Study the Effectiveness of Ceftriaxone on Biofilm Formation by Different Strains of *E. Coli* Isolated from Patients with Urinary Tract Infection.

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Abstract: Infections of the urinary tract are among the most common diseases in the world, affecting both males and females around the world. Females are more susceptible to urinary tract infections than males, owing to the female body's anatomical configuration. The pathogenic *Escherichia coli* is the most common causative agent in 80%–90% of urinary tract infections. This study was carried out in the laboratories of the Medical State University of Grodno. The study used four strains of *E. coli* from the museum of the Department of Microbiology, Virology, and Immunology at the Medical State University of Grodno to determine the features of Ceftriaxone's action on different strains of *E. coli* in the composition of biofilms. The results showed that all screened museum strains of *E. coli* isolated from pathological material of patients with urinary tract infection are able to form biofilms. The minimum inhibitory concentration of CTX for all studied *E. coli* biofilms was in the range of 35-280 mg/ml and the minimum inhibitory concentration in plankton was in the range of 0.27-0.068 mg/ml.

Keywords: urinary tract infection, *Escherichia coli*, ceftriaxone, virulence factors, biofilms.

1. Introduction

Urinary tract infection spreads to all ages and genders, and recent studies have indicated the development of these infections into more severe and dangerous infections, in addition to the high costs of treatment spent by the patient and hospitals. In 1994, the United States of America spent about one billion dollars on the treatment of UTI patients, and about 7 million patients went to hospitals, and about one million patients were already in hospitals [1].

Uropathogenic *Escherichia coli* is the most common causative agent of UTIs, 80%–90% [2]. At least once in their lives, 40% of females and 12% of males will suffer from a UTI. Up to 8.4% of females and 1.7 % of males have a UTI episode during their first six years of life. In total, 20%–40% of females and children will experience a recurring instalment of UTI in the 6 to 12 months following the first episode [3].

Age, gender, and other factors all play a role in UTI revalence can be affected by immunosuppression and urological

instruments [4]. UTIs are among the most severe health risks, accounting for 34% of all associated health care illnesses [5]. Women are usually more susceptible to infection than men because of the body's anatomical configuration, the genital opening's proximity to the anus, and the short distance between the urinary opening and the bladder [6].

Ceftriaxone is a drug that is used to treat a variety of infectious diseases, such as *E. coli*, *pneumonia*, or *meningitis* and patients who have had surgery, ceftriaxone is often used to avoid infection. antibiotics with a broad spectrum of antimicrobial action against Gram-positive and Gram-negative bacteria. Ceftriaxone overdose may increase the chance of urolithiasis and acute (PARF). Ceftriaxone was approved for medicinal use in 1982 after it was patented in 1978. It is classified as an essential medicine by the WHO [6].

2. Materials and Methods

The purpose of the study is to determine the effect of Ceftriaxone on biofilm-forming bacteria and select the minimum inhibitory concentration. The study used four strains of *E. coli* from the Department of Microbiology, Virology, and Immunology at the Medical State University of Grodno's museum: *E. coli* B12 (instead of *E. coli* 63), *E. coli* k-12 (instead of *E. coli* 65), E. coli ATCC 25923 (instead of *E. coli* 66), and a clinical pathogenic strain of *E. coli* isolated from pathological material of Diagnosed based on Bergey's Manual of Systematic Bacteriology.

2.1. Methods for determining the sensitivity of bacteria to antibiotics

To find out the sensitivity of the bacteria to antibiotics, a series of different concentrations of Ceftriaxone started at a concentration of 2.18 mg/ml. In microtiter 96 well plates, 100 µl of Mueller-Hinton Broth was placed in each hole, and added to 100 µl of the antibiotic for the first hole, After that, the process is diluted to eight concentrations. After that, each hole was filled with 10 µl of bacterial suspension, and the control was 100 µl of the Mueller-Hinton Broth with 10 µl of bacteria without any antibiotics. After that, incubation was done in the incubator at 37 oC for 24 hours. To read the results of the experiment, the sample was entered into a spectrophotometer before incubation and again after incubation, where the results were compared, and the results were also read by creating turbidity in the wells [7].

2.2. Modeling microbial biofilms in vitro

To achieve the goal of the study, a technique was developed to produce biofilms in artificial conditions from the obtained microorganisms, and biofilms were recreated in such combinations as they were in natural conditions on the inner base of the UT. To obtain biofilms in artificial conditions, a suspension of *E.coli* strains was prepared. For this, 100 µl of a suspension of *E.coli* strains

was added to each well of Microtiter 96 well plates and 100 µl of the Mueller-Hinton broth, and incubated in an incubator for 24 h at a temperature of 37°c. After 24 hours, the wells were washed by adding 100 µl of phosphate buffered saline to discard the broth. (pH 7.2–7.4). Then the phosphate buffered saline was discarded and 100 µl of broth was added and incubated for 24 h at 37 °C. The same steps were repeated three times for three days. The obtained biofilms were used in the antibiotic effect on biofilms. Through the previous process, biofilms were obtained. So it was examined under an electron microscope. To control biofilm growth, special 3.5 mm electron microscopy copper grids coated with a former film were used and some were selected for microscopy with an accelerating voltage of 80 kV using a JEM1011 electron microscope (JEOL, Japan).

As the steps of biofilm formation were: irreversible attachment, cell aggregation, accumulation in layers, formation of the matrix, surface adhesion, Biofilm maturation, biofilm growth, cell structural, metabolic heterogeneity, biofilm dispersion, cell dispensation into planktonic cells and release into the environment [8].

3.3. Determination of the sensitivity of biofilms to the antibiotic

The sensitivity of microorganisms in biofilms was studied using antibiotic in various concentrations of ceftriaxone starting at 280 mg/ml, 140 mg/ml, 70 mg/ml, 35 mg/ml, 17.5 mg/ml, 8.75 mg/ml, 4.37 mg/ml, 2.18 mg/ml.

After preparing the solution of the antibiotic, 100 µl of liquid culture medium was added to all test wells with biofilms for microorganisms and 100 µl of antibiotic. The first well was filled with solution and 8 serial dilutions of the antibiotic were made. The control was a well-biofilm and culture medium without any antibiotics. To visualise the growth of microorganisms, one drop of 0.3% resazurin was added to each well. Resazurin is an indicator used to detect changes in redox potential in a medium. As a result of the growth of microorganisms, their colour changes from blue to pink

3. Results

The determination of the minimum inhibitory concentration of ceftriaxone for various strains of *E. coli* was carried out by the method of serial dilutions of the antibiotic in Mueller-Hinton broth, first concentration 2.18 mg/ml, second 1.09 mg/ml, third 0.545 mg/ml, fourth 0.272 mg/ml, fifth 0.136 mg/ml, sixth 0.068 mg/ml, seventh 0.034 mg/ml, and eighth 0.017 mg/ml, and was taken into account by the change in the turbidity of the contents of the wells after using a spectrophotometer, table 1. The density of the solution was recorded immediately after the experiment and after 24 hours of incubation in 37^0 C.

The results showed that the bacteria resistance test to the antibiotic CTX showed that the minimum inhibitory concentration for *E. coli* 63 was 0.13 mg/ml, and the minimum inhibitory concentration for *E. coli* 65 and *E. coli* 66 was 0.068 mg/ml, while the minimum inhibitory concentration for *E. coli* isolated from the pathological material was 0.27 mg/ml, table 1, table 2 and figure 1.

Table 1: CTX resistance results by spectrophotometer.

M.O	Time	Concentrations of CTX								Control
		2.18 mg/ml	1.09 mg/ml	0.54 mg/ml	0.27 mg/ml	0.13 mg/ml	0.068 mg/ml	0.034 mg/ml	0.017 mg/ml	
E. coli 63	Before	0.099	0.098	0.109	0.107	0.110	0.110	0.097	0.101	0.098
	After	0.091	0.120	0.098	0.103	0.106	0.473	0.104	0.110	0.386
E. coli 65	Before	0.093	0.094	0.096	0.096	0.097	0.100	0.103	0.098	0.104
	After	0.089	0.093	0.094	0.098	0.103	0.100	0.278	0.243	0.375
E. coli 66	Before	0.087	0.088	0.091	0.094	0.093	0.094	0.097	0.099	0.112
	After	0.084	0.085	0.088	0.087	0.082	0.079	0.325	0.385	0.417
E. coli pathogen strain	Before	0.090	0.092	0.092	0.094	0.094	0.100	0.105	0.98	0.107
	After	0.084	0.088	0.095	0.090	0.279	0.194	0.214	0.333	0.341

Before: The results before incubation.
After: The results after 24h incubation.

Table 2: CTX resistance results depending on turbidity.

M.O	Time	Concentrations of CTX								Control
		2.18 mg/ml	1.09 mg/ml	0.54 mg/ml	0.27 mg/ml	0.13 mg/ml	0.068 mg/ml	0.034 mg/ml	0.017 mg/ml	
E. coli 63	Before	-	-	-	-	-	-	-	-	-
	After	-	-	-	-	-	+	+	+	+
E. coli 65	Before	-	-	-	-	-	-	-	-	-
	After	-	-	-	-	-	-	+	+	+
E. coli 66	Before	-	-	-	-	-	-	-	-	-
	After	-	-	-	-	-	-	+	+	+
E. coli pathogen strain	Before	-	-	-	-	-	-	-	-	-
	After	-	-	-	-	+	+	+	+	+

(+): There is a growth of microorganisms.
(-): There is no growth of microorganisms.
Before: The results before incubation.
After: The results after 24h of incubation.

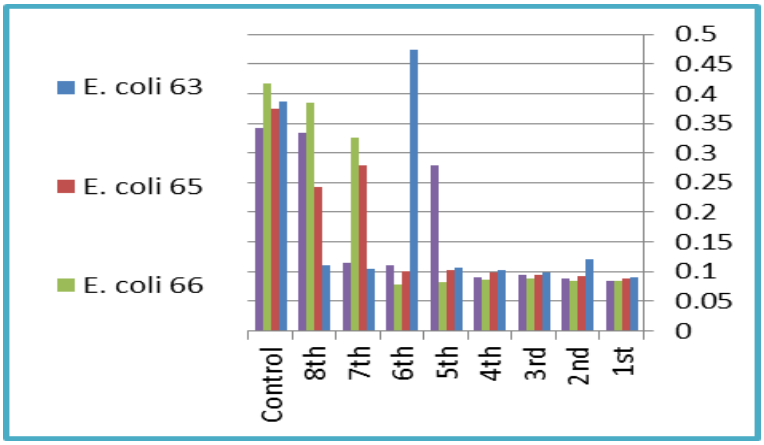

Figure 1: CTX resistance results after 24 hours.

3.1. Ceftriaxone antimicrobial action on biofilms

The results of the CTX effect on biofilms with *E. coli* showed after one hour that CTX at a concentration of 17.5 mg/ml inhibits the growth of biofilms of all *E. coli* strains, table 3. After 6 hours, the concentration of 70 mg/ml inhibits the growth of biofilms with *E. coli* 63, and the concentration of 17.5 mg/ml inhibits the growth of biofilms with *E. coli* 65 and *E. coli* isolated from the pathological material, and the concentration of 35 mg/ml inhibits the growth of biofilms with *E. coli* 66, table 4. After 24 hours of incubation, the minimum inhibitory concentration of CTX for E. coli 63 was 280 mg/ml, as for *E. coli* 65 it was 140 mg/ml, as for *E. coli* 66 it was 70 mg/ml, and for *E. coli* isolated from the pathological material it was 35 mg/ml, table 5.

Table 3: CTX Biofilms formation results after 1 hour.

The type of organism	Concentration of the antibiotic					Control M.O & broth
	280	140	70	35	17.5	
E. coli 63	-	-	-	-	-	+
E. coli 65	-	-	-	-	-	+
*E. coli*66	-	-	-	-	-	+
E. coli pathogen strain	-	-	-	-	-	+

(+): bacteria have the ability to biofilms formation.
(-): bacteria have no ability to biofilms formation

Table 4: CTX Biofilms formation results after 6 hours.

The type of organism	Concentration of the antibiotic					Control M.O & broth
	280	140	70	35	17.5	
E. coli 63	-	-	-	+	+	+
E. coli 65	-	-	-	-	-	+
*E. coli*66	-	-	-	-	+	+
E. coli pathogen strain	-	-	-	-	-	+

(+):bacteria have the ability to biofilms formation.
(-): bacteria have no ability to biofilms formation.

Table 5: CTX Biofilms formation results after 24 hours.

The type of organism	Concentration of the antibiotic					Control M.O & broth
	280	140	70	35	17.5	
*E. coli*63	-	+	+	+	+	+
*E. coli*65	-	-	+	+	+	+
*E. coli*66	-	-	-	+	+	+
E. coli pathogen strain	-	-	-	-	+	+

(+):bacteria have the ability to biofilms formation.
(-): bacteria have no ability to biofilms formation.

4. Discussion

The primary etiological agent linked with community acquired UTI is Uropathogenic *Escherichia coli* (UPEC) [9]. One of the most intriguing methods discovered so far for UPEC infection of the host [10], within the superficial bladder cells. These structures have been seen in urine from UTI patients [11,12]. Biofilm development is another essential factor in UTI pathogenesis. The genetic expression of bacteria in biofilm differs from that of planktonic bacteria, and various studies have established the significance of this difference [13].

Biofilm production is a complicated process that includes several phases, each with its own set of variables [14]. The irreversible attachment to the surface where flagella production is suppressed is a critical event in the success of biofilm growth. Adhesive organelles like as type 1 fimbriae and curli are required for *Escherichia coli* irreversible adhesion to surfaces [15].

Many persistent infections are caused by biofilm-producing bacteria, which are difficult to eliminate. *E. coli* biofilm formation enhances colonization and increases UTI. These infections may be challenging to treat due to multiple medication resistance [16].

The study showed different results for the resistance of the four types of *E. coli* to ceftriaxone in bacterial plankton and biofilms.

E. coli 63, 280 mg / ml: 0.13 mg / ml = 2153 times, this means that in order to suppress the growth of *E. coli* 63 in the composition of the biofilm, it is necessary to increase the concentration by more than 2153 times in comparison with the *E. coli* in plankton.

E. coli 65, 140 mg/ ml : 0.068 mg / ml = 2058 times it is necessary to increase the concentration.

E. coli 66, 70 mg/ ml : 0.068 mg / ml = 1029 times, it is necessary to increase the concentration.

E. coli isolated from the pathological material, 35 mg / ml : 0.27 mg / ml = 129 times it is necessary to increase the concentration.

5. Conclusion

All screened museum strains of *E. coli* isolated from pathological material of patients with urinary tract infection are able to form biofilms.

The minimum inhibitory concentration of CTX for all studied coli biofilms was in the range of 35-280 mg/mL. The most sensitive to CTX were *E. coli* isolated from pathological material. the minimum inhibitory concentration was 35 mg/ml. The most resistant was *E. coli* 63, at 280 mg/ml. The minimum inhibitory concentration of CTX for *E. coli* biofilms is more than 2000 times higher than the minimum inhibitory concentration for *E. coli* in plankton.

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