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

MSc. Yousif Sinan Taha Alhamadani

*Department of Biology and Ecology, Yanka Kapala State University of Grodno. Ozheshko St., 22, 230023, Grodno, Belarus.*

*Corresponding Author: Tel:+375256058862, E-mail: yousefalhmdany20@gmail.com*

*Received 07-04-2021, Accepted 05-12-2021, published 31-05-2022.*

**DOI:** 10.52113/2/09.01.2022/1-11

**Abstract:** Urinary tract infections are among the most frequent diseases in the world, affecting both males and women around the world. Females are more susceptible to urine tract infections than males, owing to the female body's anatomical configuration. The pathogenic *Escherichia coli* is the most common causative agent 80% - 90% of urinary tract infections. This study was carried out in the laboratories of the Medical State University of Grodno. The object of study was taken 4 strains of *E. coli* from the museum of the Department of Microbiology, Virology and Immunology Medical State University of Grodno where determine the features of the action of Ceftriaxone on various strains of *E. coli* in the composition of biofilms. The purpose of the study are determination of the effect of Ceftriaxone on biofilm-forming bacteria and select the minimum inhibitory concentration. 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 0.27-0.068 mg/ml.

**Keyword:** urinary tract infection, Uropathogenic *Escherichia coli*, Ceftriaxone, virulence factors, Pathogenicity, biofilms.

1. **Introduction**

Urinary tract infection is spreading to all ages and genders, and recent studies have indicated the development of these injuries to more severe and severe injuries in addition to the high material costs of treatment spent by the patient and the hospitals, in 1994, the United States of America spent the treatment of (UTI) about one billion dollars in one year and about 7 million patients went to hospitals and about million patients were already in hospital [1].

Uropathogenic *Escherichia coli* the most common causative agent 80% - 90% [2]. At least once in their lives, 40% of females and 12% of males will suffer from a (UTI). In the first six years of life, up to 8.4% of females and 1.7 percent of males will have a (UTI) episode. In complete, 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, (UTI) prevalence can be affected by immunosuppression and urological
instruments [4]. Catheter-related (UTI) are among the most severe health risks, accounting for 34% of all associated health care illnesses [5]. The women are usually more susceptible to infection than males because of the body's anatomical configuration and the genital opening's proximity to the anus and the short distance between the urinary opening and the bladder.

Ceftriaxone is a drug that is used to treat a variety of infectious diseases, including forms that are serious or life-threatening, such as *E. coli*, Pneumonia, or meningitis. In patients undergoing some forms of surgery, ceftriaxone is often used to avoid infection. Spectrum of antimicrobial action, antibiotics with a broad range of 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), it's a drug that's available as a generic.

1.1 Characterization of *E. coli*

They are Gram negative bacilli, it has no ability to configure spores. Their colonies are soft, smooth, slightly convex, colonies are mucous when they have a capsule, shiny pink on the MacConkey agar and Cromagar Orientation agar, green metallic sheen on Eosin (EMB) agarNon-fermented cellulobios, with fermented ramenose accounting for more than 80% of the total, and fermented sorbitol accounting for more than 90%.

It is also not analyzed of gelatin and it is not producing H2S in (TSI) agar, most of them are producers enzyme β-glucoronidase and In the presence of (KCN) it does not rise , the appropriate pH for growth is (9 - 4.4) and the optimum temperature is (36–37) oc.

Table 1: Biochemical tests of *E. coli* [6].

<table>
<thead>
<tr>
<th>Test</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Urease</th>
<th>Indole</th>
<th>Methyl red</th>
<th>Voges proskauer</th>
<th>Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) : means that the test result is positive. 
(-) : means that the test result is negative.

1.2 Virulence factors

(VF) are specialized properties that microorganisms have that allows them to bypass the host's defenses and infect the host cells [7]. Despite the discovery of multiple virulence factors in Uropathogenic *E. coli*, epidemiological and experimental results suggest that no one knows these pathogens in isolation.

Adhesions, iron acquisition systems, and other functional categories are used to group UPEC VFs Toxins, proteases, and protections are all examples of these. Specific genes on plasmids or chromosomes encode virulence factors, some of which are chromosomal-only
(e.g., hly and pap), others, primarily plasmid-related, e.g., traT and iss, and (afa). Subsequently, the multifaceted nature of understanding the pretended through explicit VF qualities in UTI pathogenesis is further enhanced by the fact that VFs can be transmitted horizontally or vertically.

1.3 Pathogenicity of E. coli in UT

It is amongst the most serious and deadly illnesses, as it is caused by bacterial infection and multiplication in the (UT), which includes the lower (urethra and bladder) and upper(ureters and kidneys). Cystitis and urethritis are two terms for inflammation that starts in the lower urinary tract [8,9].

E. coli it is among the most widely types of bacteria cause diseases in (UT), in addition to bacteria that cause other forms of infections of (UT) [7]. (UTI) pathogenesis is a multifaceted and difficult procedure that is affected by variety of host biological besides the characteristics of the infecting pathogen, as well as virulence factors. Consequently this encounter a epidemiological research pose a challenge in regards due to the effect of host factors on the role of particular (VF) in (UTI) pathogenesis. In certain cases of healthy people, the (UT) is usually sterilized, the entry of external (M.O) is difficult due to the outflow of urine, bacteriostatic activities of the immune response cells and tissue-secreting antibacterial agents. The source of infection by E. coli strains is usually the host fecal flora, which spreads across the perineal region, per urethral regions, and vaginal to the lower part of the (UT) (i.e., bladder and urethra) where bacteria can multiply and form colonies[10]. There are two theories on how (M.O) transfer from the fecal flora to the intestinal flora (UT) E. coli copies in the stool will be involved in the proliferation hypothesis, and E. coli organisms with developer virulence will be selected in the pathogenic organisms hypothesis [10]. Both of these theories may play a role in the development of urinary tract disease [11].

While the E. coli infection strain is primarily spread via the stool of the host mammals, Other (M.O) proximal external reservoirs have been discovered. A report was produced on the prevalence of (UTI) in the community [12,13], but without conclusive evidence that this infection was transmitted from one person to another, it has therefore been suggested that water and food contaminated with E. coli bacteria is the cause [12,13]. Specifically, molecular similarities are broad between Humans that are stable or contaminated with E. coli from supermarket meat products It's already been explained [12]. The transmission of E. coli has been confirmed in the home, among humans and their pets, as well as among sexual partners.
Intrusive bacteria (VF) and host defense mechanisms of the immune system decide the infection's outcome. There are a lot of factors of the host, such as gender, age, immune system condition, or pregnancy, may predispose urinary tract infection and allow microorganisms that carry the least (VF) to trigger the illness [10]. Acute cystitis is an inflammation of the lower (UT) that results in signs such as excessive urination and dysuria. Acute pyelonephritis is defined by symptoms include fever, abdominal discomfort, and malaise if the illness is found in the upper urinary tract.

1.4 Ceftriaxone

Chemical formula C18H18N8O7S3, molecular mass is 554.58 g/mol. The owner of the registration certificate: Borisov Plant of Medicines, OJSC (Republic of Belarus).

Figure 1: Ceftriaxone powder for solution for infusion.


Ceftriaxone is a drug that is used to treat a variety of infectious diseases, including forms that are serious or life-threatening, such as E.coli, Pneumonia, or meningitis. In patients undergoing some forms of surgery, ceftriaxone is often used to avoid infection.

This treatment belongs to the antibiotics for cephalosporin's class of drugs. It operates on the basis of preventing the creation of bacteria. This medicine is not for use in newborns and premature infants who have high levels of bilirubin in the blood.

Spectrum of antimicrobial action, antibiotics with a broad range of action against Gram-positive and Gram-negative bacteria.

Mechanism of action of ceftriaxone blocks the action of mucopolypeptides in the bacteria cell lipid bilayer that found wall, which is how it works. Ceftriaxone connects to carboxypeptidases, endopeptidases, and transpeptidases throughout the microbial plasma membranes with its beta-lactam moiety. These enzymes are required for cell wall synthesis and cell division.

2. Materials And Methods

The object of the study was taken 4 strains of E. coli from the museum of the Department of Microbiology, Virology and Immunology Medical State University of Grodno: 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 clinical pathogenic
strain of *E. coli* separated from the pathological material of a patient with a illness of the urinary system. 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 antibiotic, a series of different concentrations of the Ceftriaxone start in concentration 2.18 mg/ml. By microtiter 96 wells plates, 100 μl of Mueller-Hinton Broth were placed in each hole, and addition to 100 μl of the antibiotic for the each first hole, after which the process of dilution from the first hole to the second and then the third and so on 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 °c 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 are also read by creating turbidity in the wells.

### 2.2 Methods of 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 working suspension of microorganisms was prepared. For this, 100 μl of a suspension of microorganisms was added to each well of Microtiter 96 wells 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 are washed by discarding the broth and adding 100 μl of phosphate buffer d saline (pH 7.2-7.4). Then discard the phosphate buffered saline and add 100 μl of broth and incubate for 24 h, at 37°C. The same steps were repeated for 3 times, for 3 days. The obtained biofilms were used in the antibiotic effect on biofilms. As biofilms consisting of one type of microorganism were obtained. Acquired biofilms growth images using electron microscopy. Acquired biofilms growth images using electron microscopy from the Department of Microbiology, Virology and Immunology Medical State University of Grodno to control the growth of the biofilm, special copper grids for electron microscopy with a diameter of 3.5 mm coated with a former film placed in the tablets, some of them selected for microscopy using a JEM1011 electron microscope (JEOL, Japan) at an accelerating voltage of 80 KV.
As the steps of biofilm formation were: Irreversible attachment, cell aggregation and accumulation in layers formation of the matrix and surface adhesion. Biofilm maturation, biofilm growth and cells structural and metabolic heterogeneity, Biofilm dispersion, cell dispensation into planktonic cells and released in the environment.

2.3 Methods for determining the sensitivity of microorganisms in the composition of biofilms to antibiotic

The sensitivity of microorganisms in biofilms was studied using antibiotics in various concentrations of Ceftriaxone started in 280mg/ml.

After the antibiotic are prepared, 100μl of liquid culture medium was added to all test wells with biofilms for microorganisms and 100 μl of antibiotic the first well has been filled with solution and 8 serial dilutions of the antibiotic were made. The control was a well biofilms and culture medium without any antibiotics.

To visualize 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 color 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, 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. The density of the solution was recorded immediately after the experiment and after 24 hours of incubation in a thermostat at 37 °C.

The results showed that the bacteria resistance test to the antibiotic CTX that 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 2: CTX resistance results by spectrophotometer.

<table>
<thead>
<tr>
<th>M.O</th>
<th>Time</th>
<th>Concentrations of CTX</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.18 mg/ml</td>
<td>1.09 mg/ml</td>
</tr>
<tr>
<td><em>E. coli</em> 63</td>
<td>Before</td>
<td>0.099</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.091</td>
<td>0.120</td>
</tr>
<tr>
<td><em>E. coli</em> 65</td>
<td>Before</td>
<td>0.093</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.089</td>
<td>0.093</td>
</tr>
<tr>
<td><em>E. coli</em> 66</td>
<td>Before</td>
<td>0.087</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.084</td>
<td>0.085</td>
</tr>
<tr>
<td><em>E. coli</em> pathogenic strain</td>
<td>Before</td>
<td>0.090</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.084</td>
<td>0.088</td>
</tr>
</tbody>
</table>

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

### Table 3: CTX resistance results depending on turbidity.

<table>
<thead>
<tr>
<th>M.O</th>
<th>Time</th>
<th>Concentrations of CTX</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.18 mg/ml</td>
<td>1.09 mg/ml</td>
</tr>
<tr>
<td><em>E. coli</em> 63</td>
<td>Before</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em> 65</td>
<td>Before</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em> 66</td>
<td>Before</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em> pathogenic strain</td>
<td>Before</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) There are growth of microorganisms.
(-) There are no growth of microorganisms.
Before: The results before incubation.
After: The results after 24h incubation.
3.1 Antimicrobial action of Ceftriaxone with biofilms.

The results of CTX effect on biofilms with *E. coli* showed after one hour that CTX at these concentration of 17.5 mg/ml inhibits the growth of biofilms of all *E. coli* strains.

After 6 hours, the concentration of 70 mg/ml inhibit the growth of biofilms with *E. coli* 63, and the concentration 17.5 mg/ml inhibits the growth of biofilms with *E. coli* 65 and *E. coli* isolated from the pathological material, and in concentration 35 mg/ml inhibit the growth of biofilms with *E. coli* 66.

After 24 hours of incubation, the minimum inhibitory concentration of CTX for *E. coli* 63 was 280 mg/ml, as for *E. coli* 65 was 140 mg/ml, as for *E. coli* 66 was 70 mg/ml, and for *E. coli* isolated from the pathological material was 35 mg/ml.

Table 4: CTX Biofilms formation results after 1 hour.

<table>
<thead>
<tr>
<th>The type of organism</th>
<th>Concentration of the antibiotic 280mg/ml</th>
<th>Control M.O &amp; broth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>280 140 70 35 17.5</td>
<td>- - - - +</td>
</tr>
</tbody>
</table>

(+) bacteria have the ability to biofilms formation. (-) bacteria have no ability to biofilms formation.
3.2 Comparison of the antibiotic in planktonic and in composition of biofilm

1. *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.

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

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

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

4. CONCLUSION

The ineffectiveness of antibiotic therapy for diseases of the urinary system is largely associated with the resistance of the microorganisms that caused this disease. *E. coli* resistance is greatly enhanced by the ability of these microorganisms to form biofilms on the surface of the urinary tract mucosa. In this regard, for the selection of therapy, it is necessary not only to clarify the etiology of the disease, determination of the microorganism that caused the disease, and its sensitivity or resistance, but it is also necessary to determine the sensitivity of these microorganisms to antibiotics in the biofilm. Based on the Study conducted, conclusions can be drawn.

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

2. The minimum inhibitory concentration of CTX for all studied *E. 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 *E. coli* 63 was 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.

REFERENCES


