 0.000 < 0.01$), in the serum amounts of lipopolysaccharide (LPS) between samples exhibiting ESBL E.Coli infection on the suitable culture conditions. In cases where there is no observable proliferation of ESBL E. Coli on suitable culture medium, the urinary tract exhibits no discernible growth. Conversely, when samples from individuals with urinary tract infections are cultured on proper media, the presence of apparent ESBL E. Coli growth is seen.

Conclusion:

Keywords: IL-17, Escherichia coli, Extended-Spectrum β, lipopolysaccharide (LPS)

Introduction

Escherichia coli (E. coli) is a frequently seen bacteria that is present in the gastrointestinal tract of both humans and animals. Although several strains of Escherichia coli (E. coli) are innocuous and play a vital role in maintaining gastrointestinal homeostasis, some strains possess the capacity to induce pathogenicity. Of specific concern within the category of pathogenic strains are those that possess the ability to produce Extended-Spectrum β-Lactamases (ESBLs). Bacteria that produce extended-spectrum beta-lactamases (ESBLs) possess the ability to degrade a diverse array of antibiotics, resulting in challenges in the treatment of infections and contributing to heightened rates of illness and death (1–3).

Urinary tract infections (UTIs) are an often encountered clinical issue, with Escherichia coli (E. coli) being the predominant causative pathogen. The prevalence of urinary tract infections

(UTIs) caused by Escherichia coli strains that produce extended-spectrum beta-lactamases (ESBLs) has increased in recent times. This trend presents a significant obstacle for healthcare professionals since these strains exhibit resistance to a wide range of commonly used medicines. The resistance mechanisms shown by these bacteria are a direct result of horizontal gene transfer, often facilitated by plasmids, resulting in the acquisition of Extended-Spectrum Beta-Lactamase (ESBL) genes (4,5).

Nevertheless, the virulence of ESBL-producing Escherichia coli, particularly when obtained from individuals with urological conditions, extends beyond the scope of antibiotic resistance. The capacity of bacteria to attach, infiltrate, and generate toxins may have a substantial impact on the progression and resolution of an illness. There is a growing scholarly interest in comprehending the mechanisms by which these strains engage with the immune system of the host, as well as the significance of certain biomarkers in ascertaining the gravity and prognosis of the ailment (6,7).

Many research's findings have shown that certain immunological biomarkers may serve as potential indicators of the immune system's reaction to infections caused by ESBL-producing E. coli. Biomarkers have the potential to provide doctors significant insights into the evolution of the illness, facilitating the early identification of severe instances and perhaps informing treatment interventions (8,9).

Moreover, whereas the clinical ramifications of infections caused by ESBL-producing Escherichia coli in human subjects have been extensively established, there exists a must to comprehend their pathogenicity in animal models. Examining the impact of these microorganisms on animal organisms may provide valuable knowledge on the underlying processes of infection, the development of diseases, and the possible efficacy of treatment measures (10,11).

The objective of this research is to isolate and diagnose Escherichia bacteria that exhibit broad-spectrum beta-lactamase activity in female patients with urinary tract infections and to investigate the correlation between several immunological markers and infection caused by Escherichia coli strains that contain broad-spectrum beta-lactam enzymes. Aso, to examine the antibiotic resistance of isolates that produce broad-spectrum beta-lactamase-producing bacteria.

Methodology

The Accquant ELISA kit and the Human HS-IL-17(High sensitive Interleukin 17) Accquant ELISA kit and the Human LBP (Lipopolysaccharide-binding protein) enzyme-linked immunosorbent assay kit were used. ELISA Kit (Manufacturer: Wuhan Fine Biotech Co., Ltd – China) 48T.

Preparation of culture media

Ready-made cutler media preparation

Brain Heart Infusion (BHI) broth with 0.1% agar and Nutrient Agar and Trypton Soy Agar

The media utilised in this study were prepared in adherence to the guidelines provided by the manufacturer. Specifically, 37.0 grammes of the media were dissolved in 1 litre of distilled water under the application of heat until complete dissolution was achieved. Subsequently, the media were subjected to sterilisation in an incubator set at a temperature of 121°C and a pressure of 15 pounds per inch for a duration of 15 minutes. Following sterilisation, the media were transferred into sterile tubes, each of which was assigned a unique numerical identifier. These tubes were then incubated at a specified temperature for a period of 48 hours to ascertain the absence of any contamination. Finally, the tubes were stored at a temperature of 4°C until they were ready for use.

Medium Mueller-Hinton agar

The preparation of this medium was conducted in accordance with the instructions provided by the manufacturer. A quantity of 55.07 grammes of the medium was dissolved in 1 litre of water. The solution was heated until it reached its boiling point, ensuring full dissolution. Subsequently, the solution was subjected to sterilisation in an incubator at a temperature of 121°C and a pressure of 15 pounds per square inch for a duration of 15 minutes. Care was taken to prevent excessive heating during this process. Following sterilisation, the solution was kept undisturbed. Once the temperature of the medium reaches 50 degrees Celsius, it is then transferred onto sterile Petri plates and subsequently preserved at a temperature of 4°C till it is ready for utilisation.

Medium MacConkey agar

The medium was prepared in accordance with the guidelines provided by the manufacturer. Specifically, 38.0 grammes of the medium were dissolved in 1 litre of water and heated until boiling, ensuring complete dissolution. Subsequently, the medium was sterilised using an autoclave at a temperature of 121°C and a pressure of 15 pounds/ang2 for a duration of 15 minutes. Finally, the sterilised medium was transferred into sterile dishes. then, the specimen was subjected to incubation at ambient temperature for a duration of 48 hours in order to ascertain its freedom from contamination. Following this, it was then preserved at a temperature of 4°C in all three containers until it was ready for utilisation.

Laboratory culture media

Selective agar medium for bacteria causing urinary tract infections (UTI ChromoSelect Agar).

The medium was prepared in accordance with the guidelines provided by the manufacturer. Specifically, 55.4 grams of the medium were dissolved in 1 litre of sterile distilled water. The resulting solution was heated until complete dissolution was achieved. Subsequently, the medium was subjected to sterilisation in an incubator, maintained at a temperature of 121°C and a pressure of 1 bar, for a duration of 15 minutes. Following sterilisation, the medium was allowed to cool down to a The medium is thereafter transferred onto aseptic Petri plates and maintained at a temperature of 4°C until it is ready for use.

Specific chromatography of broad-spectrum beta-lactamase-producing bacteria CHROMagar ESPL

medium was supplemented by dissolving 10 grammes of the supplement powder provided by the manufacturer in 100 millilitres of purified water, followed by heating to 80 Celsius and stirring until completely dissolved. The supplemented medium was then added to the base medium, which had cooled to 45 Celsius, with gentle stirring. The resulting medium was sterilised in an autoclave at a temperature of 121 Celsius and a pressure of 1 bar for 15 minutes. To prepare the supplement, dissolve 570 mg of powder in 10 ml of sterile distilled water. Stir the mixture for several minutes until a homogenous solution is achieved. Next, the base solution is vigorously mixed with the

supplement, followed by the careful transfer of the resulting medium onto sterile Petri dishes. These dishes are thereafter kept at a temperature of 4°C, in a controlled environment devoid of light and moisture, until they are ready for utilisation.

Preparation of culture media

McFarland turbidity solution

The turbidity solution used in the experimentation of antibiotic resistance testing was created in the following manner: In the first phase, a solution denoted as Solution No. 1 was prepared by dissolving 1.175 grammes of an aqueous compound known as barium chloride (BaCl2.2H2O) in 100 millilitres of sterile distilled water.

In the second step, a solution was prepared by combining 1 millilitre of concentrated sulfuric acid (H2SO4) with 99 millilitres of distilled water. In the third step, a volume of 0.5 millilitres of solution No. 1 is combined with 99.5 millilitres of solution No. 2 in order to achieve a turbidity with a concentration of 1.5 x 810 cells per millilitre. The resulting mixture is well mixed in a glass container. In the preservation step, the solution is carefully maintained in an opaque glass container that is firmly sealed, and it is kept in a dark location (Vandepitte et al., 2003) (12).

Preparation of Kit Solution

Washing Solution

The preparation of the washing solution involves following the guidelines provided by the laboratory equipment manufacturer. This entails dissolving 15 millilitres of concentrated washing solution in 375 millilitres of distilled water. The resulting mixture is then subjected to gentle heating in a water bath, maintaining a temperature between 40 and 50 degrees Celsius. This heating process facilitates the dissolution of any remaining undissolved crystals. Subsequently, the mixture is allowed to cool down to room temperature. It is important to note that the preparation should be carried out no more than 20 minutes prior to its intended use.

Standard Solution

The preparation of standard solutions involves following the manufacturer's instructions in a two-step process. The first phase involves the preparation of tube 0: The process involves the addition of 1 ml of sample expansion buffer to a standard tube, designated as "tube 0" by the manufacturer. Subsequently, tube 0 is incubated at room temperature for a duration of 10 minutes. The subsequent phase involves the preparation of tubes 1 to 7. The Eppendorf tubes are sequentially labelled from 1 to 7 and each tube corresponds to a certain sample extension in the following order: The series of fractions 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 are being considered. Tube No. 7 is designated as the blank control tube. Subsequently, a volume of 0.3 millilitres of sample expansion cap is added to all tubes. Additionally, a volume of 0.3 millilitres is transferred from tube No. 0 to tube No. 1, and from there, the process continues. 0.3 millilitres are sequentially transported from tube number 1 to tube number 2, from tube number 2 to tube number 3, and from tube number 3 to tube number 4. In order to proceed, it is necessary to transfer a volume of 0.3 millilitres from tube No. 4 to tube No. 5. Subsequently, a volume of 0.3 millilitres from tube No. 5 should be transferred to tube No. 6. Additionally, it is advised to introduce a sample expansion cap of 0.3 millilitres into tube No. 7. It is important to note that these actions should be completed within a maximum time frame of 60 minutes.

Biotin-labeled Antibody Solution

Biotin-labelled antibody solutions are prepared according to the manufacturer's instructions in two stages: The first stage (calculating the required quantity): is done through the simple equation below:

Required volume of biotin-labeled antibody solutions = 0.1 x number of pits to be used

Then, 0.2 milliliters are added to the volume obtained in the above equation.

The second stage (preparing the required quantity): It is done by diluting the biotin-detection antibody with the Antibody Dilution Buffer in a ratio of 1/100 times, whereby every 1 microliter of the biotin-tagged detection antibody is mixed with 99 microliters of extension buffer. Opposites

Preparation of HRP-Streptavidin Conjugate (SABC) Solution

Streptavidin substrate solutions are prepared according to the manufacturer's instructions in two stages: The first stage (calculating the required quantity): is done through the simple equation below:

Required volume of streptavidin substrate solutions = 0.1 x number of pits to be used. T

hen, 0.2 milliliters are added to the volume obtained in the above equation.

The second stage (preparing the required quantity): It is done by diluting the concentrated streptavidin solutions with the streptavidin expansion buffer at a rate of 1/100 times, whereby every 1 microliter of concentrated streptavidin is mixed with 99 microliters of the streptavidin expansion buffer.

Bacterial isolates

Bacterial sample collection

40 urine samples were collected from patients suffering from urinary tract infections, under the supervision of a specialist physician, from patients hospitalized in Balad General Hospital, Al-Dujail Surgical Hospital, and some popular clinics, for the period from 01/02/2023 until 03/01/2023, as collection containers were used. containers to collect the sample from the middle of the urine in the early morning, then part of the samples was cut off and kept in Brain Heart Infusion (BHI) broth medium while the other part was cultured directly within a period not exceeding 30 minutes in UTI ChromoSelect Agar medium, and then the positive samples were cultured On the UTI ChromoSelect Agar only on the next medium, which is CHROMagar ESPL medium, and then the ELISA test and the antibiotic resistance test were performed.

Cultural identification

The phenotypic characteristics of the isolated colonies were studied after cultivation and purification of the bacterial isolates on UTI ChromoSelect Agar medium and on CHROMagar ESPL medium.

Antibiotic resistance test

The sensitivity of Escherichia coli bacteria to antibiotics was tested using the Kirby-Baure method according to Vandepitte et al (2003) (13) as follows: a (3-5) of the colonies that were grown on CHROMagar ESPL medium at 24 hours of age were transferred to a tube containing 5 milliliters of normal saline, and then the turbidity of the solution was adjusted with the turbidity of the 0.5 McFarland solution prepared previously,

which is equivalent to (1.5 x 810) cells/ml. The sterile cotton swab was inserted into the tube containing the bacterial suspension, then it was rotated and pressed against the inner wall of the tube to remove the excess amount, and then it was passed over plates containing Acar Mueller-Hinton medium several times and in different directions to obtain homogeneous growth. The antibiotic tablets were placed on the surface of the culture medium at equal distances, then the tablets were gently pressed using sterile forceps, and then the plates were incubated at 37 degrees Celsius for 24 hours. The results of the diameters of the inhibition zones around the discs were recorded in millimeters, and then the results were compared with standard tables (14)

Enzyme-linked immunosorbent assay (ELISA) test

In the initial phase of the study, blood samples were obtained from each of the individuals who were involved in the research. The blood samples were obtained and then left for two hours at room temperature, and then the samples were centrifuged at a speed of 1000 rpm for 20 minutes. The supernatant is subsequently gathered in aseptic, disposable tubes that are devoid of pyrogens and endotoxins, followed by the immediate commencement of the experiment.

Statistical analysis

The statistical analysis performed using SPSS-28. Descriptive statistics are used to identify the individuals within a sample and determine the measures of central tendency, namely the arithmetic mean, as well as the measures of dispersion, such as the standard deviation, among the population. By using line graphs to visually represent the data, individuals are able to discern the underlying patterns and interrelationships within the data. Within the field of analytical statistics, a study was undertaken to investigate the variability in mean results after the acquisition of information on the distribution features of the data. The Mann Whitney U test was used in instances when the data deviated from a normal distribution. The independent samples t-test was used to assess and interpret data that conforms to a normal distribution.

Results and Discussion

ESBL E. Coli Isolation and diagnosis

A total of forty urine samples were obtained from individuals who were hospitalised at Balad General Hospital, Dujail Surgical Hospital, and several well-known clinics, all of whom were diagnosed with urinary tract infections (UTIs). Following the conduction of culture and phenotypic testing, it was determined that 10 isolates, accounting for 25% of the total, may be classified as... The Escherichia coli bacteria that have the ability to manufacture a wide range of beta-lactamase enzymes.

Morphological identification

The identification of E. Coli isolates was conducted by assessing their phenotypic characteristics. This involved culturing the isolates on specific growth media, namely MacConkey agar medium, ChromoSelect Agar for bacteria associated with urinary tract infections (UTI), and ESBL Chrome Agar, a chromatography medium designed for bacteria that produce broad-spectrum beta-lactamases. The lactose sugar was undergoing fermentation by the bacteria, resulting in the formation of distinct colonies on the MacConkey Agar medium. These colonies had a smooth and glossy appearance with well-defined edges. Additionally, on both the UTI ChromoSelect Agar medium and the ESBL Chrome Agar medium, the colonies appeared as dark pink-reddish structures (Figure 1).

In a study conducted by Day et al. (2019) (15), a total of 20,234 stool samples collected from various regions in London, East Anglia, northwest England, and Scotland were analysed. The results revealed that 2,157 samples (11%) were identified as ESBL E.Coli. Similarly, Mitsan et al. (2020) (16) conducted a study that also reported findings related to this matter. The study included a total of 3,378 urine samples obtained from three distinct healthcare institutions in Nigeria. The findings revealed that 34% of these samples were attributed to ESBL E.Coli. In contrast to the findings of the thesis provided below, it may be posited that the dissemination of ESBL E.Coli remains somewhat regulated, therefore warranting attention. The significance of illuminating this matter and providing it significant consideration arises from its inherent danger and the need of averting the unregulated proliferation of antibiotics.

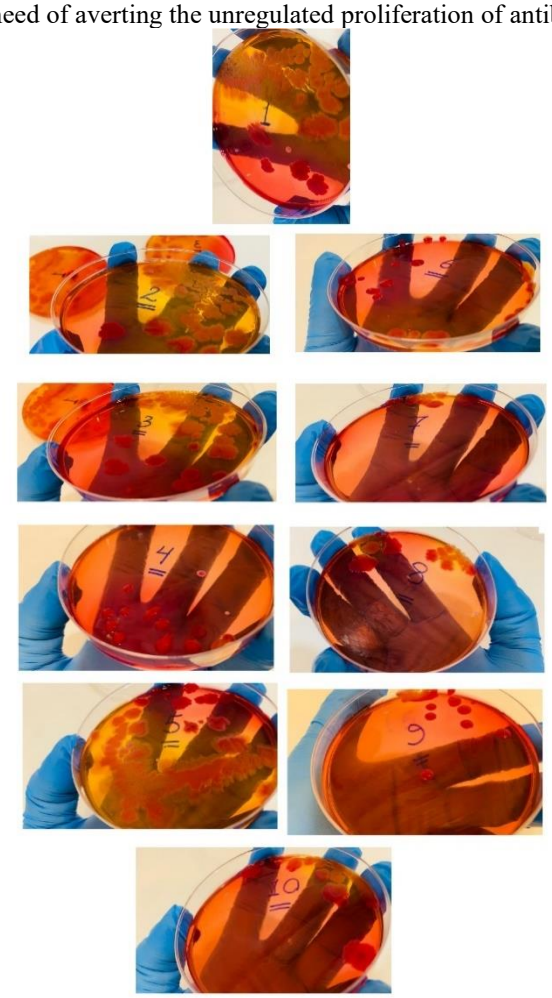

Figure (1): Bacterial growth on ESBL Chrome Agar medium

ESBL E. Coli Antimicrobial resistance

The isolates were subjected to antibiotic resistance testing for ESBL E. Coli after their classification was verified through the observation of positive bacterial growth on ESBL Chrome Agar medium. This involved cultivating the isolates on Mueller-Hinton agar medium and evaluating their response to nine distinct antibiotics, as depicted in Figure 2.

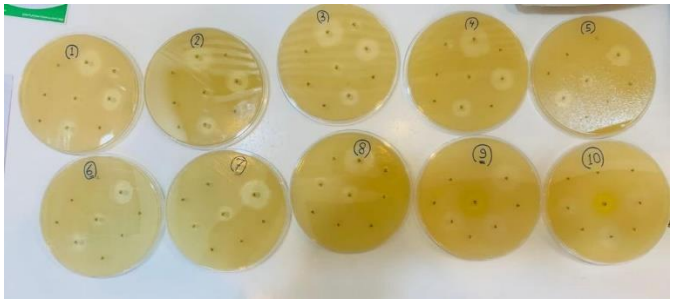

Figure 2: Bacterial growth on Mueller-Hinton agar medium

All bacterial isolates exhibited resistance to several multidrug-resistant (MDR) drugs, as shown in Table 1. All the isolates exhibited resistance to Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, and Pipracillin, while 30% of the isolates shown resistance to Ciprofloxacin. A total of 40% of the isolates exhibited resistance to both Gentamycin and Nitrofurantoin, whereas all samples shown susceptibility to Imipenem.

Table 1. Multiple antibiotic resistance patterns of ESBL E.Coli isolates

Antibiotype	1	2	3	4	5	6	7	8	9	10
Cefepime	R	R	R	R	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R	R	R	R	R
Ceftazidime	R	R	R	R	R	R	R	R	R	R
Ceftriaxone	R	R	R	R	R	R	R	R	R	R
Ciprofloxacin	S	S	S	S	R	S	R	R	S	S
Gentamycin	S	R	S	S	S	R	R	R	S	S
Imipenem	S	S	S	S	S	S	S	S	S	S
Nitrofurantoin	R	R	R	R	S	S	S	S	S	S
Pipracillin	R	R	R	R	R	R	R	R	R	R

Where: R) Resistant, S) Sensitive

In a study conducted by Du et al. (2002) (17), the resistance of ESBL E.Coli causing blood infections in 85 patients was investigated. The findings revealed that treatment with cephalosporins was associated with unfavourable outcomes and a reduced likelihood of survival, with one out of every 14 patients experiencing this outcome. Conversely, the administration of imipenem was correlated with the highest rates of survival, observed in 14 out of 40 patients. These results align with the information presented in this correspondence, as all ESBL-positive E.Coli isolates exhibited resistance to Ceftriaxone (a third-generation cephalosporin) and Cefepime (a fourth-generation cephalosporin), while all isolates demonstrated sensitivity to imipenem.

In a previous study done by Kayastha et al. (2020) (7), a sample consisting of 79 E.Coli isolates out of a total of 103 samples was examined. The findings of this investigation revealed that all bacterial strains that exhibited the ability to manufacture the broad-spectrum beta-lactamase enzyme also shown resistance to several drugs. All bacterial strains shown resistance to ampicillin, cefotaxime, ceftriaxone, and ceftazidime, but the majority demonstrated sensitivity to imipenem (89.7%), nitrofurantoin (82.8%), piperacillin/tazobactam (79.3%), and amikacin (72.4%). This pattern of susceptibility is inconsistent with this study. Based on the information presented in this correspondence, it is evident that a considerable proportion of the samples (40%) exhibited resistance to Nitrofurantoin, whereas all samples shown resistance to Pipracillin. This observation implies the potential occurrence of improper utilisation of both Nitrofurantoin and Pipracillin.

Enzyme-linked immunosorbent assay (ELISA)

Procesior characterization

The ELISA test was conducted on a sample of persons (n = 40), with a subset of individuals exhibiting the most normal clinical parameters being designated as the control group (n = 5). A decrease in clinical manifestations was detected in correlation with reduced blood levels of interleukin and lipopolysaccharide (LPS), as anticipated. This trend was evident for both IL-17 and LPS, with a noticeable distinction. In the range of concentrations encompassing the titer and the remaining members of the sample under investigation.

Sample characterization and visualizing

The mean standard for IL-17, with a sample size of 5, was calculated to be. In the subset of samples where the presence of ESBL E.Coli was verified by culture, the serum levels of IL-17 varied from 135.2 pkg/ml to 190.5 pkg/ml, whereas the serum levels of LPS ranged from 648.57 pkg/ml to 1081.02 pkg/ml. Regarding the samples in which the presence of ESBL E.Coli was not established by culture (n = 25), the serum concentrations of IL-17 exhibited a range of 67.64 pkg/ml to 135.36 pkg/ml, while the serum concentrations also fell within the range of 67.64 pkg/ml to 135.36 pkg/ml. The following table, Table 2, presents the measurements of central tendency and measures of dispersion for enzyme-linked immunosorbent tests.

Table 2. Measures of central tendency and measures of dispersion for enzyme-linked immunosorbent assays

Parameter / Group	IL - 17			LPS		
	Max	Min	$\bar{X}$ ($\pm SD$)	Max	Min	$\bar{X}$ ($\pm SD$)
Contol (n = 5)	65.50	51.25	59.158 (±5.882)	422.48	305.36	344.284 (±47.226)
UTI (n = 25)	135.36	67.64	113.488 (±16.145)	910.22	435.42	679.812 (±113.666)
ESBL E.Coli (n = 10)	190.5	135.2	150.740 (±16.859)	1081.02	648.57	817.730 (±133.786)

In general, it is observed that the concentrations of ESBL E.Coli are typically higher compared to non-broad-spectrum beta-lactamase producing bacteria in urinary tract infections. This observation holds true for IL-17, as depicted in the figure 3. The diagram shown in Figure 4 illustrates the configuration of LPS.

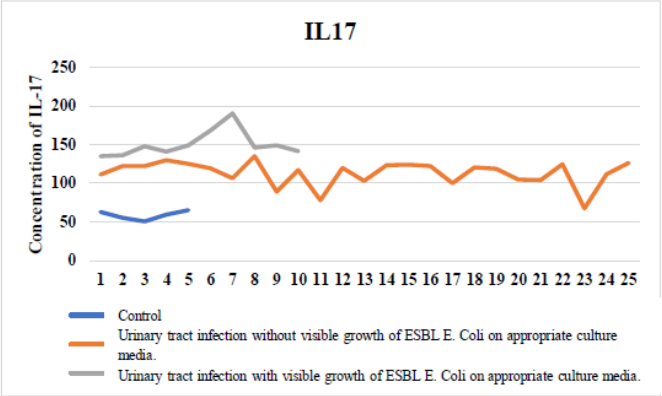

Figure 3. Graph illustrating IL-17 concentrations

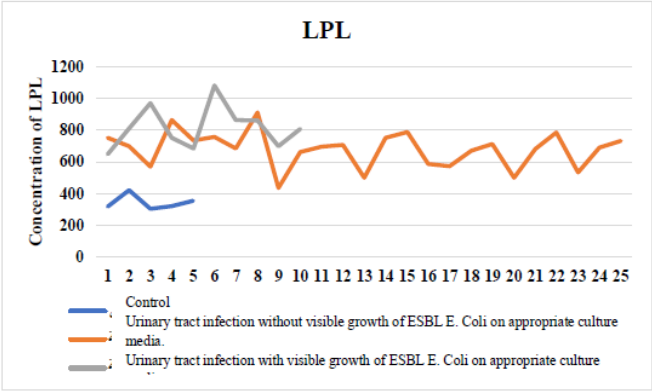

Figure 4. Graph illustrating LPS concentrations

It is worth mentioning that elevated levels of interleukin are often seen with increased concentrations of lipopolysaccharide, suggesting the potential for an upregulation of IL-17 levels due to alterations in LPS. The present study conducted by Leclercq et al. (2021) (18) aimed to investigate the correlation between diversity and alteration in lipopolysaccharide (LPS) on the virulence of Escherichia coli. Through the examination of 500 distinct genetic sequences from 499 Escherichia coli samples, the study confirmed the presence of an association between LPS modification and the virulence and pathogenicity of Escherichia coli. Furthermore, the study established a link between LPS alteration and the emergence of the LPS K12 type in Escherichia coli of the STc131 type (belonging to group B2), which coincided with the development of antibiotic resistance.

It is worth mentioning that elevated levels of LPS do not consistently coincide with elevated levels of IL-17. However, it is important to note that the sample exhibiting greater IL-17 concentration also exhibited high LPS concentrations (QTAIL-17 = 190.483, QTALPL = 862). Indeed, sample E5 was included in the analysis; however, it did not exhibit the greatest levels of LPL concentration. Nevertheless, the sample F2 had the most elevated LPL content (QTALPL = 1081.023), which coincided with an IL-17 value beyond the range seen in samples without apparent development but with urinary system infection. The presence of numerous pathways involved in the stimulation of IL-17 can be observed in ESBL E. Coli when cultured on suitable media. These pathways include, but are not restricted to, the activation of T cells of the gamma-delta type (T cellδγ). Shibata et al. (2007) (19) have reported on the stimulation of IL-17 in this context.

In order to find out whether there is a statistically significant difference between the levels of IL-17 between the standard and samples with infection in the urinary system without visible growth of ESBL E.Coli on the appropriate culture media and samples with infection in the urinary system and with visible growth of ESBL E. Coli on the appropriate culture media. We initially went to the normal distribution test to determine whether the data was normally distributed as shown in Table 3.

Table 3. Test of normal distribution of serum concentrations of IL-17

Tests of Normality							
	Sample Type	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Concentration of IL - 17	Control	.177	5	.200*	.952	5	.752
	Urinary tract infection without visible growth of ESBL E.Coli on appropriate culture media.	.193	25	.017	.868	25	.004
	Urinary tract infection with visible growth of ESBL E.Coli on appropriate culture media.	.329	10	.003	.799	10	.014
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Based on the findings presented in Table 5, it is evident that the standard concentrations exhibit a normal distribution. This conclusion is supported by a p value of less than 0.05, leading to the rejection of the null hypothesis, which posits that the data does not follow a normal distribution. Consequently, the alternative hypothesis, which asserts that the data is normally distributed, is accepted. Nevertheless, the concentrations do not exhibit a normal distribution, as shown by the p-value being more than 0.05. The data shown in Figure 5 demonstrates a notable trend in patient samples, whereby the median concentration tends to approach the third quartile. This observation suggests a definite inclination towards greater concentrations in the presence of ESBL E. Coli on adequate culture conditions.

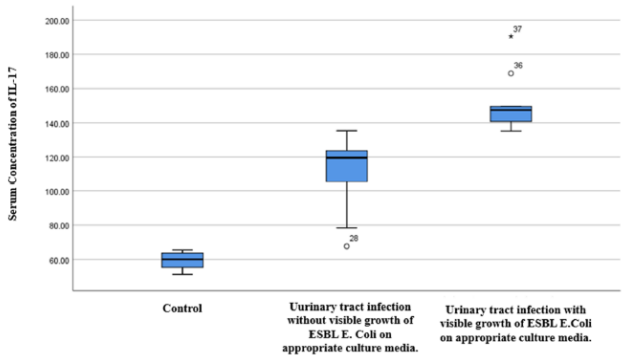

Figure 5. Boxplot of the serum concentration of IL-17.

Given the non-normal distribution of the data, as shown in Table 4, the Mann Whitney U test was used to assess the absence of a significant difference in median values between samples with urinary tract infection and those without obvious growth of ESBL E.Coli. In the context of culture media and samples pertaining to urinary system infections, it is shown that ESBL E.Coli exhibits noticeable growth on the proper culture media. This growth tendency is depicted in Figure 6, where the medium approaches the third quartile in patient samples.

Table 4 presents the outcomes of the Mann-Whitney U test conducted on the serum concentrations of IL-17 derived from various pathological specimens.

	Serum concentration of IL-17
Mann-Whitney U	1.000
Wilcoxon W	326.000
Z	-4.528
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]
a. Grouping Variable	
b. Not corrected for ties.	

According to the findings shown in Table 4, a significant difference was seen in the serum concentrations of interleukin between samples with urinary system infection but no evident development of ESBL E.Coli on the media. This difference was determined to be statistically significant at a confidence level of 99% ($p = 0.000 < 0.01$). The selection of suitable cultures and samples for the detection of urinary tract infection and the presence of ESBL E. Coli on the proper culture medium is of utmost importance.

The purpose of this study was to assess whether there existed a statistically significant disparity in the levels of LPS between the standard and infected samples in the urinary system, where no visible growth of ESBL E. Coli was observed on the appropriate culture media, and samples in the urinary system with visible growth of ESBL E. Coli on the appropriate culture media. The normal distribution test was conducted first to assess the normality of the data, as shown in Table 5.

Table 5. Testing the normal distribution of serum concentrations of LPS

Tests of Normality							
	Sample Type	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Concentration of IL - 17	Control	.287	5	.200*	.837	5	.157
	Urinary tract infection without visible growth of ESBL E.Coli on appropriate culture media.	.159	25	.105	.962	25	.462
	Urinary tract infection with visible growth of ESBL E.Coli on appropriate cultu zsawx Zre media.	.169	10	.200*	.945	10	.610
*. This is a lower bound of the true significance.							
a. Lilliefors Significance Correction							

Based on the findings presented in Table 6, it is evident that the concentrations of LPS exhibit a normal distribution. This conclusion is supported by the statistical analysis, as indicated by the p-value being less than 0.05. Consequently, we reject the null hypothesis, which posits that the data does not conform to a normal distribution, and accept the alternative hypothesis, which asserts that the data is indeed normally distributed. Given the assumption of normal distribution of the data, a t-test for independent samples was performed to examine if there were any statistically significant variations in LPS concentrations across samples exhibiting urinary tract infection without any observable development of ESBL E.Coli on the culture medium. The appropriateness of specimens with urinary tract infection and the presence of visible growth of ESBL E. Coli on suitable culture medium.

Table 6. the outcomes of an independent samples t-test conducted to analyse the serum amounts of lipopolysaccharide (LPS) in various pathological samples.

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Serum Concentration of LPS	Equal variances assumed	.262	.612	3.085	33	.004	137.91780	44.70911	46.95642	228.87918
	Equal variances not assumed			2.872	14.494	.012	137.91780	48.02784	35.23702	240.59858

According to the data shown in Table 6, the computed F value does not meet the criteria for statistical significance at a 95% confidence level ($F = 0.262$, $p = 0.612 > 0.05$). Hence, the alternative hypothesis, positing that the sample variance is not homogenous, is rejected in favour of the null hypothesis, which asserts that the variance is indeed homogeneous. The presence of variation among samples; Hence, the obtained T value ($t = 3.085$, $p = 0.004 < 0.01$, CI: 46.95 – 228.87) indicates a statistically significant difference, with a confidence level over 99%, in the blood concentrations of LPS between instances of urinary tract infection without growth. The manifestation of ESBL E. coli on suitable culture medium and its levels in instances of urinary system infection accompanied with observable growth of ESBL E. coli on acceptable culture media.

Conclusion

The findings of the present study indicate that the mean prevalence of Escherichia coli strains that produce beta-lactamase enzyme among individuals admitted to hospitals with urinary tract infections is 25%. This number is within the range of worldwide prevalence reported in earlier research. All Escherichia coli isolates obtained from patients diagnosed with urinary tract infection exhibited multidrug resistance (MDR) against various antibiotics. Among the tested antibiotics, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, and Pipracillin demonstrated the highest levels of resistance, whereas all isolates displayed susceptibility to Imipenem. The findings from the immunosorbent assay revealed statistically significant variations ($p < 0.01$) in the serum levels of interleukin IL-17 and lipopolysaccharide (LPS) between samples exhibiting urinary tract infection without observable growth of ESBL E.Coli on the appropriate culture media, and samples displaying urinary tract infection with visible growth of ESBL E.Coli on the appropriate culture media.

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