STUDY THE MUTAGENIC EFFECTS OF ULTRAVIOLET (UV) ON VIABILITY AND ANTIBIOTIC SENSITIVITY OF ENTEROPATHOGENIC ESCHERICHIA COLI ISOLATED FROM INFANTS DIARRHEA


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ABSTRACT

During this study a total of (28) diarrheal samples were collected from infants and young children younger than (3) years in (2) hospitals in Baghdad, during the period from 1/4/2014 to 30/8/2014. Diagnosis of Escherichia coli is depends upon isolation and laboratory identification of the bacterium. Bacterial isolates were identified by gram stain and API 20E system. Results showed that among the total of (28) diarrheal samples that were collected, only (19) isolate (67.85%) were gave typical morphological characteristics and biochemical test that related to Escherichia coli while the rest (9) isolates may belong to other pathogenic bacteria from different genera. Results of Serotyping reveals that only (17) isolates from (19) isolates were give positive results with polyvalent antisera on slide agglutination test. Results of antibiotic sensitivity test for (10) EPEC isolates reveals that EPEC isolates were 100% sensitive to trimethoprim/ sulphamethoxazole, ciprofloxacin and gentamycin, 80% of isolates were sensitive to Nalidixic acid and amoxicillin. Results also reveals that 90% of isolates are resistant to ampicillin, bacitracin and Erythromycin. Results of mutation to Escherichia coli isolates (E1) reveals that exposure to UV irradiation from (1-5) mint leads to change in the sensitivity of isolates to, amoxicillin, chloramphenicol, gentamicin and trimethoprim/ sulphamethoxazole from (25,24,20,20) mm zone of inhibition respectively to resistant isolates while the same isolate reveal changes in results from resistant to sensitive to cefixime and bacitracin which have (22,20) mm zone of inhibition respectively after mutation but the same results were remained for the other antibiotic (ciprofloxacin, Erythromycin, ampicillin, nitrofurantion, and Nalidixic acid. Also results of mutation to Escherichia coli (E1) isolates reveals that exposure...
to UV irradiation from (1-5) mint leads to change in the viability of E1 isolates in which increased exposure time to UV irradiation leads to decrease in viable cell count from too numerous to count in control plate to (80x10^6), (50x10^6) CFU/ml in M1, M2 plates after exposure to UV light for 1,2 mint, then increased exposure time to (3,4,5) mint lead to decrease viable cell count to (25,14,2) x 10^6 CFU/ml in (M3, M4, M5) plates respectively.

**KEYWORDS:** Bacterial isolates, UV irradiation, sulphamethoxazole.

**INTRODUCTION**

Diarrhea means an increased frequency or decreased consistency of bowel movements (Rollhion & Darfeuille, 2007). Diarrhea is classified by physicians into acute, which lasts one or two weeks, and chronic, which continues for longer than 2 or 3 weeks (Eckburg et al., 2005).

Diarrhea caused by enteric infections is a major factor of morbidity and mortality worldwide. The diarrheagenic *E.coli* pathotypes that caused diarrhea include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), and enteroaggregative *E. coli*. These pathotypes are defined by the presence or absence of one or more definable *E. coli* virulence factors (Kennth, 2014).

Baby's diarrhea could be caused by a viral or bacterial infection. It might also be the result of a parasite, a course of antibiotics, or something ate (Prober & Larry, 2010). *Enteropathogenic Escherichia coli* (EPEC) is a major cause of endemic infantile diarrhea exacerbated by seasonal outbreaks in developing countries (Dennehy, 2000). An estimated 2–4 billion episodes of infectious diarrhea occur each year and are especially prevalent in infants. EPEC have certain serotypes, which are much more likely to be encountered in infant diarrhea more than others. It had been demonstrated that the incidence of community-acquired EPEC infection was highest in the six-month period following childbirth and that the infection was more severe in younger children (Robins & Browne, 2007).

Pathogenesis of *Enteropathogenic E. coli* depend on adherence of EPEC strains to the intestinal mucosa resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "attachment and effacing" of cells. EPEC strains are said to be "moderately-invasive (Baumgart & Dogan, 2007). first is intimate contact, sometimes characterized as pedestal formation. second is loss of microvilli which is the result of
rearrangement of the host cell cytoskeleton (Rendon, 2007). Loss of microvilli leads to malabsorption and osmotic diarrhea. Diarrhea is persistent, often chronic, and accompanied by fever. EPEC are negative for ST and LT, but most strains produce relatively small diarrhea is caused by increased secretion or decreased absorption of fluids and electrolytes (Viswanathan et al., 2009). Certain ion transport processes are particularly associated with diarrhea activity (Moyenuddin et al., 2004). E. coli are resistant to many antibiotics that are effective against gram-positive organisms (Johnson et al., 2006).

Mutations are a heritable change in the base sequence of DNA. Some mutations can be neutral or beneficial to an organism, but most are actually harmful because the mutation will often result in the loss of an important cellular function (Kendric, 2010). There are two forms of electromagnetic radiation that are mutagenic; ionizing radiation and non-ionizing radiation. Ionizing radiation, such as x-rays or gamma radiation carries enough energy to remove electrons from molecules in a cell. Non-ionizing radiation, such as ultraviolet (UV) light, exerts its mutagenic effect by exciting electrons in molecules. The excitation of electrons in DNA molecules often results in the formation of extra bonds between adjacent pyrimidines (specifically thymine) in DNA (Mark, 2000).

MATERIAL AND METHODS

Isolation and identification of bacteria

A total of (28) diarrheal samples were collected from infant and young children younger than three years suffering from diarrhea in (2) hospitals in Baghdad. Samples were directly streaked on MacConkey agar and eosin methylene blue agar, then incubated at 37°C for 24 hrs. The diagnostic procedures consisted of direct microscopy observation, Gram staining, Biochemical tests.

Identification of bacteria by API 20E system

Identification of the isolates was carried out by subculturing suspected colonies from MacConkey Agar plates on API 20E microtubes system. This system is designed for the performance of 20 standard biochemical tests. Each test in this system is preformed within a sterile plastic microtube which contains the appropriate substrates that fixed to an impermeable plastic strip (gallery). Each gallery contains 20 microtubes (each of which consist of a tube and a couple selection).

Serotyping
Three to five colonies from each isolates that were identified by API 20E as \textit{E. coli} were selected for subculture on Blood agar medium for serotyping. Determination of the EPEC serotypes was performed by slide agglutination test using antiserum \textit{E. coli} somatic trivalent coli 1 (O125, O114, O44, O158, O142, O26) and coli 2 (O119, O111, O086, O55, O127, O126) according to the instructions of the manufacturer.

**Antibiotic sensitivity Test**
All aspects of this procedure are standardized according to NCCLS to ensure consistent and accurate results.

**A- Preparation of bacterial samples**
Inoculum from the test bacteria (\textit{EPEC}) were cultured in nutrient broth and incubated at 37°C for 24hrs, then the broth culture was diluted to \(10^5\) McFarland turbidity standard, which is roughly equivalent to 150 million cells/mL.

**B- Incubation Procedure**
Media used in this test must be Mueller-Hinton agar, poured into Petri dishes at only 4 mm depth. The pH level of the agar must be between 7.2 and 7.4. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism then streak on the Mueller-Hinton agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in other direction. Repeat this rotation 3 times. Allow the plate to dry for approximately 5 minutes. Using a flame-sterilized forceps, gently press each disc containing specific antibiotics to the agar to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature 37°C before reading the results.

**Determination of viable cell count**
For determination of viable cell count, (8) tube containing (9) ml of normal saline were labeled from 1 to 8. Prepare nutrient agar, then after autoclaving, poured in to (8) petriplates and labeled from 1 to 8 respectively. \textit{EPEC} was cultivated in brain heart infusion broth at 37°C for 24 hrs, then followed by centrifugation at (4000) rpm for(10) min, the pellet was diluted in sterile phosphate buffer PH 7. Bacterial suspension were shacked to ensure even distribution of microorganism. Aseptically remove (1) ml of bacterial suspension with a sterile pipette and transfer it to the \(10^{-2}\) dilution, then shake the tube vigorously to distribute the bacteria evenly. Using a new sterile pipette, aseptically transfer (1) ml from the \(10^{-2}\)
dilution tube to the $10^{-3}$ dilution. Vortex the sample. Complete the same procedure until reach to dilution $10^{-8}$ tube. Using a sterile pipette transfer (1) ml from each dilution tubes to agar plates respectively. Sterilize the glass spreader by dipping it in alcohol and flaming it, after that cooled, spread the sample over the surface of the plates by touching the rod to the agar then rotating the plate and spread other parts. Repeat the procedure for all plates then incubated at 37°C for 24hrs. After the incubation count the colonies on each plates.

**Mutagenesis by UV irradiation**

Mutagenesis by UV irradiation was done by subjecting fresh culture of EPEC in petriplates to UV radiation in a dark place using the UV transilluminator. The tray of the irradiation was approximately (15x25) cm that exposes to a sample in Petriplates in which the distance between the UV source and irradiated suspension was 11(cm).

Bacterial suspension at concentration $10^6$ CFU/ml were choose to exposed to UV light. From bacterial concentration $10^6$ CFU/ml, 1 ml of cell suspension were transferred into (6) sterilized Petri plates contains EMB agar that labeled from 1 to 6, the last Petri plates left as control that don’t exposed to UV irradiation while the other Petri plates exposed to UV irradiation for (1,2,3,4,5) min respectively at wave length (254 ) nm and energy (60 ) J/cm² under sterile condition, then all Petri plates were incubated at 37 °C for 24 hrs. To determine the viable count and survivals of E.coli, bacterial cells subjected to the UV and remain viable were considered as mutants and used for further study.

**Antibiotic sensitivity Test for mutant EPEC Escherichia coli isolate**

Antibiotic sensitivity Test for mutant EPEC isolate that exposed to UV irradiation at different time (1,2,3,4,5) min were done according to NCCLS in which muted bacteria at each time were cultured in nutrient broth tube labeled from 1 to 5 respectively, then incubated at 37°C for 24hrs. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism at each time then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube. Using the swab, streak on the Mueller-Hinton agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in other direction. Allow the plate to dry for approximately 5 minutes. Using a flame-sterilized forceps, gently press each disc containing specific antibiotics to the agar to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature 37°C before reading the results.

**RESULTS & DISCUSSION**
Isolation and Identification of *Escherichia coli* isolates

Results showed that among the total of (28) diarrheal samples that were collected from infants and young children, only (19) isolate (67.85%) were gave typical morphological characteristics and biochemical test that related to *Escherichia coli* while the rest (9) isolates may belong to other pathogenic bacteria from different genera as shown in Table (1.1). *EPEC* have certain serotypes, which are much more likely to be encountered in infant diarrhea more than others (Aslani & Ali, 2009). Studies in Brazil, Mexico, and South Africa have shown that 30–40% of infant diarrhea can be accredited to EPEC. These strains are also an important cause of disease in nosocomial outbreaks (Estrada et al., 2009).

Diagnosis of *Escherichia coli* is depends upon isolation and laboratory identification of the bacterium. The samples were directly streaked on MacConkey and EMB agar and incubated at 37°C for 24 hrs. On MacConkey agar, deep red colonies are produced, as the organism is lactose-positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on EMB agar produces black colonies with a greenish-black metallic sheen. This results has been agreed with (Evans et al., 2007).

Table (1.1): Number of *Escherichia coli* isolated from infants and young children and their percentage.

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No of samples</th>
<th>No of positive strains</th>
<th>Others</th>
<th>Percentage %</th>
<th>Percentages of negative strain %</th>
<th>Total Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant</td>
<td>15</td>
<td>11</td>
<td>4</td>
<td>73.33</td>
<td>26.67</td>
<td>100</td>
</tr>
<tr>
<td>Young children</td>
<td>13</td>
<td>8</td>
<td>5</td>
<td>61.53</td>
<td>38.46</td>
<td>100</td>
</tr>
<tr>
<td>Total Number</td>
<td>28</td>
<td>19</td>
<td>9</td>
<td>67.85</td>
<td>32.14</td>
<td>100</td>
</tr>
</tbody>
</table>

Microscopic examination

Microscopic examination of *Escherichia coli* revealed gram negative bacilli pink colored with rod shaped appearance that arranged in single or in pair.

API 20E system

In API 20E system, *Escherichia coli* colonies were subjected to further identifications. It's give negative results in Arginine Dihydrolase (ADH), Citrate utilization (CIT), H₂S production, urease production (URE), Tryptophane deaminase (TDA), Voges Proskauer (VP), Gelatin liquefaction (GEL). Isolates were give positive results in Indole production.
(IND), B-lactamase test (ONPG), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), Glucose, mannitol, , Sorbitol, rhaminose, melibiose fermentation but give negative results in sucrose, inositol ,amylase fermentation. This results has been agreed with (Levinson & Janet, 2000). The result of API 20E system reveal that only 19 isolates from 28 isolates were identified as *Escherichia coli*.

**Serotyping**

For detection *Enteropathogenic Escherichia coli*, 19 isolates that related to *Escherichia coli* were subjected to slide agglutination test with standard polyvalent antisera (coli 1,coli 2). Results reveals that only 17 isolates were give positive results on slide agglutination test.

**Antibiotic sensitivity Test**

From total of (17) *EPEC* isolates only (10) isolates were subjected to antibiotic sensitivity test. Results of this test reveals that 100% of EPEC isolates were sensitive to trimethoprim/sulphamethoxazole, gentamycine and ciprofloxacin , 80% of them were sensitive to Nalidixic acid and amoxicillin while 70% of isolates were sensitive to chloramphenicol and oxytetracycline as shown in table (1.2).

Results also reveals that 90% of isolates are resistant to ampicillin ,bacitracin and Erythromycine while 60% of isolates were resistant to , cefixime and Nitrofurantion).

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal feeds (Johnson et al .,2006). The rate of adaptive mutations in *E. coli* is "on the order of $10^{-5}$ per genome per generation, Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer(Afset etal.,2004). Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against gram-positive organisms (Barros ,2013) .*E. coli* bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species (Salyers & Gupta ,2004).

Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more
common. These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy (Hayward & Griffin, 2004). Extended-spectrum beta-lactamase–producing E. coli (ESBL E. coli) are highly resistant to an array of antibiotics, and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective (Partington et al., 2004).

When bacteria which carry transmissible R factors (R+ bacteria) are ingested by a human host, the R-factors may transfer into commonly occurring bacteria of the gastrointestinal tract. These organisms may subsequently transfer this resistance to pathogenic organisms, resulting in reduced efficacy of antimicrobial chemotherapy in the event of an infection (Rolhion & Darfeuille, 2007). In vivo studies have shown that when individuals carrying R+ bacteria are subjected to antibiotic therapy, these organisms flourish and transfer their resistance to other bacteria (Webb & Starr, 2005).

Table (1.2): Antibiotic sensitivity Test of EPEC isolates.

<table>
<thead>
<tr>
<th>NO of bacteria</th>
<th>SX T 25 mg/ml</th>
<th>CIP 5 mg/ml</th>
<th>C 10 mg/ml</th>
<th>E 15 mg/ml</th>
<th>AM 25 mg/ml</th>
<th>F 100/ mg/ml</th>
<th>AX 25 mg/ml</th>
<th>T 30 mg/ml</th>
<th>NA 30 mg/ml</th>
<th>B 10 mg/ml</th>
<th>CFM 5 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E2</td>
<td>I</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E3</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>T</td>
<td>I</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>E4</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E5</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E6</td>
<td>I</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>E7</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>E8</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>E9</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>E10</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

NO. number ,E. Escherichia coli, S. sensitive, R .resistant, I. intermediate

Mutation of Enteropathogenic Escherichia coli isolate

From total of (10) EPEC isolates, E1 were selected for mutation by UV irradiation. Results of mutation to Escherichia coli isolates (E1) reveals that exposure to UV irradiation from (1-5) mint leads to change the sensitivity of isolates to, amoxicillin, chloramphenicol, gentamicin and trimethoprim/sulphamethoxazole from (25,24,20,20)mm zone of inhibition respectively to resistant isolates which have zone of inhibition (10)mm or less while the same isolate reveal changes in results from resistant to sensitive to cefixime and bacitracin which have (22,20)mm zone of inhibition respectively.
Also results of mutation to E1 isolates reveals the same results for the antibiotic (ciprofloxacin, Erythromycin, ampicillin, nitrofurantion, and Naldixic acid as shown in table (1.3).

Radiation carries enough energy to remove electrons from molecules in a cell. When electrons are removed from molecules, ions called free radicals. Free radicals can damage most other molecules in a cell, such DNA or RNA, by oxidizing them (Evelyn, 2001). UV lead to excitation of electrons in DNA molecules often results in the formation of extra bonds between adjacent pyrimidines (specifically thymine) in DNA. When two pyrimidines are bound together in this way, it is called a pyrimidine dimer. These dimmers often change the shape of the DNA in the cell and can cause problems during replication (Mark ,2009).

Table (1.3): Antibiotic sensitivity test for mutated Enteropathogenic Escherichia coli E1 isolates .

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Code</th>
<th>Concentration</th>
<th>Zone of inhibition(mm)</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim/sulphamethoxazole</td>
<td>SXT</td>
<td>25mg/ml</td>
<td>20</td>
<td>17</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>5mg/ml</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>CN</td>
<td>10mg/ml</td>
<td>20</td>
<td>20</td>
<td>14</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>C</td>
<td>30mg/ml</td>
<td>24</td>
<td>20</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E</td>
<td>15mg/ml</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AM</td>
<td>25mg/ml</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nitrofurantion</td>
<td>F</td>
<td>100mg/ml</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>6</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>AX</td>
<td>25mg/ml</td>
<td>25</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>T</td>
<td>30mg/ml</td>
<td>18</td>
<td>12</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>NA</td>
<td>30mg/ml</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>B</td>
<td>10mg/ml</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Cefixime</td>
<td>CFM</td>
<td>5mg/ml</td>
<td>R</td>
<td>R</td>
<td>10</td>
<td>12</td>
<td>16</td>
<td>22</td>
</tr>
</tbody>
</table>

Also results of mutation to Escherichia coli isolates ( E1) reveals that exposure to UV irradiation from (1-5) mint leads to change the viability of E1 isolates from too numerous to count in control plate to (80x10^6), (50x10^6) in M1,M2 plates after exposure to UV light for 1,2 mint respectively as shown in figure (1.1). Results also reveals that increase exposure time to UV irradiation to (3,4,5)mint leads to decrease viable cell count to (25,14,2) x 10^6 CFU/ml in M3, M4, M5 plates respectively as shown in figure (1.2,1.3,1.4) respectively.
In the presence of a mutagen, the rate of mutation can increase dramatically. Mutagens can be in the form of a chemical, such as nicotine, or in the form of electromagnetic radiation (Kendric, 2010). On the other hand (Mark, 2000) showed that *Escherichia coli* reduced mutability in response to UV with increased time of exposure.

Certain mutations in *Escherichia coli* cause increased sensitivity to ultraviolet light (UV) dramatically, also changed the UV mutability of the sensitive strain producing induced mutations at smaller doses of UV. Also change viability in which its decrease with increased time (Ksh, 2000).

The percentage of the total surviving coliform population resistant to tetracycline or chloramphenicol was significantly higher than the percentage of the total coliform population resistant to those antibiotics before UV irradiation. This finding was attributed to the mechanism of R-factor mediated resistance to tetracycline (Bach et al., 2002).

**Figure (1.1):** *Enteropathogenic Escherichia coli* control isolates (left) and muted *enteropathogenic E1 isolates after (1) mint (right).*

**Figure (1.2):** Muted *Enteropathogenic Escherichia coli E1 isolates after (3) mint.*
Figure (1.3): Muted Enteropathogenic *Escherichia coli* E1 isolates after (4) mint.

Figure (1.4): Muted Enteropathogenic *Escherichia coli* E1 isolates after (5) mint.

REFERENCES


