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Pharmacy

COLIFORM BACTERIA AS THE SIGN OF SWAGE POLLUTION DISTURBING FACTOR TO THE WATER QUALITY IN AL-RUMAITHA REGION HOUSES TAP- WATER

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ABSTRACT Water quality assessment mechanisms vary according to continuous water quality testes and examinations; therefore. A coliform bacterium is an indicator of water pollution by sewage, and proves the presence of microorganisms also pathogenic, which coexist with the bacteria of the group mentioned. Micro-organism had been counted using most probable number method, after it's been collected from the houses directly from faucets. The contaminated samples cultivated in nutrient broth, then by using streak plate method applied to differential and selective MacConkey agar, blood agar, and EMB, then conformational test of hemolytic in Blood agar, and Indole citrate. The biochemical tests had done by APITM tests. Current WHO guidelines recommend Fecal Coliform as indicator of the effectiveness of disinfection processes, and as index organisms for potential prescience of fecal contamination and waterborne pathogens. The results were positives for nutrient agar, blood agar, MacConkey agar, respectively. Metallic sheen growth on EMB as selective media for E.coli confirmed the results and proved the contamination of drinking water. the samples showed positive results for the biochemical tests, the diagnosis was confirmed by API20 tests. Yet, It is a part of new market era, especially after the system collapse and the underground biases of cities were destroyed, the merchants encourage the neglect of government departments to increase their profits from water bottled. For some, it's used as a political era to compare the healthy status of poor people before and after the war.

KEYWORDS : Faecal coliform, drinking water, contamination.

INTRODUCTION

Water quality assessment mechanisms vary according to continuous water quality testes, and the prevalence of pollutants that depend on population density and the abundance of services1. Continuous maintenance is one of the most important factors that maintain water quality. The quality of raw water, which deteriorates quality and beyond the day, added another burden on the sterilization and purification, even that the means and the methods used are not eligible to reach the required values and global measurements within the international quality standard of water[2,3,4].

Aquatic organisms show a high sensitivity to changes in the physical and chemical properties of the aquatic environment in which they live. Some gases, such carbon dioxide and oxygen, play a key role in maintaining life in aquatic systems. Dissolved oxygen levels change due to temperature changes and organic matter increases in water5,6. Temperature has a major role in influencing metabolic processes in aquatic plants and animals and competing with one another over food sources. The density of the population varies according to the temperature fluctuation in the environment in which they live. The degree of pH has a significant impact on aquatic organism through its effect on a various biochemical processes in water. Many soluble elements can be precipitated into high pH hydroxide, and are once again degraded at very low pH, because they are closely related to the concentration of carbon dioxide in water[4,7,8].

In case of disease, especially in areas that lack sanitary facilities and extensions, in such a case must be isolated the specific pathogen of water. There are three types of bacteria as an indicator of water pollution are Coliform group, Faecal streptococcus, and Clostridium perfringes, while the Streptococcus indicate the modern pollution of water from the source of fecal, the presence of Coliform confirms the contamination of drinking water and mixing with sewage pipes[9,10].

Coliform bacteria are present in large numbers in human faeces at percentage 200x109 coilform per person per day. Therefore, the presence of these bacteria in water is a definitive evidence of pollution in sewage water, and proves the presence of microorganisms also pathogenic, which coexist with the bacteria of the group mentioned 11. Escherichia coli, which is E.coli, is a good indicator of the presence of microorganisms in water that is dehydrated in drinking water12. One of the conditions that must be met in drinking water should be free of coliform bacteria within 100 ml of sample water studied. In the absence of E. coli in the sample, 99% of the pathogenic microorganisms also do not exist13. The characteristics of these bacteria can be characterized as a bacterium, gram negative, not oxidative, because it does not contain oxidase enzyme, aerobic selectively, does not form spores, develop selectively in the nutrient medium containing yellow salts, and fermentation of lactose within 48 hours producing gas[14,15,16.]

Since coliform bacteria are found in large numbers in faeces and can be detected in low concentrations of up to 1 in 100ml, they are considered to be sensitive to the presence of fecal contamination17. There are two basic procedures for detecting the bacteria that are scattered in the water and the number is the multi-tube method and membrane filtration technique. Using multi-tube method, an estimate of the number of uncertain colorectal coliforms present in a given volume of water can be obtained by appropriate size stapes in a number of culture tubes. This method is applicable to water of all types, especially turbid water, easy reading the positive ones. The number of coliform bacteria in water can be determined by filtering measured sizes through membrane filters, usually made of cellulose esters, with diameters of 0.45 micrometer , where the coliforms are held and the membranes are incubated face-to-face on the selective culture. Aldehyde then prepares those colonies. As the gas is not noticed on the membranes, its assumed that all colonies that produce acid or aldehyde also produce gas, and use the latter to quickly obtain results in some tests[13,18].

The main reason for drinking water purification are to ensure the destruction of pathogenic microbes and the establishment of a barrier that prevents access to distribution networks, and prevent the proliferation of germs in the pipes. The importance of disinfection is to ensure the healthy quality of drinking water supply. It was necessary to measure the concentration of water disinfectants. The relative efficiency of disinfectants produced from the same concentration of disinfectants can be expressed. Only generalized data on the relative efficacy of different disinfectants can be given because of the different nature of microorganisms and the difficulty of calibrating tests conditions such as pH, temperature, and chemical properties of water. Chlorine or chlorine dioxide can be used in the decontamination process, where the latter works at a pH of less than 8. Chlorine amine compounds cannot be used in primary sterilization because they are slow biocides and are nor recommended as primary disinfectants for treatment purposes. It may be used to maintain the residual concentrations in distribution networks and water reservoirs in cities[19].

The aim of the study is to evaluate the quality of potable (Potus) water of Al-Rumaitha area by investigating the presence and estimation of the intestinal coliform bacteria, which is an indicator of the spread of pathogenic bacteria and the damage of the transmission networks and their contamination with sewage water.

MATERIALS AND METHODS

Collection of Samples: The collection of water samples requires expertise and care, taking into account the quantity of sample water and the method of storage of water in the houses. The samples are taken directly from the liquid water and not from the stored water. Then, fill the sample bottle after opening the faucet for at least 30 seconds to get rid of the accumulations in the pipe. Fill the class bottle completely to

prevent growth of microorganisms inside the bottle during the storage period. Preferably the color of the bottle is dark and the light free to prevent the growth of algae and micro-organism that use photosynthesis, to make sure we wrap the cans tin leaves, and close them, then keep the bottles at the temperature relatively cool 10 C during the storage period. A number of samples were collected for drinking water reaching the houses of Al-Rumaitha for the period 1/11/2011-1/4/2012.

Most probable number method: In 1914, the first US Public Health Service Drinking Water Standard adopted a bacteriological standard that was applicable to any water supply provided by an interstate common carrier. It specified that not more than one out of five 10 ml portions of any sample examined should show the presence of the B. coli group by the specified Multiple-Tube Fermentation procedure (now referred to as the Most Probable Number or MPN procedure). Although this test is simple to perform, it is time-consuming, requiring 48 hours for the presumptive results. Following presumptive isolation of coliforms, further testing is required for confirmation of the coliform type- Confirmation of E. coli with the indole test was undertaken in the UK, but lactose fermentation at 44°C alone was used in the US13-. Thus over a period of some 50 years, water bacteriologists developed the concept of B. coli (later E. coli) as the indicator of faecal pollution, but continued to attach significance to the total lactose fermenters, known variously as 'coli-aerogenes' group, Escherichia-Aerobacter group, colon group or generally referred to as the 'total coliforms' group.

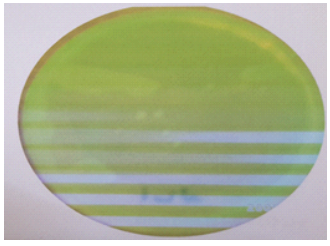
Morphology and Identification of Micro-organisms: In order to identify the type of bacteria in a water sample, it's had cultivated by nutrient broth for 24 h. at 37C. The original suspension can be streaked on an agar plate with a wire loop (streak-plate techniques). As the streaking continues, fewer and fewer cells are left on the loop, and finally the loop may deposit single cells on the agar; The plate is incubated 37C for 48h., and any well-isolated colony is then removed, resuspended in water and again streaked on MacConkey agar and blood agar and EMB agar. Conformation test used the spread plate technique, a small volume of diluted microbial suspension containing ca 30-300 cells in transferred to the center of an agar plate and spread evenly over the surface with a sterile bent-glass rod. The dispersed cells develop into isolated colonies, because the number of colonies should equal the number of viable organisms in a sample spread plates can be used to count the microbial population. The most prevalence micro-organisms is Escherichia coli typical colonial morphology with a iridescent metallic sheen on differential media such EMB agar., motile, flat, non viscous colonies[2,6,21.]

Microscopical Gram-stain: Its begin with application of a basic dye, crystal violate. A solution of iodine is then applied; all bacteria will be stained blue at this point in a procedure. The cells are then treated with alcohol, gram-positive cells retain the crystal violate-iodine complex, remaining blue; gram negative cells are completely decolorized by alcohol. As a last step, a counter stain (e.g. the red dye sefranin) is applied so that the decolorized gram-negative cells will take on contrasting color. Swage Enteric is a gram negative rod[20].

Biochemical Tests: Various classification schemes for coliforms have emerged. The earliest were those of MacConkey (1909) which recognised 128 different coliform types, while Bergey and Deehan (1908) identified 256. By the early 1920s, differentiation of coliforms had come to a series of correlations that suggested indole production, gelatin liquefaction, sucrose fermentation and the Voges–Proskauer reaction were among the more important tests for determining faecal contamination . These developments culminated in the IMViC (Indole, Methyl red, Voges–Proskauer and Citrate) tests for the differentiation of so-called faecal coliforms, soil coliforms and intermediates; these tests are still in use today. by using API-20 (Analytical Profile Index) consist of number of plastic strips, each small compartment contain powder that can be inculcated from the bacterial culture, the colorimetric changes can be scored numerically to produce a number that matches a specific bacterial species. API-20 method used to demonstrate the indole-citrate and kligler test (mannitol fermentation) E.coli typically produce positive results for indole, lysing decarboxylase, and production of gas by mannitol fermentation. E. coli from the swage confirmedly recognized by its hemolysis on blood agar20.

The Results

Microbial Diagnosis Results: It's not feasible to test water for all known waterborne pathogens to assess whether it is safe for drinking. Instead, it has been heavy reliance on fecal indicator organisms as measures of drinking water quality. Current WHO guidelines recommend Fecal Coliform as indicator of the effectiveness of disinfection processes, and as index organisms for potential prescience of fecal contamination and waterborne pathogens. The study was carried out by taking several samples of the water of houses in Al-Rumaitha district. The results showed that E. coli found in large number about 70% of total samples by MPN method. The samples were transferred by the sterile container with one use to the laboratory. The samples were grown on the nutrient agar, incubated 37C for 48 h.(Fig.1) the results was positive. Consequently, the samples were separated and diagnosed on blood agar and MacConkey agar at 37C for 48h. the results also positive by appearing the growth on both media (Fig.2).



Fig(1). illustrate the presence of E. coli bacteria in houses water samples cultivated on Nutrient agar. Positive results

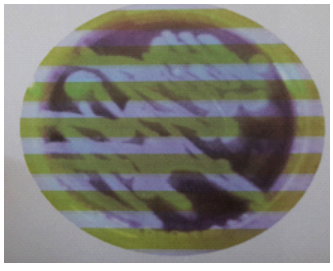


Fig 2). Illustrate the growth of E.coli bacteria on blood agar with hemolytic action as diagnostic media. Positive results.

The bacteria appeared to be negative rods, red color when stained by gram stain as it's examined under the oil 100 objective lens, Fig (3). Cell wall contains a thin layer of peptidoglycan.

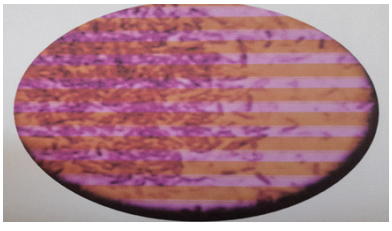
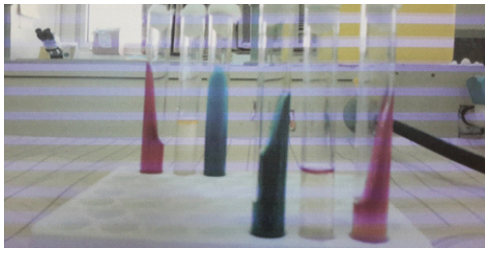


Fig (3). Exhibit facultatively anaerobic gram negative rods, red, E.coli bacteria . 100x

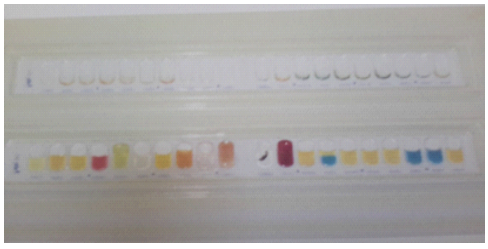
Cultivation traits: Aerobic or facultative aerobic germs develop rapidly on the normal culture media at the optimum 37C temperature. The colonies are bright smooth on nutrient agar. Their colonies on MacConkey agar are red, fermented the lactose sugar, appeared large in size on blood agar with grey- tinted. The appearance of the bacteria on the EMB medium is distinctive where the colonies have a metallic sheen luster, Fig.4.

Fig(4). Exhibit the metallic sheen colonies of E.coli on EMB medium.

Biochemical traits: the samples showed positive results for the biochemical tests, producing the Indol and positive for methyl red, and negative in reducing citrate tests, Urease, and Voges-Proskauer tests. However, in the kligler test, the results was positive without gas production. The samples were diagnosed as Fecal Cloiform. The diagnosis was confirmed by API20 tests, Fig.5,6.



Fig(5). Show the biochemical tests for E.coli, Indole, citrate, mannitol fermentation.



Fig(6). Show the API-20 complete confirmation biochemical tests for E.coli.

The Discussion:

Traditionally, indicator micro-organism have been used to suggest the presence of pathogens. Today, between we understand a myriad of possible reasons for indicator presence and pathogen absence, or vice versa. Since, Escherich described Bacillus coli (Escherich, 1885) renamed Escherichia coli by Castellani and Chalmers (1919) from the faeces of breast-fed infants, therefore, the sanitary significance of finding various coliforms along with streptococcus and C. perfringens was recognized by bacteriologists by the start of the twentieth century[22].

Various classification schemes for coliform have emerged. The earlest were those of MacConkey followed by Bergey and Deehan, that they suggested IMViC (Indole, Methyl red, Vogas-Proskauer and Citrate) tests for differentiation of so called faecal coliform, these tests are still in use today.

In this study, the results show that E.Coli was found in large number about 70% of the samples, which mean a clear evidence of water contamination. The samples were positive when cultivated on nutrient agar. Consequently, it was separated on differential media MacConkey and blood agar to give a positive results too. Wisely, it was cultivated on EMB to give conformational positive results. The biochemical tests pointed a positive, in IMViC and API20.

Yet, It is a part of new market era, especially after the system collapse, and swage nets, water nets, underground biases of cities were destroyed, the merchants encourage the neglect of government departments to increase their profits from water bottled. For some, it's possible due to high rate income, but to the most is a big problem. Rather than, its used as a political era to compare the healthy status of poor people before and after the war. Drinking water is exposed to many pollutants when pumped from the treatment plant to the homes, and most important pollutant are exposure to dust and swages nets and contaminate due to tampering and improper pipline and pipe holes and non-provisions, allowing access to pollutants, in addition to the poor used pipes, which is rapidly damaged. The study was consistent with other studies over world, mentioned the prevalence of faecal coliform from swage, result in drinking water contamination23,7.

E.coli was chosen as the biological indicator of water treatment safety. Accordingly, E.Coli survives in drinking water for between 4-12 weeks, depending on the environmental conditions. Therefore, under most circumstances, it is possible to design a monitoring program that permits public health protection at a modest cost. In chlorine disinfectant resistance experiment, it was found that E.coli survived at least as long as all bacterial pathogens. Globally, drinking water has been established as primary transmission pathway for diarrhea pathogens25,26. Yet, evidence directly linking diarrheal illness to measured faecal contamination in drinking water remains in conclusive.

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