

# The Study on the Survival of *Escherichia Coli* in Water at Room Temperature

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## ABSTRACT

*Escherichia coli* (*E. coli*) is one of the most frequent causes of many bacterial infections especially gastroenteritis in developing countries. It is also used as an indicator for faecal pollution in the surveillance of bacteriological quality of drinking water. This study was conducted to determine the survival of *E. coli* in water at room temperature (27°C). *E. coli* which is cultured in Lactose Peptone Broth was inoculated into 8 bottles each containing 10 millilitres of distilled water. They were kept at 27°C. Starting from the day 1, ten-fold dilutions were made from each bottle number and *E. coli* count was done from each dilutions by using pour plate method. The colony forming unit/ millilitre (CFU/ml) was calculated. The same procedure was carried out from bottles number 2 to 8 from day 2 to day 8 consecutively. CFU/ml of *E. coli* in dilution 10<sup>-5</sup> was markedly decreased from 3.9 x 10<sup>6</sup> in day 1 to 0 in day 8. The findings suggest that if the water is contaminated with low number of *E. coli*, it can be eliminated by keeping water at room temperature for only few days.

**Key words:** *E. coli*; water; room temperature

## INTRODUCTION

*Escherichia coli* (*E. coli*), is one of the most frequent causes of many bacterial infections, including gastroenteritis in developing countries. It is also an important cause of haemorrhagic colitis and haemolytic uremic syndrome in the United States<sup>1</sup>. It also causes urinary tract infection (UTI), neonatal meningitis, bacteraemia and intra-abdominal infections<sup>1</sup>. *E. coli* strains such as enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) produce potent toxins that cause serious gastrointestinal infections<sup>2</sup>. *E. coli* is also used as an indicator of faecal pollution in the microbiological surveillance of drinking water. Detection of *E. coli* indicates, necessary for an immediate investigation of the water supply system in order to identify and eliminate the source of pollution<sup>3</sup>.

*E. coli* is a common member of normal intestinal flora. These *Enterobacteriaceae* family live in the gut of warm blooded animals so they grow best in temperature same as body temperature<sup>4</sup>. *E. coli* is a gram-negative bacilli, facultative anaerobic and non-spore forming bacteria<sup>5</sup>. Coliforms can found in

the aquatic environment, in soil and on vegetation where they are universally present in large numbers in the faeces of warm-blooded animals. Typical genera include *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Serratia*, and faecal coliform such as *Escherichia*<sup>6</sup>. Unlike the general coliform group, *E. coli* are almost exclusively of faecal origin and their presence is thus an effective confirmation of faecal contamination<sup>7</sup>. *E. coli* counts in household drinking water may or may not reliably indicate the presence of diarrhoeagenic pathogens originating in faeces but the extent which a bacterial indicator like *E. coli* predict risks from all classes of pathogens<sup>8</sup>.

The aim of this study was to find out the duration of survival of *E. coli* in distilled water at room temperature and also to understand if *E. coli* can survive in unfavourable nutrient condition.

## **MATERIALS AND METHODS**

*E. coli* control strain (ATCC25922) was used for this study. First, identification of stock culture of *E. coli* strain was done to exclude any contamination. *E. coli* colonies grown on MacConkey agar (Oxoid, Malaysia) plate incubated 24 hours at 37°C incubator were inoculated into Lactose Peptone Broth (BD Difco, USA) and incubated 24 hours at 37°C incubator. The next day 5 µl of Lactose Peptone Broth in which *E. coli* were grown overnight was added into each of 8 bijou bottles that contained 10 millilitres of sterile distilled water and kept at room temperature. The bottles were labelled as bottle number 1 to 8. In day one serial 10 fold dilution were made from bottle number 1 up to 10<sup>-5</sup> dilution, using distilled water. One ml from each dilution was added onto Plate Count agar (Oxoid, Malaysia) using pour plate method and incubated at 37°C incubator for 24 hours. The plate count was made in the next day from agar plates which contained 1-300 colonies or colony forming units (CFU). The agar plate with 30-300 CFU are usually selected for the plate count method<sup>9</sup>. CFU were multiply by dilution factor to know the CFU/ml on the respective agar plate and the data was recorded. The same procedure was done on the consecutive days daily from bottle number 2 to bottle number 8 from day 2 to day 8 and CFU/ml were noted.

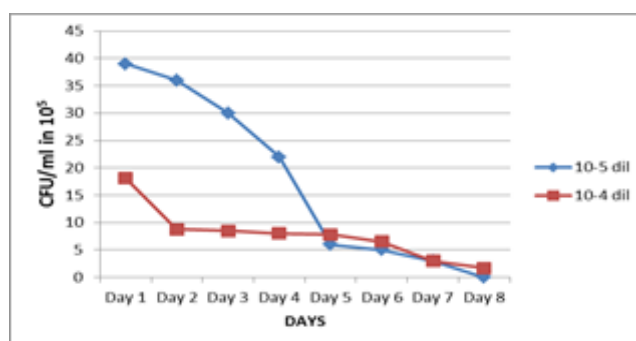
## **RESULTS**

Colony count with more than 300 CFU on the plates of dilution 1: 10, and 1: 100 in all the eight bottles was not considered. But in dilution 10<sup>-3</sup> of 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> bottles at 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> days of reading, the CFU decreased to 272, 181 and 168 respectively. It was found that the CFU at dilution 10<sup>-4</sup> decreased markedly from day 1 to day 8 from 181 to 17 and also CFU at dilution 10<sup>-5</sup> decreased from 39 to 0 from day 1 to day 8 respectively (Table 1).

**Table 1:** Colony forming unit (CFU) of *E. coli* on plate count agar in tenfold dilutions from day 1 to 8

Dilution Days	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>
1 (Bottle No.1)	>300	>300	>300	181	39
2 (Bottle No.2)	>300	>300	>300	88	36
3 (Bottle No.3)	>300	>300	>300	85	30
4 (Bottle No.4)	>300	>300	>300	80	22
5 (Bottle No.5)	>300	>300	>300	78	6
6 (Bottle No.6)	>300	>300	272	65	5
7 (Bottle No.7)	>300	>300	181	30	3
8 (Bottle No.8)	>300	>300	168	17	-

The CFU/ml of dilution 10<sup>-4</sup> in day 1, 2, 3, 4, 5, 6, 7, and 8 was 1.81 x10<sup>6</sup>, 8.8 x10<sup>5</sup>, 8.5 x10<sup>5</sup>, 8.0 x10<sup>5</sup>, 7.8 x10<sup>5</sup>, 6.5 x10<sup>5</sup>, and 3.0 x10<sup>5</sup> and 1.7 x10<sup>5</sup> CFU/ml respectively. Similarly CFU/ml of dilution 10<sup>-5</sup> in day 1, 2, 3, 4, 5, 6, 7, and 8 was 3.9 x10<sup>6</sup>, 3.6 x10<sup>6</sup>, 3.0 x10<sup>6</sup>, 2.2 x10<sup>6</sup>, 6 x10<sup>5</sup>, 5 x10<sup>5</sup>, and 3.0 x10<sup>5</sup> and zero CFU/ml respectively. The graphs show that CFU/ml decreased constantly from day 1 to day 8 in both dilutions (Figure 1).



**Figure 1:** The graph showing decline in average CFU/ml of dilution 10<sup>-4</sup> and 10<sup>-5</sup> from day 1 to 8.

There were marked differences between CFU/ml of dilution 10<sup>-4</sup> in day 1 and day 8, which were 1.81 x 10<sup>6</sup> and 1.7 x 10<sup>5</sup> respectively. Likewise CFU/ml for day 1 and day 7 of dilution 10<sup>-5</sup> showed markedly decrease from 3.9 x 10<sup>6</sup> to 3 x10<sup>5</sup> and even CFU/ml of day 8 was noted as zero.

## DISCUSSION

The survival of bacteria depends on a number of environmental factors such as moisture and holding capacity, temperature, pH, presence of oxygen and nutrients, the availability of organic matter and

antagonism from micro-flora. Although many factors have been identified, it appears that many factors are still unknown<sup>10</sup>.

Persistence and survival of bacteria in water has been linked to water temperature. In this study, it was found that *E. coli* could survive in room temperature, 27°C for up to 8 days and may be more. Several studies reported that cooler water temperatures can increase the ability of *E. coli* to survive in a variety of aquatic conditions. According to Wcislo & Chrost (2000), most of the studies stated that the survival of *E. coli* in water is enhanced at lower temperature<sup>11</sup>. Bogosian et al (1996) stated that in non-sterile river water, *E. coli* was able to survive for up to 6 days at 37°C, for 8 days at 20°C, and for 12 days at 4°C<sup>12</sup>. In the study by Sampson et al (2006), *E. coli* could survive for up to 30 days in non-sterile lake water without sand and up to 40 days in the presence of sand at 48°C<sup>10</sup>.

In our study it was shown that *E. coli* can survive at least 8 days even in unfavourable nutrient condition in the sterile water. Flint (1987) stated that survival times were less in water which was only filtered through either a Whatman filter paper or a 0.45 micron Millipore filter or in untreated water, suggesting that competition with the natural microbial flora of the water was the primary factor in the disappearance of the introduced bacteria<sup>13</sup>. Wcislo & Chrost (2000) study found that better nutritional condition and the presence of native heterotrophic microflora significantly prolonged the survival time of *E. coli* in the studied environment<sup>11</sup>.

In our method 5 µl of *E. coli* was added into 10ml of sterile water to prepare an initial suspension. It is known that cell density and access to nutrients have significant effects on the growth rate of *E. coli*<sup>14</sup>. The variation in survival rates of *E. coli* among different studies might be due to difference in inoculum dose.

Better results could be obtained if the plate counts were performed in triplicate to control against the inherent variability in growth assays. The current experiment was carried out for only 8 days due to limited time and resources. Further studies should be considered at different temperatures eg. 4°C, 27°C, 37°C and 44°C and for longer period. This study used *E. coli* control strain ATCC25922 as a test organism and it will be better to use *E. coli* isolated from the field for future studies to simulate the real situation.

In conclusion, *E. coli* survive in water at least up to 8 days in room temperature. But CFU/ml of *E. coli* markedly decreased from day 1 to day 8. Thus keeping contaminated water in the water container at room temperature for few days can eliminate *E. coli* if level of contamination is low.

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**CONFLICT OF INTEREST:** None.

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