

Assessment of Bacteriological Quality of Drinking Water in Some Primary and Secondary Schools in Mukalla City-Hadhramout/Yemen

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Abstract

Assessment of faecal contamination and detection enumeration of coliforms and other microorganisms in water is one of the measurements to determine hygienic quality of water. This study aimed to assess the drinking water in some primary and secondary schools in Mukalla city-Hadhramout/Yemen to estimate the bacterial contamination of drinking water and its safety to public. The water samples were studied for bacteriological analysis in which inoculated into culture media using two methods, the presumptive test for the most probable number (MPN) and confirmatory test for bacterial identification. Also, some physiochemical properties of water such as temperature, total dissolved salts (TSS), electrical conductivity, hydrogen ion concentration (pH) and turbidity were measured and compared with criteria of Yemeni and World Health Organization (WHO) qualifications. This study revealed that the bacterial pollution of drinking water was showed in 8 schools with *Escherichia coli* 33%, coliforms of *Proteus spp.* 25% and *Citrobacter spp.* 17% and *Staphylococcus spp.* 25%. In conclusion, this study showed that if the values of total dissolved salts, electrical conductivity, temperature, pH and turbidity increased in water, the bacterial number in water also increased, and the bacterial pollution indicates inadequate drinking water and the need to be treated and filtered.

Key words: Drinking water, *Escherichia coli*, Coliforms. Mukalla, Hadhramout.

Introduction:

Water is one of the most important elements for all forms of life and is indispensable in the maintenance of life on Earth and essential for the composition and renewal of cells. Water represents 70% of human body, participates in the composition of tissues and transports the most diverse substances throughout organism [16], and when polluted it may become dangerous to humans health [10].

Water pollution is a global problem and poses a serious threat to human life. The World Health Organization (WHO) estimated that there are four billion cases of diarrhea each year in addition to millions of other cases of illness associated with the lack of access to clean water. More than three million people in the world die of water-related diseases due to contaminated water each year, including 1.2 million children [22].

Many infectious diseases are transmitted by water through the fecal-oral route. It is seen that chemical and physical pollution of water is not less important but the deadly pollutants present in drinking water are of biological origin [5]. Microorganisms play a major role in water quality and the microorganisms that are concerned with water borne diseases are *Salmonella spp.*, *Shigella spp.*, *Escherichia coli* and *Vibrio cholera*. All these cause typhoid fever, diarrhoea, dysentery, gastroenteritis and

cholera [1]. Therefore, water is examined microbiologically to determine its sanitary quality and its suitability for general use [13].

The presence of faecal coliforms of *Escherichia coli* is used as an indicator for the presence of any of these water borne pathogens [1]. Children are generally more vulnerable to intestinal pathogens and it has been reported that about 1.1 million children die every year due to diarrheal diseases [18]. The disinfection treatment of drinking water has important role in reducing the waterborne epidemics. The inadequate disinfection treatment of municipal water results in the provision of unsafe drinking water to the people which can pose a great threat and risk of waterborne epidemics by bacterial pathogens to the population consuming it [3].

It is therefore important to determine the quality, microbial diversity from water sources consumed by the people, especially used by children, because they are vulnerable to different kinds of diseases since their immune systems are still developing. The conventional methods of water microbiological analysis based on presumptive coliforms, faecal coliforms, standard plate counts and further confirmatory tests are cumbersome, time consuming and expensive. Therefore, this study aimed to assess the bacterial contamination of drinking water and its safety to public in some primary and secondary schools in Mukalla city - Hadhramout/Yemen.

Materials and Methods:

Study design and study area:

A cross-sectional based study was carried out in

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2016 for bacteriological analysis of drinking water of 16 primary and secondary schools in the following zones of Mukalla city (Fowa, Sharaj, Mukalla, Al-Dis and Rokub).

Samples collection:

Drinking water samples were aseptically collected in sterilized 300ml capacity glass stoppered bottles from main direct tap and refrigerator tank of water supply for each school according to methods of the American Public Health Association (APHA) [4]. Care was taken not to allow air bubbles into the bottles during collection. Duplicate samples were taken from each sampling point aseptically into sterilized glass bottles, kept in an ice box, and transported immediately to the microbiology laboratory at the Faculty of Environmental and Marine Biology/Hadhramout university for analysis. The biological tests were carried out on fresh samples since samples for bacteriological should not be stored longer than six hours.

Physical and chemical properties measurement:

The samples were kept in ice due to the importance of temperature. The total dissolved salts (TDS), electrical conductivity (EC), Hydrogen ion concentration (pH) and turbidity of water were measured according to the American Public Health Association (APHA) [4], and the results were compared with criteria of Yemeni and World Health Organization (WHO) qualifications.

Presumptive test (The Most Probable Number MPN):

Presumptive test was performed by a method of most probable number (MPN). Following strict aseptic procedures, the sample was shaken vigorously. MacConkey broth (Oxoid, England) was used (for total coliform bacteria), inoculation each with 5ml of sample and 5ml of the medium with inverted Durham tubes were incubated at 37°C for 48 hours and examined for acid and gas production. Acid production was

determined by colour change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tube [4,6].

Confirmatory test of bacteria:

Confirmatory test of bacteria was carried out by streaking a loopful of broth from a positive tube in presumptive test onto MacConkey agar plate for pure colonies. The plates were incubated at 37°C for 24-48 hours. Colonies developing on MacConkey agar medium were further identified as faecal coliforms [1].

Characterization of isolates:

The macroscopic examination for colonies morphology and microscopic examination through Gram staining and biochemical tests (catalase, coagulase, oxidase, urease, indole, citrate utilization and sugar fermentation tests) were used in identifying all isolates [19].

Data analysis:

SPSS (version 19.0 software) was used for data analysis. The frequencies, percentages, mean, and standard deviation were measured. Correlation was used to define a difference between the variables. Statistical significance was defined at P-values less than 0.05.

Results:

Physiochemical investigations:

The results of physical and chemical investigations showed that the average of temperature of all samples getting over allowed limit of Yemeni and WHO qualifications, but the average of total dissolved salts was 1280.3 mg/L in main water supply and 1078.4 mg/L in the water tank and it's in the allowed limit of Yemeni qualifications and over limit of WHO qualifications, whereas the average of electrical conductivity, pH and turbidity got in the limited of Yemeni and WHO qualifications. The difference statistically of water sources and variables TDS, temperature, turbidity and EC was significant (P-value > 0.05) (Table 1).

Table (1): The physiochemical properties of drinking water

No.	School name	Water source	Temperature °C	TDS	EC	pH	Turbidity
Yemeni qualifications			25	1000	1500	6.5 - 8.5	5
WHO qualifications			25	1500	2500	6.5 - 9.0	5
1	Al-Saidah Zinab primary school (Girls)	Tape	37.5	954	1048	7.8	2.9
		Tank	37.5	951	1044	8	1.2
2	Al-Shaikh Naser primary school (Boys)	Tape	37.8	1034	1125	8	4.7
		Tank	31.3	314	373	8.2	4.6
3	Fowah complex for girls (primary and secondary)	Tape	31.7	1007	1101	7.6	2.5
		Tank	31.2	951	997	7.1	2.3
4	Ibn Sina secondary school (Boys)	Tape	31.8	130	137.7	7.7	4.4
		Tank	32	130	137.8	7.2	2.4
5	Fatima Al-Nakhibi primary school (Girls)	Tape	37.1	998	1090	7.8	3.6
		Tank	31.7	1070	1165	7.7	3.4
6	Al-Mina secondary school (Girls)	Tank	37.3	1502	1615	8.1	2.2
7	Al-Noor primary school (Boys)	Tape	27.6	1660	1779	8	1.8
		Tank	37.5	1648	1770	7.9	1.6
8	Al-Saidah Aaiesha primary school (Girls)	Tape	27	1708	1823	8.5	1.8
9	Bidairy primary school (Boys and girls)	Tape	21	1526	1635	8.3	2.2
		Tank	21.5	1645	1761	8.1	2
10	Aaisha Bahadhiq primary school (Boys and girls)	Tape	21.5	1600	1705	8.1	2.7
		Tank	21.5	1597	1708	8.1	2.5
11	Balqees secondary school (Girls)	Tape	37	1643	1763	8	1.3
12	Shihab primary school (Boys)	Tape	37	1616	1736	8.1	1.5
		Tank	37	1658	1780	8.1	1.4
13	14 th October primary school (Boys)	Tape	37	1595	1715	8.2	3.8
		Tank	37	1624	1745	8	3.6
14	Saba secondary school (Girls)	Tape	27	45	44	7.6	1.7
		Tank	27.2	37	37.6	7	1.5
15	Saba secondary school (Boys)	Tape	27.2	1840	1959	7.7	2
		Tank	27.2	109	114.2	7.9	1.8
16	Omer Bin Abdul-Aziz primary school (Boys)	Tape	27.1	1849	1967	8	2.2
		Tank	27.5	1862	1986	7.7	2
Correlation			0.588	0.750	0.748	0.320	0.627
Significant			0.027*	0.002*	0.002*	0.265	0.016*

*Correlation is significant at the 0.05 level

Measurement's unit:

TDS: mg/l, EC: micromhos/cm, Turbidity: nephelometric turbidity unit (n.t.u)

Presumptive test for coliforms (MPN):

A total of 145 untreated water samples were examined by presumptive test for coliforms, (75 tape water sample and 70 tank water samples). Twenty six (34.6%) and 27(38.5%) of tape water and tank water samples were found positive

respectively.

Confirmatory test for coliforms and other bacteria:

Twenty four (45%) out of 53 water samples were found positive for faecal *Escherichia coli*, coliforms and other bacteria. The bacteriological

analysis showed that the bacterial growth of samples taken from tank water were 14(51.8%) and 10(38.4%) for samples from tape water supply. The results revealed that if the values of TDS, EC, temperature, pH and turbidity

increased, the bacterial number in water increased with no statistically significant difference of bacterial growth and variables TDS, EC, temperature, pH and turbidity (P-value < 0.05).

Table (2) Confirmatory test of bacterial species isolated

No.	School name	District	No. of isolated bacteria from		Bacterial species isolates		
			Tape	Tank	<i>Escherichia coli</i>	Coliforms	Other bacteria
1	Al-Saidah Zinab primary school (Girls)	Fowah	2	1	Not detected	Positive	Not detected
2	Fowah complex for girls (primary and secondary)		1	1	Not detected	Positive	Not detected
3	Ibn Sina secondary school (Boys)		4	2	Positive	Positive	Positive
4	Fatima Al-Nakhibi primary school (Girls)		—	4	Positive	Not detected	Positive
5	Al-Mina secondary school (Girls)	Mukalla	—	5	Positive	Positive	Positive
6	Al-Noor primary school (Boys)		1	1	Positive	Not detected	Not detected
7	Aaisha Bahadhiq primary school (Boys and girls)	Sharaj	1	—	Positive	Not detected	Not detected
8	Saba secondary school (Boys)	Rokub	1	—	Not detected	Positive	Not detected

Four types of bacteria were isolated, which are *Escherichia coli*, coliforms of *Proteus spp.* and *Citrobacter spp.*, and *Staphylococcus spp.* (Table 3).

Table (3) Bacterial species isolated from drinking water

No.	Bacterial type	Frequency	Percentage
1	<i>Escherichia coli</i>	8	33%
2	<i>Proteus spp.</i>	6	25%
3	<i>Citrobacter spp.</i>	4	17%
4	<i>Staphylococcus spp.</i>	6	25%
Total		24	100%

Discussion:

Drinking water should be free from known pathogenic microorganisms and indicator bacteria which is a symptom for fecal contamination of water. Coliforms are the most important indicator bacteria which are considered in the bacteriological examination of water [23]. Water may contain poisonous chemical substances, pathogenic organisms (infective and parasitic agents), industrial or other wastes or sewage and is referred to as being contaminated or polluted. Most of the infections in developing countries can be

attributed to lack of safe drinking water, like cholera, typhoid and hepatitis [13, 17].

Microbiological quality of drinking water is primarily determined by enumeration of indicator organisms, whose presence indicates faecal contamination. The presence of the indicators is often a key in assessing potential public health risks due to bacterial pathogens and is used in drinking water quality regulations and guidelines in many countries [3].

Faecal contamination of water is established by the isolation of an organism that occurs only in faeces, never free-living in nature. There are

several such organisms like *Escherichia coli*, *Clostridium perfringens* and *Streptococcus faecalis*. The finding of *Escherichia coli*, *Clostridium perfringens* and *Streptococcus faecalis* are sufficient evidence that the water in question is not safe, since enteric pathogens may be presumed present [13].

Coliforms is considered a bacterial types that indicates human or animal pollution of drinking water [21]. In this study, twenty four (45%) found positive for faecal coliforms and other bacteria in water samples. *Escherichia coli* (33%), coliforms of *Proteus spp.* (25%) and *Citrobacter spp.* (17%) were isolated, as well as *Staphylococcus spp.* (25%). This study showed that bacterial pollution of tank water is more than tape water samples (51.8% and 38.4% respectively). This may be due to the turbidity of accumulation of gelatinous layers in tank water walls [11]. Other studies showed that 36.5% bacterial pollution of drinking water of some primary and secondary schools in Aden Governorate were caused by *Escherichia coli* and other coliforms [7]. High counts of faecal coliforms (*Escherichia coli*) and pathogens (*Bacillus spp.*, *Proteus spp.*, *Enterobacter spp.* and *Salmonella spp.*) were isolated from borehole water samples in Nigeria, and this indicator bacteria constitutes a threat to public health [14], and the majority of the drinking water sources are either of unacceptable quality or grossly polluted because they are contaminated with *Escherichia coli* [2].

The water is commonly contaminated with microbes and this contamination may play a role in the transmission of potentially harmful bacterial species, *Escherichia coli*, *Klebsiella*

pneumoniae, *Vibrio cholerae*, *Proteus spp.*, *Pseudomonas aeromonas* and *Staphylococcus aureus* [15]. Another study revealed that *Escherichia coli* (60%), *Klebsiella spp.* (40%), *Enterobacter spp.* (20%), *Pseudomonas spp.* (70%), *Proteus spp.* (10%), *Staphylococcus spp.* (40%) and *Salmonella spp.* (0%) were determined by presumptive coliform count from different sources of treated tap water [9]. For sachet water, *Escherichia coli* and *Streptococcus faecalis* were isolated with colony forming units (CFU), and for tap water, isolates included coliform, *Clostridium spp.* and *Penicillium spp.* [13].

A significantly high level of contamination was observed in samples collected from submersible pumps (47.6%) and water tanks (47.3%), as these sources of water are more exposed and liable to contamination in India [12]. Coliforms specially *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella spp.* and *Helicobacter pylori* were isolated from different drinking water sources in Pakistan [8]. Also, *Escherichia coli* was predominant (75%) in tape water, *Salmonella spp.* and *Enterobacter spp.* (4%), followed by *Klebsiella spp.* (3%), *Proteus spp.* and *Bacillus spp.* with (2%) [20].

Conclusion:

This study showed that if the values of total dissolved salts, electrical conductivity, temperature, pH and turbidity increased, the bacterial number in water increased. Also, the bacterial pollution of drinking water showed in 50% of schools with *Escherichia coli*, coliforms and *Staphylococcus spp.*, which prove that the water is not suitable for drinking and need to be filtered and treated.

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التقييم البكتيري لجودة مياه الشرب في بعض مدارس التعليم الأساسي والثانوي في مدينة المكلا – حضرموت / اليمن

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الملخص

تقييم التلوث البرازي والكشف عن عدد بكتيريا القولون وشبه عصيات القولون وأنواع الأحياء الدقيقة الأخرى هو أحد القياسات المستخدمة لتحديد الجودة الصحية للمياه . هدفت هذه الدراسة لتقييم جودة مياه الشرب في بعض مدارس التعليم الأساسي والثانوي في مدينة المكلا – حضرموت / اليمن لتحديد التلوث البكتيري لمياه الشرب ومدى سلامتها للناس. تمت دراسة عينات المياه للتعرف على البكتيريا التي تم تزييعها على الأوساط الزراعية باستخدام طريقتي الاختبار الافتراضي للعدد الأكثر احتمالاً والاختبار التأكيدي، بالإضافة إلى قياس بعض الخصائص الفيزيوكيميائية للمياه مثل درجة الحرارة، الأملاح الذائبة الكلية، الناقلية الكهربائية، تركيز أيون الهيدروجين والعمارة ومقارنتها بالموصفات القياسية اليمنية والعالمية. بينت الدراسة وجود تلوث للمياه في 8 مدارس بنسبة إجمالية بلغت 50%، حيث شكّلت بكتيريا الإيشريشية القولونية 33% وعصيات شبه القولون وهي جنس المتقلبات 25% وجنس الستروباكترا 17% وجنس المكورات العنقودية 25%. وأوضحت الدراسة أنه كلما زادت قيم الأملاح الذائبة الكلية، الناقلية الكهربائية، درجة الحرارة، تركيز أيون الهيدروجين والعمارة زادت أعداد البكتيريا في المياه، وأن وجود هذه الأنواع من البكتيريا في المياه يدل على عدم صلاحيتها للشرب مما يتطلب ذلك انتباهاً شديداً لتنقيتها ومعالجتها.

الكلمات المفتاحية : مياه الشرب، الإيشريشية القولونية، شبه عصيات القولون، المكلا، حضرموت.