

BACTERIOLOGICAL ANALYSIS AND ISOLATION OF ESCHERICHIA COLI OF LOCAL WATER SUPPLY TANKERS IN DIYALA PROVINCE

**Seminar submitted to the college council of veterinary medicine in partial fulfillment of the requirement of bachelor degree in veterinary medicine and surgery**

**By**

**Sura shaker Mahmoud**

**Supervised by**

**Assistant lecture**

**Samer Raad Abdul Hussain**

**List of contents**

**Contents pages**

**1- Abstract………………………………………………………….4**

**2-introduction……………………………………………………...6**

**3- Literature review……………………………………………..8-17**

**4- Material and methods……………………………………..…19-23**

**5- Result………………………………………………………….25-26**

**6- Discussion ……………………………………………………...28**

**7- Conclusion and recommendation…………………………......29**

**8- References………………………………………………….….30-39**

**9- Arabic abstract …………………………………………...……40**

**LIST OF PICTURES AND PHOTOS**

**ITEM PAGE**

**1- Figure 1, 2 showing the special sterile screw capped collecting bottle……………………………………………………………………... 20**

**2- Figure 3, 4 showing the actual bacterial growth on nutrient agar... 21**

**3- Figure (5): coliform growth on MacConkey agar……………….......22**

**4- Figure 6: gram staining of positive coliform culture………………..23**

**5- Figure 7: conformation gram staining showing *E coli* bacteria…….23**

**LIST OF TABELS**

**TITLES PAGE**

**1- Table (1): determination of total bacterial viable count…..……..25**

**2- Table (2): differential coliform test…………...…………………..26**

**Abstract**

The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. It is important to note that *E. coli* and waste can get in our water in many different ways.

In this study the Presence of *Escherichia coli* in water supplied by water tanker suppliers in some cities of Diyala province was determined. Out of 15 different water supply tanker examined which were designated alphabetically as A, B, C, D, E, F, and O. Escherichia coli was isolated from five samples. The viable count showed numbers of bacteria present in the sample were seen growing in the nutrient agar. The pink colonies on MacConkey plates were isolated and subculture are made in fresh agar plates incubated at 37c for 24-hrs. The pure cultures were then identified using gram stain and biochemical tests.

**Chapter one**

**Introduction**

**1-Introduction**

Safe water is one of the most important felt needs in public health in the twenty first century [1]. Visually clear and colorless drinking water is acceptable. However, it should also be safe and free from chemical toxin and pathogenic microorganism [2]. *Escherichia coli* are widely distributed in the gastro-intestine tract of humans, pests, ruminants, non-ruminants and wild animal, where they are known to live as commensals [3] [4]. They are gram negative facultative anaerobic bacteria [3] [5]. They are also from the family *Enterobacteriaceae* and ferments glucose or lactose [3] [6]. Although most E. coli live in commensalism with their host, pathogenic E. coli strains exit and normally cause hemolytic uremic syndrome that can be fatal [3][ 5].

Escherichia coli have been isolated from humans, farm animals, wild animals, milk, water and environmental samples some of which have been responsible for foodborne illnesses and deaths [7][8][16]. Through poor processing and handling of foods or farm animals *E. coli* can cross contaminate a variety of sources including drinking water. Humans and farm animals can get *E. coli* infection by drinking water from such sources.

No work has been done on the occurrence of *E. coli* in water supply vehicle in poor water supply regions. Therefore, this work was carried out to find out whether E. coli is present or absent in that kind of drinking water sources for humans and farm animals in some regions of Diyala province.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2-Literature review**

***Escherichia coli* 2.1**

Escherichia coli (also known as *E. coli*) is a [gram-negative](https://en.wikipedia.org/wiki/Gram-negative), [facultatively anaerobic](https://en.wikipedia.org/wiki/Facultative_anaerobic_organism), [rod-shaped](https://en.wikipedia.org/wiki/Bacillus_(shape)), [coliform bacterium](https://en.wikipedia.org/wiki/Coliform_bacteria) of the [genus](https://en.wikipedia.org/wiki/Genus) [*Escherichia*](https://en.wikipedia.org/wiki/Escherichia) that is commonly found in the lower [intestine](https://en.wikipedia.org/wiki/Intestine) of [warm-blooded](https://en.wikipedia.org/wiki/Warm-blooded) organisms (endotherms).[[17]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-2)[[18]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Singleton-3) Most *E. coli* [strains](https://en.wikipedia.org/wiki/Strain_(biology)) are harmless, but some [serotypes](https://en.wikipedia.org/wiki/Serotype) can cause serious [food poisoning](https://en.wikipedia.org/wiki/Foodborne_illness) in their hosts, and are occasionally responsible for [product recalls](https://en.wikipedia.org/wiki/Product_recall) due to [food contamination](https://en.wikipedia.org/wiki/Food_contamination).[[19]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-CDC-4)[[20]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Vogt-5) The harmless strains are part of the [normal flora](https://en.wikipedia.org/wiki/Human_flora) of the [gut](https://en.wikipedia.org/wiki/Gut_(zoology)), and can benefit their hosts by producing [vitamin K2](https://en.wikipedia.org/wiki/Vitamin_k),[[21]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Bentley-6) and preventing colonization of the intestine with [pathogenic bacteria](https://en.wikipedia.org/wiki/Pathogenic_bacteria).[[22]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Hudault-7) [[23]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Reid-8) E. coli is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.[[24]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Russell2001-9)

E. coli and other facultative [anaerobes](https://en.wikipedia.org/wiki/Anaerobic_organism) constitute about 0.1% of [gut flora](https://en.wikipedia.org/wiki/Gut_flora),[[25]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid15831718-10) and [fecal–oral transmission](https://en.wikipedia.org/wiki/Fecal%E2%80%93oral_route) is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential [indicator organisms](https://en.wikipedia.org/wiki/Indicator_organism) to test environmental samples for [fecal contamination](https://en.wikipedia.org/wiki/Feces).[[26]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Feng_2002-11) [[27]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Thompson-12) A growing body of research, though, has examined environmentally persistent E. coli which can survive for extended periods outside of a host.[[28]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid21558695-13)

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is a [chemoheterotrophic](https://en.wikipedia.org/wiki/Chemotroph#Chemoheterotroph) whose chemically defined medium must include a source of carbon and energy.[[29]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-:0-14) *E. coli* is the most widely studied [prokaryotic](https://en.wikipedia.org/wiki/Prokaryote) [model organism](https://en.wikipedia.org/wiki/Model_organism), and an important species in the fields of [biotechnology](https://en.wikipedia.org/wiki/Biotechnology) and [microbiology](https://en.wikipedia.org/wiki/Microbiology), where it has served as the [host organism](https://en.wikipedia.org/wiki/Host_organism) for the majority of work with [recombinant DNA](https://en.wikipedia.org/wiki/Recombinant_DNA). Under favorable conditions, it takes only 20 minutes to reproduce.[[30]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-15)

**2.2 Biology and biochemistry**

**2.2.1Type and morphology**

*E. coli* is a Gram-negative, facultative anaerobic (that makes [ATP](https://en.wikipedia.org/wiki/Adenosine_triphosphate) by [aerobic respiration](https://en.wikipedia.org/wiki/Aerobic_respiration) if [oxygen](https://en.wikipedia.org/wiki/Oxygen) is present, but is capable of switching to [fermentation](https://en.wikipedia.org/wiki/Fermentation_(biochemistry)) or [anaerobic respiration](https://en.wikipedia.org/wiki/Anaerobic_respiration) if oxygen is absent) and [nonsporulating](https://en.wikipedia.org/wiki/Endospore) bacterium.[[31]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-16) Cells are typically rod-shaped, and are about 2.0 [μm](https://en.wikipedia.org/wiki/Micrometers) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm3.[[32]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-17) [[33]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid24287933-18) [[34]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-19)

*E. coli* stains Gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, E. coli picks up the color of the counterstain [safranin](https://en.wikipedia.org/wiki/Safranin) and stains pink. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that E. coli is not damaged by penicillin.[[29]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-:0-14)

Strains that possess [flagella](https://en.wikipedia.org/wiki/Flagellum) are [motile](https://en.wikipedia.org/wiki/Motility). The flagella have a [peritrichous](https://en.wikipedia.org/wiki/Flagellum#Flagellar_arrangement_schemes) arrangement.[[35]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid17189361-20)

**2.2.2. Culture growth**

Optimum growth of E. coli occurs at 37 °C (98.6 °F), but some laboratory strains can multiply at temperatures up to 49 °C (120 °F).[[36]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-22) *E. coli* grows in a variety of defined laboratory media, such as [lysogeny broth](https://en.wikipedia.org/wiki/Lysogeny_broth), or any medium that contains glucose, ammonium phosphate, monobasic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water. Growth can be driven by [aerobic](https://en.wikipedia.org/wiki/Aerobic_respiration) or [anaerobic respiration](https://en.wikipedia.org/wiki/Anaerobic_respiration), using a large variety of [redox pairs](https://en.wikipedia.org/wiki/Redox), including the oxidation of [pyruvic acid](https://en.wikipedia.org/wiki/Pyruvic_acid), [formic acid](https://en.wikipedia.org/wiki/Formic_acid), [hydrogen](https://en.wikipedia.org/wiki/Hydrogen), and [amino acids](https://en.wikipedia.org/wiki/Amino_acid), and the reduction of substrates such as [oxygen](https://en.wikipedia.org/wiki/Oxygen), [nitrate](https://en.wikipedia.org/wiki/Nitrate), [fumarate](https://en.wikipedia.org/wiki/Fumarate), [dimethyl sulfoxide](https://en.wikipedia.org/wiki/Dimethyl_sulfoxide), and [trimethylamine N-oxide](https://en.wikipedia.org/wiki/Trimethylamine_N-oxide).[[37]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Ingledew-23) *E. coli* is classified as a facultative anaerobe. It uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates.[[29]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-:0-14)

**2.3Diversity**

*E. coli* encompasses an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its medical importance, [[31]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-30) and *E. coli* remains one of the most diverse bacterial species: only 20% of the genes in a typical *E. coli* genome is shared among all strains.[[32]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-comparison-31)

In fact, from the evolutionary point of view, the members of genus *Shigella (S. dysenteriae, S. flexneri, S. boydii, and S. sonnei*) should be classified as *E. coli* strains, a phenomenon termed [taxa in disguise](https://en.wikipedia.org/wiki/Taxa_in_disguise).[[33]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid12361912-32) Similarly, other strains of E. coli (e.g. the [K-12](https://en.wikipedia.org/wiki/E._coli_K-12) strain commonly used in [recombinant DNA](https://en.wikipedia.org/wiki/Recombinant_DNA) work) are sufficiently different that they would merit reclassification.

A [strain](https://en.wikipedia.org/wiki/Strain_(biology)) is a subgroup within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain [pathogenic capacity](https://en.wikipedia.org/wiki/Pathogenicity), the ability to use a unique carbon source, the ability to take upon a particular [ecological niche](https://en.wikipedia.org/wiki/Ecological_niche), or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of fecal contamination in environmental samples.[[26]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Feng_2002-11) [[27]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Thompson-12) For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another [mammal](https://en.wikipedia.org/wiki/Mammal), or a [bird](https://en.wikipedia.org/wiki/Bird)

**2.3.1Serotypes**

A common subdivision system of *E. coli*, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of [lipopolysaccharide](https://en.wikipedia.org/wiki/Lipopolysaccharide) layer; H: [flagellin](https://en.wikipedia.org/wiki/Flagellin); K antigen: capsule), e.g. O157:H7).[[41]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid334154-33) It is, however, common to cite only the serogroup, i.e. the O-antigen. At present, about 190 serogroups are known.[[42]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-34)

**2.4 Normal microbiota**

*E. coli* belongs to a group of bacteria informally known as [coliforms](https://en.wikipedia.org/wiki/Coliforms) that are found in the gastrointestinal tract of [warm-blooded animals](https://en.wikipedia.org/wiki/Warm-blooded_animals).[[43]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Bergey2B-41) *E. coli* normally colonizes an infant's [gastrointestinal tract](https://en.wikipedia.org/wiki/Gastrointestinal_tract) within 40 hours of birth, arriving with food or water or from the individuals handling the child. In the bowel, *E. coli* adheres to the [mucus](https://en.wikipedia.org/wiki/Mucus) of the [large intestine](https://en.wikipedia.org/wiki/Large_intestine). It is the primary [facultative anaerobe](https://en.wikipedia.org/wiki/Facultative_anaerobic_organism) of the human gastrointestinal tract.[[44]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Todar-60) ([Facultative anaerobes](https://en.wikipedia.org/wiki/Facultative_anaerobic_organism) are organisms that can grow in either the presence or absence of oxygen.) As long as these bacteria do not acquire [genetic elements](https://en.wikipedia.org/wiki/Bacteriophage) encoding for [virulence factors](https://en.wikipedia.org/wiki/Virulence_factor), they remain benign [commensals](https://en.wikipedia.org/wiki/Commensalism).[[45]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Evans-61)

**2.4.1Therapeutic use**

Nonpathogenic E. coli strain Nissle 1917, also known as [Mutaflor](https://en.wikipedia.org/wiki/Mutaflor), and *E. coli* O83:K24:H31 (known as Colinfant[[46]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-62)) are used as [probiotic](https://en.wikipedia.org/wiki/Probiotic) agents in medicine, mainly for the treatment of various gastroenterological diseases,[[47]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid15292145-63) including [inflammatory bowel disease](https://en.wikipedia.org/wiki/Inflammatory_bowel_disease).[[48]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid15867585-64)

**2.5 Role in disease**

Most *E. coli* strains do not cause disease,[[49]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-65) but virulent strains can cause [gastroenteritis](https://en.wikipedia.org/wiki/Gastroenteritis), [urinary tract infections](https://en.wikipedia.org/wiki/Urinary_tract_infection), [neonatal](https://en.wikipedia.org/wiki/Neonatal) [meningitis](https://en.wikipedia.org/wiki/Meningitis), hemorrhagic colitis, and [Crohn's disease](https://en.wikipedia.org/wiki/Crohn%27s_disease). Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to [hemolytic-uremic syndrome](https://en.wikipedia.org/wiki/Hemolytic-uremic_syndrome), [peritonitis](https://en.wikipedia.org/wiki/Peritonitis), [mastitis](https://en.wikipedia.org/wiki/Mastitis), [septicemia](https://en.wikipedia.org/wiki/Septicemia), and gram-negative [pneumonia](https://en.wikipedia.org/wiki/Pneumonia). Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome; however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected *with E. coli*.[[44]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Todar-60) [[50]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-66)

There is one strain, E. coli 0157:H7, that produces the [Shiga toxin](https://en.wikipedia.org/wiki/Shiga_toxin) (classified as a bioterrorism agent). This toxin causes premature destruction of the red blood cells, which then clog the body's filtering system, the kidneys, causing hemolytic-uremic syndrome (HUS). Signs of hemolytic uremic syndrome include decreased frequency of urination, lethargy, and paleness of cheeks and inside the lower eyelids. In 25% of HUS patients, complications of nervous system occur, which in turn causes [strokes](https://en.wikipedia.org/wiki/Stroke) due to small clots of blood which lodge in capillaries in the brain. This causes the body parts controlled by this region of the brain not to work properly. In addition, this strain causes the buildup of fluid (since the kidneys do not work), leading to edema around the lungs and legs and arms. This increase in fluid buildup especially around the lungs impedes the functioning of the heart, causing an increase in blood pressure.[[51]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-69) [[52]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-70) [[53]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-71) [[54]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-72)

Uropathogenic *E. coli* (UPEC) is one of the main causes of [urinary tract infections](https://en.wikipedia.org/wiki/Urinary_tract_infection).[[50]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pre-eminent-73) It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce UPEC to the female urogenital system.[[55]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pre-eminent-73) For more information, see the databases at the end of the article or [UPEC pathogenicity](https://en.wikipedia.org/wiki/Pathogenic_Escherichia_coli#Urinary_tract_infection).

In May 2011, one *E. coli* strain, [O104:H4](https://en.wikipedia.org/wiki/E._coli_O104:H4), was the subject of a [bacterial outbreak](https://en.wikipedia.org/wiki/2011_E._coli_O104:H4_outbreak) that began in [Germany](https://en.wikipedia.org/wiki/Germany). Certain strains of *E. coli* are a major cause of [foodborne illness](https://en.wikipedia.org/wiki/Foodborne_illness). The outbreak started when several people in Germany were infected with [enterohemorrhagic E. coli (EHEC)](https://en.wikipedia.org/wiki/Enterohemorrhagic) bacteria, leading to hemolytic-uremic syndrome (HUS), a medical emergency that requires urgent treatment. The outbreak did not only concern Germany, but also 11 other countries, including regions in North America.[[citation needed](https://en.wikipedia.org/wiki/Wikipedia:Citation_needed)] On 30 June 2011, the German Bundesinstitut für Risikobewertung (BfR) (Federal Institute for Risk Assessment, a federal institute within the German [Federal Ministry of Food, Agriculture and Consumer Protection](https://en.wikipedia.org/wiki/Federal_Ministry_of_Food,_Agriculture_and_Consumer_Protection)) announced that seeds of [fenugreek](https://en.wikipedia.org/wiki/Fenugreek) from [Egypt](https://en.wikipedia.org/wiki/Egypt) were likely the cause of the EHEC outbreak.[[56]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-74)

**2.5.1 Incubation period**

The time between ingesting the STEC bacteria and feeling sick is called the "incubation period". The incubation period is usually 3–4 days after the exposure, but may be as short as 1 day or as long as 10 days. The symptoms often begin slowly with mild belly pain or non-bloody diarrhea that worsens over several days. HUS, if it occurs, develops an average 7 days after the first symptoms, when the diarrhea is improving.[[57]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-75)

**2.5.2 Treatment**

The mainstay of treatment is the assessment of [dehydration](https://en.wikipedia.org/wiki/Dehydration) and replacement of fluid and electrolytes. Administration of [antibiotics](https://en.wikipedia.org/wiki/Antibiotics) has been shown to shorten the course of illness and duration of excretion of enterotoxigenic *E. coli* (ETEC) in adults in endemic areas and in traveler's diarrhea, though the rate of resistance to commonly used antibiotics is increasing and they are generally not recommended.[[58]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-76) The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are [fluoroquinolones](https://en.wikipedia.org/wiki/Fluoroquinolone) or [azithromycin](https://en.wikipedia.org/wiki/Azithromycin), with an emerging role for [rifaximin](https://en.wikipedia.org/wiki/Rifaximin). [Oral rifaximin](https://en.wikipedia.org/w/index.php?title=Oral_rifaximin&action=edit&redlink=1), a semisynthetic rifamycin derivative, is an effective and well-tolerated antibacterial for the management of adults with non-invasive traveler's diarrhea. Rifaximin was significantly more effective than placebo and no less effective than ciprofloxacin in reducing the duration of diarrhea. While rifaximin is effective in patients with E. coli-predominant traveler's diarrhea, it appears ineffective in patients infected with inflammatory or invasive [enteropathogens](https://en.wikipedia.org/wiki/Enteropathogen).[[59]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-77)

**2.6 Bacteriological water analysis**

Bacteriological water analysis is a method of analyzing water to estimate the numbers of [bacteria](https://en.wikipedia.org/wiki/Bacteria) present and, if needed, to find out what sort of bacteria they are. It represents one aspect of [water quality](https://en.wikipedia.org/wiki/Water_quality). It is a [microbiological](https://en.wikipedia.org/wiki/Microbiology) [analytical](https://en.wikipedia.org/wiki/Analysis) procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations. This process is used, for example, to routinely confirm that water is safe for human consumption or that bathing and [recreational](https://en.wikipedia.org/wiki/Recreation) waters are safe to use.

The interpretation and the action trigger levels for different waters vary depending on the use made of the water. Whilst very stringent levels apply to [drinking water](https://en.wikipedia.org/wiki/Drinking_water), more relaxed levels apply to marine bathing waters, where much lower volumes of water are expected to be ingested by users.

**2.6.1 Methodologies**

The most reliable methods are direct plate count method and membrane filtration method.mEndo Agar is used in the membrane filtration while VRBA Agar is used in the direct plate count method.VRBA stands for violet red bile agar. A media that contain bile salts which promote the growth of gram negative and has inhibitory characteristic to gram positive although not complete inhibitory. These media contain lactose which is usually fermented by lactose fermenting bacteria producing colonies that can be identified and characterized. Lactose fermenting produce colored colonies while non lactose fermenting produce colorless ones.

Because the analysis is always based on a very small sample taken from a very large volume of water, all methods rely on statistical principles.

**2.6.2 Multiple tube method**

One of the oldest methods is called the multiple tube method.[[60]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-2) In this method a measured sub-sample (perhaps 10 ml) is diluted with 100 ml of sterile growth medium and an [aliquot](https://en.wikipedia.org/wiki/Chemistry) of 10 ml is then decanted into each of ten tubes. The remaining 10 ml is then diluted again and the process repeated. At the end of 5 dilutions this produces 50 tubes covering the dilution range of 1:10 through to 1:10000.

The tubes are then incubated at a pre-set temperature for a specified time and at the end of the process the number of tubes with growth in is counted for each dilution. Statistical tables are then used to derive the concentration of organisms in the original sample. This method can be enhanced by using indicator medium which changes color when acid forming species are present and by including a tiny inverted tube called a Durham tube in each sample tube. The Durham inverted tube catches any gas produced. The production of gas at 37 degrees Celsius is a strong indication of the presence *of* [*Escherichia coli*](https://en.wikipedia.org/wiki/Escherichia_coli_(molecular_biology)) [60].

**2.6.3 ATP Testing**

An [ATP test](https://en.wikipedia.org/wiki/ATP_test) is the process of rapidly measuring active microorganisms in water through detection [adenosine triphosphate](https://en.wikipedia.org/wiki/Adenosine_triphosphate) (ATP). ATP is a molecule found only in and around living cells, and as such it gives a direct measure of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally occurring enzyme [firefly luciferase](https://en.wikipedia.org/wiki/Firefly_luciferase) using an [illuminometer](https://en.wikipedia.org/wiki/Luminometer). The amount of light produced is directly proportional to the amount of biological energy present in the sample.

Second generation ATP tests are specifically designed for water, [wastewater](https://en.wikipedia.org/wiki/Wastewater) and industrial applications where, for the most part, samples contain a variety of components that can interfere with the ATP assay[60].

**2.6.4 Plate count**

The plate count method relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted. To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of the target bacterium is grown. Fewer than 30 colonies makes the interpretation statistically unsound whilst greater than 300 colonies often results in overlapping colonies and imprecision in the count. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured. This approach is widely utilized for the evaluation of the effectiveness of water treatment by the inactivation of representative microbial contaminants such as *E. coli* following ASTM D5465.[[61]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-3) [[62]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-4)

The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000, etc.) in sterile water and cultivating these on [nutrient](https://en.wikipedia.org/wiki/Nutrient) agar in a dish that is sealed and incubated. Typical media include [plate count agar](https://en.wikipedia.org/wiki/Plate_count_agar) for a general count or [MacConkey agar](https://en.wikipedia.org/wiki/MacConkey_agar) to count [Gram-negative bacteria](https://en.wikipedia.org/wiki/Gram-negative_bacteria) such as E. coli. Typically one set of plates is incubated at 22°C and for 24 hours and a second set at 37°C for 24 hours. The composition of the nutrient usually includes [reagents](https://en.wikipedia.org/wiki/Reagent) that resist the growth of non-target organisms and make the target organism easily identified, often by a color change in the medium. Some recent methods include a fluorescent agent so that counting of the colonies can be automated. At the end of the incubation period the colonies are counted by eye, a procedure that takes a few moments and does not require a [microscope](https://en.wikipedia.org/wiki/Microscope) as the colonies are typically a few millimeters across.

**2.6.5 Membrane filtration**

Most modern laboratories use a refinement of total plate count in which serial dilutions of the sample are vacuum filtered through purpose made [membrane filters](https://en.wikipedia.org/wiki/Membrane_filter) and these filters are themselves laid on nutrient medium within sealed plates.[[63]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-5) The methodology is otherwise similar to conventional total plate counts. Membranes have a printed millimeter grid printed on and can be reliably used to count the number of colonies under a binocular microscope.

**2.6.6 Pour plate method**

When the analysis is looking for bacterial species that grow poorly in air, the initial analysis is done by mixing serial dilutions of the sample in liquid nutrient agar which is then poured into bottles which are then sealed and laid on their sides to produce a sloping agar surface. Colonies that develop in the body of the medium can be counted by eye after incubation.

The total number of colonies is referred to as the [Total Viable Count](https://en.wikipedia.org/wiki/Total_Viable_Count) (TVC). The unit of measurement is cfu/ml (or colony forming units per milliliter) and relates to the original sample. Calculation of this is a multiple of the counted number of colonies multiplied by the dilution used.

**2.7 Pathogen analysis**

When samples show elevated levels of indicator bacteria, further analysis is often undertaken to look for specific pathogenic bacteria. Species commonly investigated in the temperate zone include [Salmonella typhi](https://en.wikipedia.org/wiki/Salmonella_typhi) and [Salmonella Typhimurium](https://en.wikipedia.org/wiki/Salmonella_Typhimurium). Depending on the likely source of contamination investigation may also extend to organisms such as [Cryptosporidium](https://en.wikipedia.org/wiki/Cryptosporidium) spp. In tropical areas analysis of [Vibrio cholerae](https://en.wikipedia.org/wiki/Vibrio_cholerae) is also routinely undertaken.

**2.7.1 Types of nutrient media used in analysis**

[MacConkey agar](https://en.wikipedia.org/wiki/MacConkey_agar) is culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), [lactose](https://en.wikipedia.org/wiki/Lactose) and [peptone](https://en.wikipedia.org/wiki/Peptone). Alfred Theodore MacConkey developed it while working as a bacteriologist for the Royal Commission on Sewage Disposal in the [United Kingdom](https://en.wikipedia.org/wiki/United_Kingdom).

[Endo agar](https://en.wikipedia.org/wiki/Endo_agar) contains peptone, lactose, [dipotassium phosphate](https://en.wikipedia.org/wiki/Dipotassium_phosphate), agar, sodium sulfite, basic [fuchsin](https://en.wikipedia.org/wiki/Fuchsin) and was originally developed for the isolation of Salmonella typhi, but is now commonly used in water analysis. As in MacConkey agar, coliform organisms ferment the lactose, and the colonies become red. Non-lactose-fermenting organisms produce clear, colorless colonies against the faint pink background of the medium.[[64]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-6)

mFC medium is used in membrane filtration and contains selective and differential agents. These include [rosolic acid](https://en.wikipedia.org/wiki/Rosolic_acid) to inhibit bacterial growth in general, except for faecal coliforms, [bile](https://en.wikipedia.org/wiki/Bile) salts inhibit non-enteric bacteria and [aniline blue](https://en.wikipedia.org/wiki/Aniline_blue) indicates the ability of faecal coliforms to ferment lactose to acid that causes a pH change in the medium.[[65]](https://en.wikipedia.org/wiki/Bacteriological_water_analysis#cite_note-7)

TYEA medium contains [tryptone](https://en.wikipedia.org/wiki/Tryptone), [yeast extract](https://en.wikipedia.org/wiki/Yeast_extract), common salt and [L-arabinose](https://en.wikipedia.org/wiki/L-arabinose) per liter of glass distilled water and is a non-selective medium usually cultivated at two temperatures (22 and 36°C) to determine a general level of contamination (a.k.a. colony count).

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3. Materials and methods**

**3.1 MATERIALS**

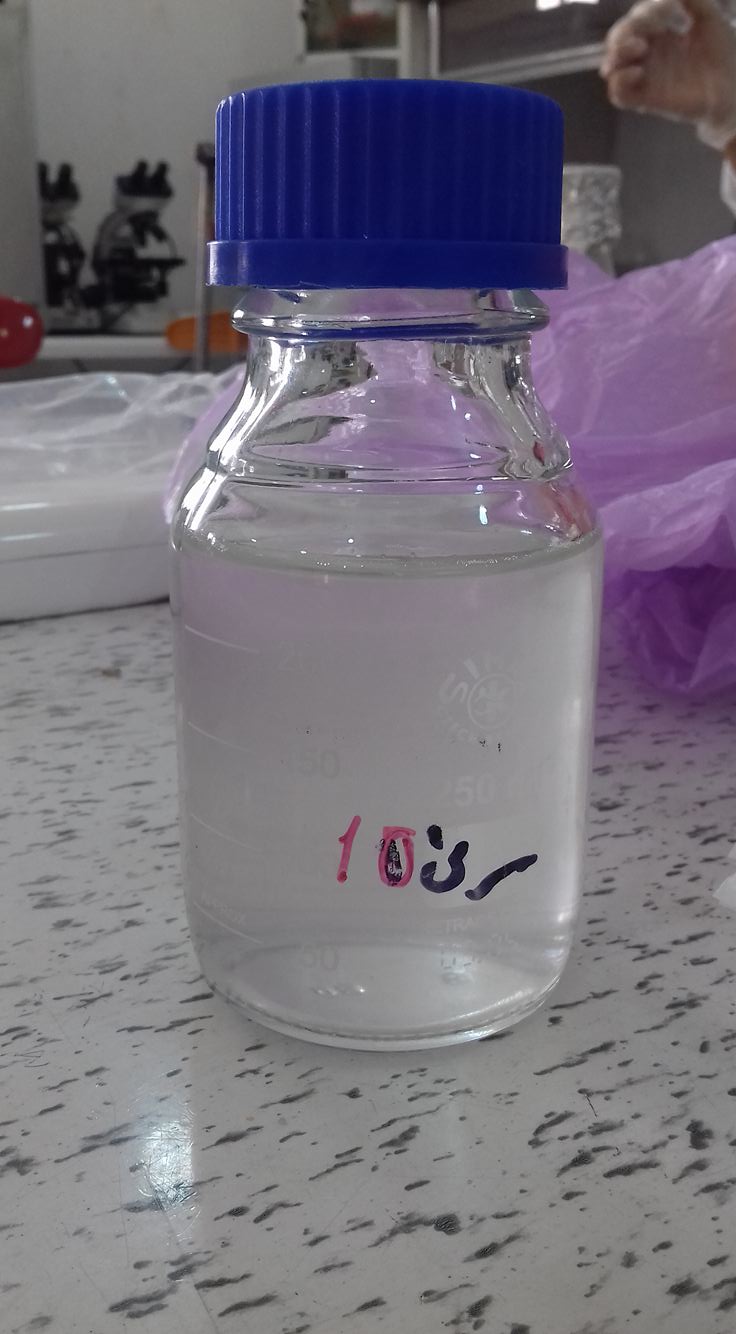
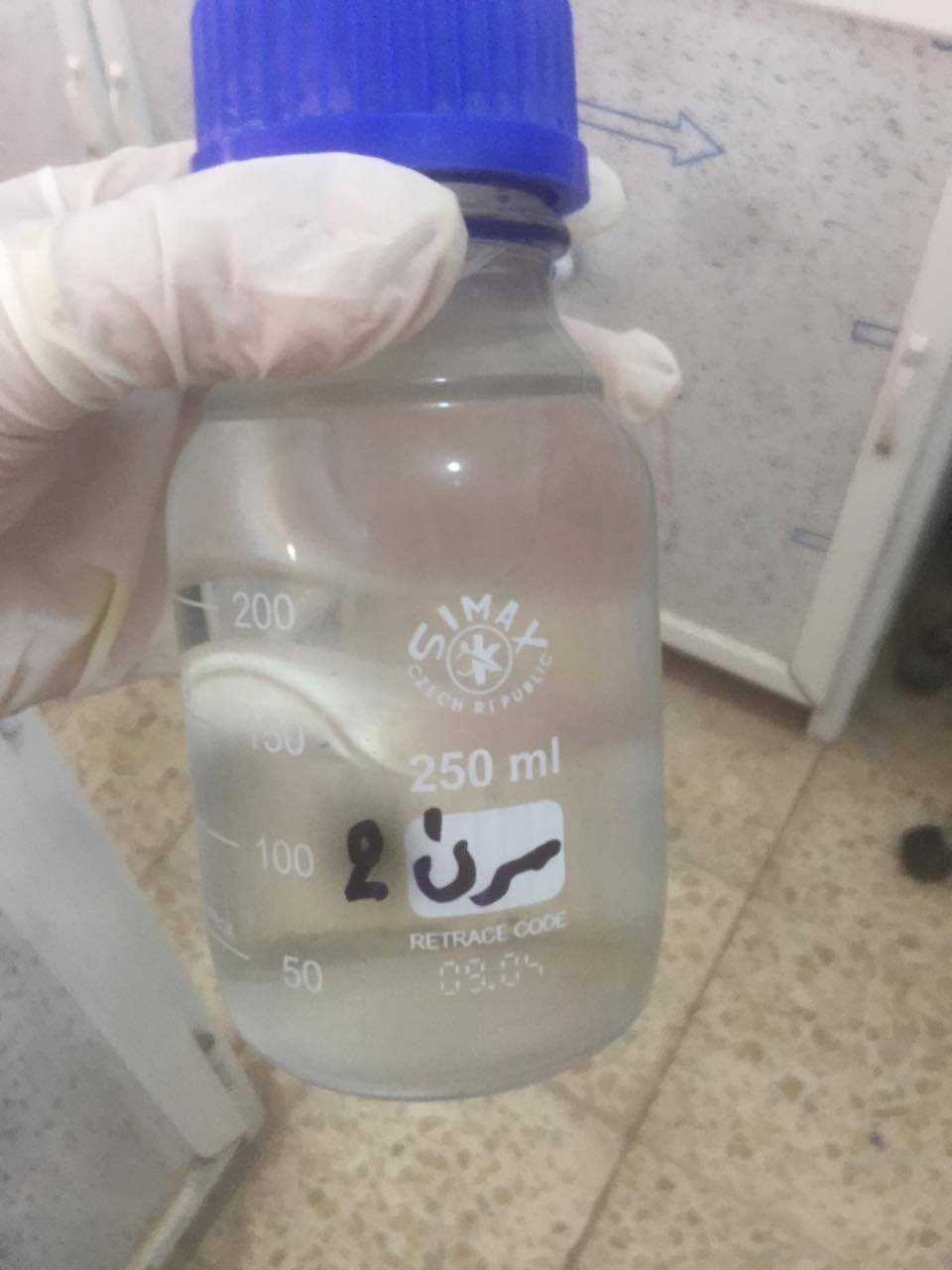
The following materials were used in this study

1. Set of 250 ml container for sampling
2. Test tube
3. Distilled water
4. Disposable petri dishes
5. Bunsen burner
6. Disposable 3ml pipette
7. Autoclave
8. incubator
9. refrigerator
10. gram stain
11. nutrient agar
12. MacConkey agar
13. Microscope

**3.2 Methods**

**3.2.1 Sample collection**

A total of 15 samples were collected from different demographic location of Diyala, Rural and Urban areas. 250 ml water sample was collected from each water supply tankers aseptically by pre sterilized screw capped bottle and transport to the laboratory as early as possible. After collection of sample test tubes were tightly closed to avoid any contamination and protection to make it protected from environmental pathogen contamination.



**Figure (2)**

**Figure (1)**

**Figure 1, 2 showing the special sterile screw capped collecting bottle**

**3.2.2 STERILIZATION**

At first collection bottles, test tubes and other instruments like flasks etc. were sterilized using autoclaved at 1210C. After that was dried in laminar flow hood in presence of UV light.

**3.2.3 Types of examination**

1. Viable count to determine number of bacteria per ml
2. Differential coliform test
3. Gram stain

**3.2.4 Viable count**

This consists of growing the organism present in the water on standard nutrient agar. 3 agar deeps are made ready for planting in 3 petri dishes. One ml of 3 decimal dilution of the sample of water (1:10, 1:100, and 1:1000) is mixed with 9 ml of nutrient agar, cooled to 45 C and plates are prepared after thorough mixing,

The plates are then incubated at 37Cº for two days. The number of isolated colonies is counted. The number of colonies is multiplied by the dilution factor gives the plate count per ml

****



**Figure (3)**

**Figure (4)**

**Figure 3, 4 showing the actual bacterial growth on nutrient agar**

**3.2.5 Differential coliform test**

To determine whether the organism including *Escherichia coli* or not.

Small shiny circular with entire margin colonies are picked up then cultivated on MacConkey agar by streaking method using sterile loop.

Plates are incubated for 24 hours. Plates are stored upside down in order to prevent environmental contamination. Pink colonies are picked up for identification either by gram stain or biochemical test or both if needed.



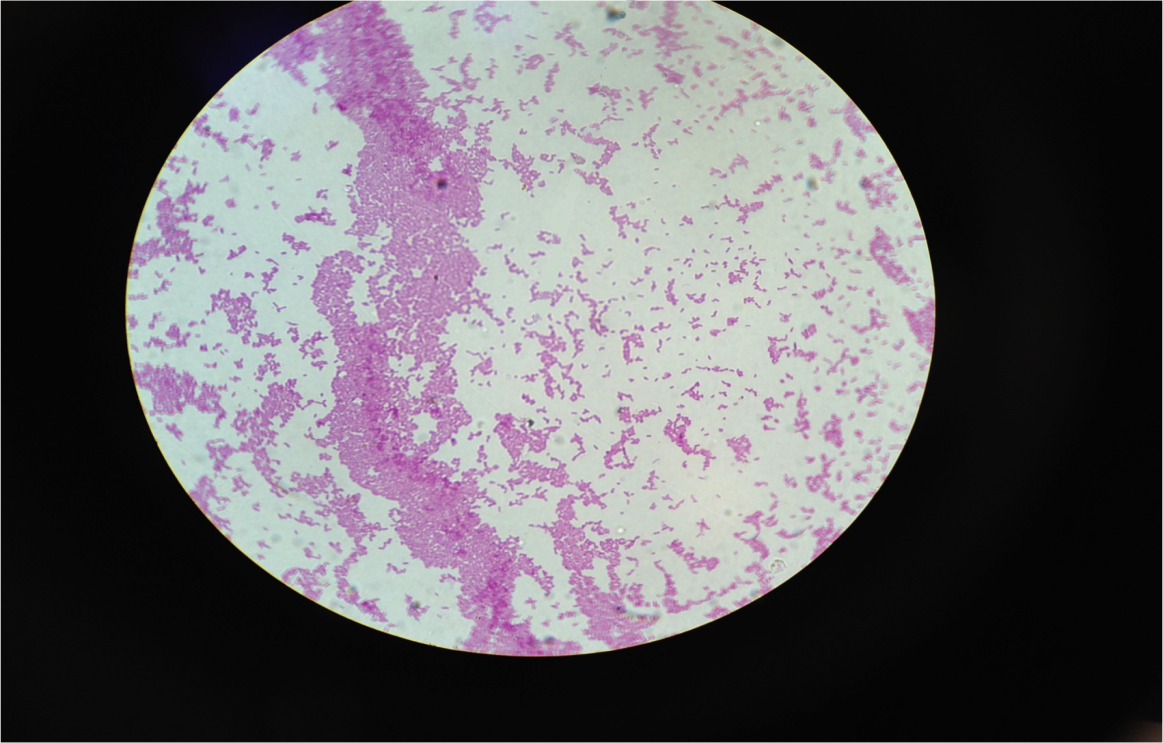
**Figure (5)**

**Figure (5): coliform growth on MacConkey agar**

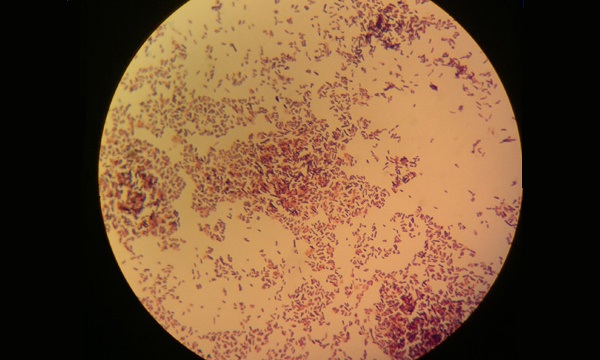
**3.2.6 Gram staining**

Bacterial growth appeared were obtained and fixed on glass slide and stained using crystal violet for 1 minute and then washed using distilled water. After that Gram Iodine was applied for 2 minutes and used 95% Acetone alcohol as decolorizing agent for couple second not more than 20 sec and finally safranin were applied for two minute and wash slide with water dried and observed using microscope

**Figure (6)**



**Figure 6: gram staining of positive coliform culture**



**Figure (7)**

**Figure 7: conformation gram staining showing *E coli* bacteria**

**CHAPTER FOUR**

**RESULT**

**4. Result**

A total of 15 samples were collected from different demographic locations of Diyala province. Rural and urban areas are included in selected regions.

In the total viable count three dilution (1:10, 1:100, 1:1000) of the origin sample were used to insure that appropriate number of colonies to be counted

**Table (1): determination of total bacterial viable count**

|  |  |  |  |
| --- | --- | --- | --- |
| NO | sample | dilution | Total bacterial viable count CFU\* /100 ml |
| 1 | A | 102 | 9.5×10ᵌ |
| 2 | B | TNTC\* |  |
| 3 | C | 101 | 8.6×102 |
| 4 | D | 103 | 8.0×104 |
| 5 | E | 101 | 1.1×103 |
| 6 | F | TNTC |  |
| 7 | G | 101 | 2.5×102 |
| 8 | H | TNTC |  |
| 9 | I | TNTC |  |
| 10 | J | 102 | 3.9×103 |
| 11 | K | 101 | 2.7×102 |
| 12 | L | TNTC |  |
| 13 | M | 101 | 2.7×102 |
| 14 | N | TNTC |  |
| 15 | O | 103 | 4.7×104 |

CFU: colony forming unites

TNTC: too numerous to count

**While in the differential coliform test**

The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the [pathogens](https://en.wikipedia.org/wiki/Pathogen) that might cause concern. Indicator organisms are bacteria such as non-specific [coliforms](https://en.wikipedia.org/wiki/Coliform), [Escherichia coli](https://en.wikipedia.org/wiki/Escherichia_coli)

As it showed in the viable count result most of the sample showed bacterial growth

In the differential coliform test we want to insure weather Escherichia coli is the included in that bacteriological growth

Out of 15 samples collected 5 (33.3%) samples were positive for Escherichia coli

Final identification of the organism done first by microscopically then followed by some biochemical test

**Table (2): differential coliform test**

|  |  |  |  |
| --- | --- | --- | --- |
| NO | sample | Presence of E coli | No of colonies |
| 1 | A | + | 7 |
| 2 | B | - | - |
| 3 | C | - | - |
| 4 | D | - | - |
| 5 | E | - | - |
| 6 | F | - | - |
| 7 | G | + | 3 |
| 8 | H | - | - |
| 9 | I | - | - |
| 10 | J | + | 11 |
| 11 | K | - | - |
| 12 | L | - | - |
| 13 | M | + | 6 |
| 14 | N | - | - |
| 15 | O | + | 2 |

**CHAPTER FIVE**

**DISCUSSION**

**5.1 Discussion**

[WHO (2002)](http://scialert.net/fulltext/?doi=jm.2015.126.131&org=10#78206_an) stated that the level of *E. coli*or thermo-tolerant bacteria should be zero in a 100 mL sample of water directly intended for drinking, as such 5 water sample out 15 analyzed are not suitable for human consumption.

Levels of E. coli cannot exceed 575 colony forming units (CFU) per 100 mL of water for partial body contact (67). The term CFU refers to the number of living bacterial cells in a water sample. Therefore, this measure is used to tell us the degree of contamination in samples of water or the degree of the infection in humans and animals. For full body contact, E. coli levels cannot exceed 235 CFU per 100 mL of water. Full-body contact refers to the human body being completely underwater in activities such as swimming or other recreational activity (67).

Coliform such as *E. coli*have been widely used as indicator of the [microbiological quality](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=microbiological+quality) of surface and ground waters ([66](http://scialert.net/fulltext/?doi=jm.2015.126.131&org=10#1406096_ja)), thus the presence of coliform is an index of bacteriological quality of water. The isolation of coliform especially *E. coli* from water sources is attributable to contamination by human and animal origin and this is of health significance as these organisms have generally been agent of gastroenteritis in humans ([66](http://scialert.net/fulltext/?doi=jm.2015.126.131&org=10#1406096_ja)). The source of contamination might have come from the water tank itself since it has been used over and over many times without proper sterilization. Or it could be point of collecting, point of distribution.

A total of 15 samples were collected from different demographic locations of Diyala province. Rural and urban areas are included in selected regions.

The total bacterial count range of (1.1×103- 9.5×103 CFU/100 ML)

Minimum viable count (1.1×103 CFU/100ML) was conducted in sample (E), whereas maximum bacterial count (9.5×103 CFU/100 ML) was found in sample (A) which is university water delivery truck.

While in differential coliform test where free from E.coli presence. Even though five sample where positive for E.coli presence (A, G, J, M, and O) the highest sample where sample (j) at 11 colonies in 1ML. and the minimum was in sample (O) at 2 colonies in 1ML of sample

Similar studies were carried out by Franciska et al. (2005) analyzed the quality of drinking water from private water supplies in Netherlands. Total 144 samples were collected for bacteriological analysis. Their results show that 10.9% samples were contaminated due to E. coli and Enterococci presence. Present study results indicate that 33% samples were coliform positive

WHO permissible limits for coliform, fecal coliform and E. coli is 0 MPN/100 mL. In present study one three tube wells water samples free from bacterial contamination, while other all samples cross the permissible limits of WHO and not fit for drinking purpose.

Similarly studies were conducted by yahya A. Shaker , Hero M. Ismael and Akhter A. Ahmed (2013) for bacteriological and microbiological Assessment for water quality of Duhok Reservoir, Iraq. They reported presence of E.coli Almost in during winter and summer season from most of the samples collected

[Khalid K. Al-Bayatti](https://www.ncbi.nlm.nih.gov/pubmed/?term=Al-Bayatti%20KK%5BAuthor%5D&cauthor=true&cauthor_uid=23365587),(2012) carried out studies for Bacteriological and Physicochemical Studies in drinking Water within Baghdad Province the study showed Treated water quality are suitable but not good for public consumption in Sharq Diglla station (55%) efficiency and in Al-Qadisia station (41%) efficiency while it showed unacceptable water quality (14%) efficiency in Al-Karama station.

**5.2 Conclusion and recommendations**

This study gives base line information about the occurrence of *E. coli* in drinking water sources (water supply tanker) for humans and farm animals in some city of Diyala province which suffer from poor water supply. It is recommended that consider water supply vehicle Are not suitable for water consumption for both human and animal. Beside and we recommend regular disinfection of drinking water sources, periodic bacteriological appraisal of drinking water sources.

**References**

1. Sobsey, M.D. and S. Bartram, (2003). Water quality and health in the new millennium: The role of the World Health Organization guidelines for drinking-water quality. Forum Nutr., 56: 396-405
2. Maheshwari, N., (2008). Clinical Microbiology and Parasitology. 2nd Edn., Jaypee Brothers Medical Publishers, New Delhi, India, ISBN-13: 978-8184483314, Pages: 272.
3. Feng, P. and S.D. Weagant, (2009). Bacteriological analytical manual.http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.
4. Frederick, A., (2011). Escherichia coli, it prevalence and antibiotic resistant in Malaysia: A mini review. Microbiol. J., 1: 47-53
5. Anonymous, (2012). Escherichia coli infection from food or water.http://www.healthlinkbc.ca/healthtopics/content.asp?hwid=hw133795.
6. CDC., (2014). Multistate outbreak of Shiga toxin-producing Escherichia coli O157:H7 infections linked to ground beef (final update). Centers for Disease Control and Prevention, Atlanta, GA., USA. http://www.cdc.gov/ecoli/2014/O157H7-05-14/index.html.
7. El Zubeir, I.E.M. and M.I.A. Ahmed, (2007). The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. Res. J. Microbiol., 2: 988-991.
8. Surendraraj, A., K.H.S. Farvin, R. Yathavamoorthi and N. Thampuran, (2009). Enteric bacteria associated with farmed freshwater fish and its culture environment in kerala, India. Res. J. Microbiol., 4: 334-344.
9. Adzitey, F., A. Abdul-Aziz and O. Moses, (2014). Microbial quality of beef in the yendi municipality of Ghana. Global J. Anim. Scient. Res., 2: 10-17.
10. Adzitey, F., C.Y. Liew, A.P. Aronal and N. Huda, (2012). Isolation of Escherichia coli from ducks and duck related samples. Asian J. Anim. Vet. Adv., 7: 351-355.
11. Adzitey, F., G.A. Teye, A.G. Ayim and S. Adday, (2010). Microbial quality of chevon and mutton sold in Tamale Metropolis of Northern Ghana. J. Applied Sci. Environ. Manage., 14: 53-55.
12. Adzitey, F., G.A. Teye, W.N. Kutah and S. Adday, (2011). Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. Livest. Res. Rural Dev., Vol. 23.
13. Adzitey, F., G.R.R. Ali, N. Huda and S.L. Ting, (2013). Antibiotic resistance and plasmid profile of Escherichia coli isolated from ducks in Penang, Malaysia. Int. Food Res. J., 20: 1473-1478.
14. Islam, M., S. Hussin and M.M. Rahman, (2011). Respiratory bacterial flora from healthy as well as respiratory symptoms' subjects. Pak. J. Biol. Sci., 14: 456-460.
15. Geidam, Y.A., Z. Zakaria, S.A. Aziz, S.K. Bejo, J. Abu and S. Omar, 2012. High prevalence of multi-drug resistant bacteria in selected poultry farms in Selangor, Malaysia. Asian J. Anim. Vet. Adv., 7: 891-897.
16. Carnot, A., J.S. Guerra, T.S. Souza and L.C. Carneiro, (2014). Antimicrobial resistance and plasmid characterization of Escherichia coli isolated in in natura water. Am. J. Drug Discov. Dev., 4: 80-84.
17. CDC., (2014). Carbapenem-resistant Enterobacteriaceae in healthcare settings. Centersfor Disease Control and Prevention,Atlanta,GA.,USA.<http://www.cdc.gov/HAI/organisms/cre/>.
18. Tenaillon, Olivier; Skurnik, David; Picard, Bertrand; Denamur, Erick (2010-03-01). ["The population genetics of commensal Escherichia coli"](http://www.nature.com/nrmicro/journal/v8/n3/abs/nrmicro2298.html). Nature Reviews Microbiology. 8 (3): 207–217. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1038/nrmicro2298](https://doi.org/10.1038%2Fnrmicro2298). [ISSN](https://en.wikipedia.org/wiki/International_Standard_Serial_Number) [1740-1526](https://www.worldcat.org/issn/1740-1526)
19. Singleton P (1999). Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley. pp. 444–454. [ISBN](https://en.wikipedia.org/wiki/International_Standard_Book_Number) [0-471-98880-4](https://en.wikipedia.org/wiki/Special:BookSources/0-471-98880-4).
20. ["Escherichia coli"](http://www.cdc.gov/ecoli/index.html/). CDC National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2012-10-02.
21. Vogt RL, Dippold L (2005). ["Escherichia coli O157:H7 outbreak associated with consumption of ground beef, June-July 2002"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1497708). Public Health Reports. 120 (2): 174–8. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [1497708](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1497708). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [15842119](https://www.ncbi.nlm.nih.gov/pubmed/15842119).
22. Bentley R, Meganathan R (Sep 1982). ["Biosynthesis of vitamin K (menaquinone) in bacteria"](http://mmbr.asm.org/cgi/pmidlookup?view=long&pmid=6127606). Microbiological Reviews. 46 (3): 241–80. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [281544](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC281544) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [6127606](https://www.ncbi.nlm.nih.gov/pubmed/6127606).
23. Hudault S, Guignot J, Servin AL (Jul 2001). ["Escherichia coli strains colonising the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1728375). Gut. 49 (1): 47–55. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1136/gut.49.1.47](https://doi.org/10.1136%2Fgut.49.1.47).[PMC](https://en.wikipedia.org/wiki/PubMed_Central) [1728375](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1728375) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [11413110](https://www.ncbi.nlm.nih.gov/pubmed/11413110).
24. Reid G, Howard J, Gan BS (Sep 2001). "Can bacterial interference prevent infection?". Trends in Microbiology. 9 (9): 424–428. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1016/S0966-842X(01)02132-1](https://doi.org/10.1016%2FS0966-842X%2801%2902132-1). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [11553454](https://www.ncbi.nlm.nih.gov/pubmed/11553454).
25. Russell JB, Jarvis GN (2001). "Practical mechanisms for interrupting the oral-fecal lifecycle of Escherichia coli". Journal of Molecular Microbiology and Biotechnology. 3 (2): 265–72. [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [11321582](https://www.ncbi.nlm.nih.gov/pubmed/11321582).
26. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (Jun 2005). ["Diversity of the human intestinal microbial flora"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1395357). Science. 308(5728):1635–8.[Bibcode](https://en.wikipedia.org/wiki/Bibcode):[2005Sci...308.1635E](http://adsabs.harvard.edu/abs/2005Sci...308.1635E). [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1126/science.1110591](https://doi.org/10.1126%2Fscience.1110591). [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [1395357](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1395357) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [15831718](https://www.ncbi.nlm.nih.gov/pubmed/15831718)
27. Feng P; Weagant S; Grant, M (2002-09-01). ["Enumeration of Escherichia coli and the Coliform Bacteria"](http://www.cfsan.fda.gov/~ebam/bam-4.html). Bacteriological Analytical Manual (8th ed.). FDA/Center for Food Safety & Applied Nutrition. Retrieved 2007-01-25.
28. Thompson, Andrea (2007-06-04). ["E. coli Thrives in Beach Sands"](http://www.livescience.com/health/070604_beach_ecoli.html). Live Science. Retrieved 2007-12-03.
29. Ishii S, Sadowsky MJ (2008). "Escherichia coli in the Environment: Implications for Water Quality and Human Health". Microbes and Environments / JSME. 23 (2): 101–8. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1264/jsme2.23.101](https://doi.org/10.1264%2Fjsme2.23.101). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [21558695](https://www.ncbi.nlm.nih.gov/pubmed/21558695).
30. Tortora, Gerard (2010). Microbiology: An Introduction. San Francisco, CA: Benjamin Cummings. pp. 85–87, 161, 165. [ISBN](https://en.wikipedia.org/wiki/International_Standard_Book_Number) [0-321-55007-2](https://en.wikipedia.org/wiki/Special:BookSources/0-321-55007-2).
31. ["Bacteria"](http://www.microbiologyonline.org.uk/about-microbiology/introducing-microbes/bacteria). Microbiologyonline. Retrieved 27 February( 2014).
32. ["E.Coli"](http://www.redorbit.com/education/reference_library/health_1/bacteria/2584144/escherichia_coli/). Redorbit. Retrieved 27 November (2013).
33. ["Facts about E. coli: dimensions, as discussed in bacteria: Diversity of structure of bacteria: – Britannica Online Encyclopedia"](http://www.britannica.com/science/bacteria/Diversity-of-structure-of-bacteria). Britannica.com. Retrieved (2015-06-25).
34. Yu AC, Loo JF, Yu S, Kong SK, Chan TF (2014). "Monitoring bacterial growth using tunable resistive pulse sensing with a pore-based technique". Appl Microbiol Biotechnol. 98 (2): 855–862. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1007/s00253-013-5377-9](https://doi.org/10.1007%2Fs00253-013-5377-9). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [24287933](https://www.ncbi.nlm.nih.gov/pubmed/24287933).
35. Kubitschek HE (Jan 1990). ["Cell volume increase in Escherichia coli after shifts to richer media"](http://jb.asm.org/cgi/pmidlookup?view=long&pmid=2403552). Journal of Bacteriology. 172 (1): 94–101. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [208405](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC208405) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [2403552](https://www.ncbi.nlm.nih.gov/pubmed/2403552).
36. Darnton NC, Turner L, Rojevsky S, Berg HC (Mar 2007). ["On torque and tumbling in swimming Escherichia coli"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1855780). Journal of Bacteriology. 189 (5): 1756–64. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1128/JB.01501-06](https://doi.org/10.1128%2FJB.01501-06). [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [1855780](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1855780) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [17189361](https://www.ncbi.nlm.nih.gov/pubmed/17189361).
37. Fotadar U, Zaveloff P, Terracio L (2005). "Growth of Escherichia coli at elevated temperatures". Journal of Basic Microbiology. 45 (5): 403–4. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1002/jobm.200410542](https://doi.org/10.1002%2Fjobm.200410542). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [16187264](https://www.ncbi.nlm.nih.gov/pubmed/16187264).
38. Ingledew WJ, Poole RK (Sep 1984). ["The respiratory chains of Escherichia coli"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC373010). Microbiological Reviews. 48 (3): 222–71. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [373010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC373010) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [6387427](https://www.ncbi.nlm.nih.gov/pubmed/6387427).
39. Krieg, N. R.; Holt, J. G., eds. (1984). Bergey's Manual of Systematic Bacteriology. 1 (First ed.). Baltimore: The Williams & Wilkins Co. pp. 408–420. [ISBN](https://en.wikipedia.org/wiki/International_Standard_Book_Number) [0-683-04108-8](https://en.wikipedia.org/wiki/Special:BookSources/0-683-04108-8).
40. Lukjancenko O, Wassenaar TM, Ussery DW (Nov 2010). ["Comparison of 61 sequenced Escherichia coli genomes"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2974192). Microbial Ecology. 60 (4): 708–20. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1007/s00248-010-9717-3](https://doi.org/10.1007%2Fs00248-010-9717-3). [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [2974192](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2974192) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [20623278](https://www.ncbi.nlm.nih.gov/pubmed/20623278).
41. . Lan R, Reeves PR (Sep 2002). "Escherichia coli in disguise: molecular origins of Shigella". Microbes and Infection / Institut Pasteur. 4 (11): 1125–32. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1016/S1286-4579(02)01637-4](https://doi.org/10.1016%2FS1286-4579%2802%2901637-4). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [12361912](https://www.ncbi.nlm.nih.gov/pubmed/12361912).
42. Orskov I, Orskov F, Jann B, Jann K (Sep 1977). ["Serology, chemistry, and genetics of O and K antigens of Escherichia coli"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC414020). Bacteriological Reviews. 41 (3): 667–710. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [414020](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC414020) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [334154](https://www.ncbi.nlm.nih.gov/pubmed/334154).
43. Stenutz R, Weintraub A, Widmalm G (May 2006). "The structures of Escherichia coli O-polysaccharide antigens". FEMS Microbiology Reviews.30(3):382 403.[doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1111/j.1574-6976.2006.00016.x](https://doi.org/10.1111%2Fj.1574-6976.2006.00016.x). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [16594963](https://www.ncbi.nlm.nih.gov/pubmed/16594963)
44. Brenner DJ, Krieg NR, Staley JT (July 26, 2005) [1984 (Williams & Wilkins)]. George M. Garrity, ed. [The Gammaproteobacteria](http://www.springer.com/life+sciences/book/978-0-387-24144-9). Bergey's Manual of Systematic Bacteriology. 2B (2nd ed.). New York: Springer. p. 1108. [ISBN](https://en.wikipedia.org/wiki/International_Standard_Book_Number) [978-0-387-24144-9](https://en.wikipedia.org/wiki/Special:BookSources/978-0-387-24144-9). British Library no. GBA561951.
45. Todar, K. ["Pathogenic E.coli"](http://www.textbookofbacteriology.net/e.coli.html).Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. Retrieved (2007-11-30).
46. Evans Jr., Doyle J.; Dolores G. Evans. ["Escherichia Coli"](https://web.archive.org/web/20071102062813/http:/www.gsbs.utmb.edu/microbook/ch025.htm). Medical Microbiology, 4th edition. The University of Texas Medical Branch at Galveston. Archived from [the original](http://www.gsbs.utmb.edu/microbook/ch025.htm) on 2007-11-02. Retrieved (2007-12-02).
47. Lodinová-Zádníková R, Cukrowska B, Tlaskalova-Hogenova H (Jul 2003). "Oral administration of probiotic Escherichia coli after birth reduces frequency of allergies and repeated infections later in life (after 10 and 20 years)". International Archives of Allergy and Immunology. 131 (3): 209–11. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1159/000071488](https://doi.org/10.1159%2F000071488). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [12876412](https://www.ncbi.nlm.nih.gov/pubmed/12876412).
48. Grozdanov L, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Dobrindt U (Aug 2004). ["Analysis of the genome structure of the nonpathogenic probiotic Escherichia coli strain Nissle 1917"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC490877). Journal of Bacteriology. 186 (16): 5432–41. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1128/JB.186.16.5432-5441.2004](https://doi.org/10.1128%2FJB.186.16.5432-5441.2004).[PMC](https://en.wikipedia.org/wiki/PubMed_Central) [490877](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC490877). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [15292145](https://www.ncbi.nlm.nih.gov/pubmed/15292145).
49. Kamada N, Inoue N, Hisamatsu T, Okamoto S, Matsuoka K, Sato T, Chinen H, Hong KS, Yamada T, Suzuki Y, Suzuki T, Watanabe N, Tsuchimoto K, Hibi T (May 2005). "Nonpathogenic Escherichia coli strain Nissle1917 prevents murine acute and chronic colitis". Inflammatory Bowel Diseases.11(5):455–63. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1097/01.MIB.0000158158.55955.de](https://doi.org/10.1097%2F01.MIB.0000158158.55955.de). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [15867585](https://www.ncbi.nlm.nih.gov/pubmed/15867585).
50. ["E. coli - Mayo Clinic"](http://www.mayoclinic.org/diseases-conditions/e-coli/basics/definition/con-20032105). mayoclinic.org. Retrieved 10 January 2017.
51. Lim, Ji Youn; Yoon, Jang W.; Hovde, Carolyn J. (20 April 2017). ["A Brief Overview of Escherichia coli O157:H7 and Its Plasmid O157"](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645889/). J Microbiol Biotechnol. 20 (1): 5–14. [PMC](https://en.wikipedia.org/wiki/PubMed_Central) [3645889](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645889) . [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [20134227](https://www.ncbi.nlm.nih.gov/pubmed/20134227) – via PubMed Central.
52. "E. Coli Food Poisoning." About. N.p., n.d. Web. 13 Dec. (2014). <<http://www.about-ecoli.com/>>.
53. "LungCongestion."TheFreeDictionary.com.N.p.,n.d.Web.13Dec.(2014).http://medical dictionary.thefreedictionary.com/Lung+Congestion>.
54. Pulmonary Edema: Get the Facts on Treatment and Symptoms." MedicineNet.N.p.,n.d.Web.13Dec.(2014). <<http://www.medicinenet.com/pulmonary_edema/article.htm>>.
55. Staff, Mayo Clinic. "Hemolytic Uremic Syndrome (HUS)." Mayo Clinic. Mayo Foundation for Medical Education and Research, 03 July 2013. Web. 13 Dec. (2014). <<http://www.mayoclinic.com/health/hemolytic-uremic-syndrome/DS00876>>.
56. ["Uropathogenic Escherichia coli: The Pre-Eminent Urinary Tract Infection Pathogen"](https://www.novapublishers.com/catalog/product_info.php?products_id=25500&osCsid=3712df5600f98259a8bdc1d9baf202e9). Nova publishers. Retrieved 27 November (2013).
57. ["Samen von Bockshornklee mit hoher Wahrscheinlichkeit für EHEC O104:H4 Ausbruch verantwortlich in English: Fenugreek seeds with high probability for EHEC O104: H4 responsible outbreak"](http://www.bfr.bund.de/cm/343/samen_von_bockshornklee_mit_hoher_wahrscheinlichkeit_fuer_ehec_o104_h4_ausbruch_verantwortlich.pdf)(PDF)(inGerman). Bundesinstitut für Risikobewertung (BfR) in English: Federal Institute for Risk Assessment. 30 June 2011. Retrieved 17 July 2011.
58. [General Information| E.coli | CDC"](https://www.cdc.gov/ecoli/general/). www.cdc.gov. Retrieved (2017-04-19).
59. US Centers for Disease Control and Prevention. ["Enterotoxigenic E. coli (ETEC)"](http://www.cdc.gov/ecoli/etec.html). Retrieved (2016-07-21).
60. Al-Abri SS, Beeching NJ, Nye FJ (June 2005). "Traveller's diarrhoea". The Lancet Infectious Diseases. 5 (6): 349–360. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1016/S1473-3099(05)70139-0](https://doi.org/10.1016%2FS1473-3099%2805%2970139-0). [PMID](https://en.wikipedia.org/wiki/PubMed_Identifier) [15919621](https://www.ncbi.nlm.nih.gov/pubmed/15919621).
61. U.S. Environmental Protection Agency (EPA), Washington, D.C. (October 2002). ["Method 1680: Fecal Coliforms in Biosolids by Multiple-Tube Fermentation Procedures."](http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2008_11_25_methods_method_biological_1680-bio.pdf) Draft. Document no. EPA-821-R-02-026.
62. Hanaor, Dorian A. H.; Sorrell, Charles C. (2014). ["Sand Supported Mixed-Phase TiO2 Photocatalysts for Water Decontamination Applications"](http://onlinelibrary.wiley.com/doi/10.1002/adem.201300259/full). Advanced Engineering Materials. 16 (2): 248–254. [doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1002/adem.201300259](https://doi.org/10.1002%2Fadem.201300259).
63. Hanaor, D.; Michelazzi, M.; Leonelli, C.; Sorrell, C.C. (2011). ["The effects of firing conditions on the properties of electrophoretically deposited titanium dioxide films on graphite substrates"](http://www.sciencedirect.com/science/article/pii/S0955221911003281). Journal of the European CeramicSociety.31(15):2877–2885.[doi](https://en.wikipedia.org/wiki/Digital_object_identifier):[10.1016/j.jeurceramsoc.2011.07.007](https://doi.org/10.1016%2Fj.jeurceramsoc.2011.07.007).
64. EPA (2002). ["Method 1106.1: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus-Esculin Iron Agar (mE-EIA)."](http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/method_1106_1.pdf) Document no. EPA 821-R-02-021.
65. Neogen Corporation, Lansing, MI (2011). ["m-Endo Agar (7724)."](http://www.neogen.com/Acumedia/pdf/ProdInfo/7724_PI.pdf) Product information sheet no. PI 7724, Rev 1
66. U.S. Geological Survey. Ohio Water Microbiology Laboratory, Columbus, OH. (January 2007). ["mFC agar method for fecal coliforms."](http://oh.water.usgs.gov/micro_methods_mFC_agar.htm) Analytical Methods.
67. Ahmed, W., R. Neller and M. Katouli, 2005. Host species-specific metabolic fingerprint Database for Enterococci and Escherichia coli and its application to identify sources of fecal contamination in surface waters. Applied Environ. Microbiol., 71: 4461-4468.
68. U.S. Environmental Protection Agency. 2009 Water Quality Standards [Online]http://www.epa.gov/waterscience/standards/wqslibrary/az/az9wqs.pdf

الخلاصة

وجود بكتريا الاشريكية القولونية في المياه هو دليل واضح على تلوث المياه اما بمياه المجاري او فضلات الحيوانات

لذلك من المهم ان يتم التأكد من عدم تلوث المياه المخصصة للاستهلاك البشري بالبكتريا القولونية.

في هذه الدراسة تم عزل الاشريكية القولونية من عينات مياه مأخوذة من مركبات نقل المياه (التنكر) التي تزود سكان بعض مناطق ومدن محافظة ديالى الشحشحة بمياه الاسالة.

مجموع (15) عينة مأخوذة من مركبات نقل المياه من مختلف مناطق ومدن الماحفظة تم ترتيبها اجديأ (A,B,C,…..O) الاشريكية القولونية تم عزلها من خمس عينات.

اختبار عد البكتريا القابلة للنمة ((VIABLE COUNT اضهر اعداد البكتريا الكلي الموجودة في العينة التي تم رؤيتها على الاجار المغذي( NUTRIENT AGAR)

بعدها تم تنمية البكتريا على اجار الماكونكي لمدة 24 ساعة بدرجة حرارة 37 درجة مؤية

حيث تم تاكيد عزل الاشريكية القولونية بواسطة التصبيغ بصبغة غرام ((GRAM STAIN والفحص المجهري بالاضافة الى الاختبارات البايوكيميائية ( BIOCHEMICAL TEST)

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب البيطري

**التحليل الجرثومي وعزل لبكتريا الاشريكية القولونية من مياه مركبات نقل المياه في محافظة ديالى**

مشروع تخرج

مقدم الى كلية الطب البيطري كجزء من متطلبات نيل شهادة البكالوريوس في الطب والجراحة البيطرية

بواسطة الطالبة

سرى شاكر محمود

تحت اشراف

المدرس المساعد سامر رعد عبد الحسين

2017