Potential Risk Assessment of Drinking Water Source Exposed to Contamination Using Microbial Indicators and Multiple Antibiotic Resistance Index

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P A P E R I N F O	*A B S T R A C T*
	In this study, drinking water sources including drilled wells (DW), water tankers (WT), stabilized water tanks (SWT), and plastic bottles (PB) as sequential sample were assessed at Dhamar City, Yemen to investigate the microbial contamination and the potential risk of contamination using microbial indicators and multiple antibiotic resistance index. The sequential sample involving 5 drilled wells, 10 water tankers, 20 water tanks, and 100 plastic bottles. The study encompassed five sequential samples. Each water sample was collected in triplicate and analyzed for *Escherichia coli* as a microbial indicator and antibiotic susceptibility testing. Results indicated that all the water samples were contaminated by *E. coli* and total coliform exceeded the acceptable levels recommended by WHO of microbial quality of drinking water. *E. coli* isolates (240) showed high resistance to the tested antibiotics comprising 79.82% to ampicillin, 78.32% to gentamicin, 67.5% to ceftriaxone, 19.98% to ciprofloxacin, 18.32% to amoxiclav, and 33.34% were resistant to tetracycline. The multiple antibiotic resistance index of *E. coli* that showed resistant to three antibiotics ranged from 0.19 to 0.24 for all the tested samples and exceeded the threshold value of 0.2 for all samples except sequential sample 4, indicating a high risk of contamination for drinking water effected by anthropogenic activities related to urbanization, accumulation of microbial contamination during the various transferring processes of water from the source into households, as well as misuse and greater exposure to antibiotics in humans and poultry farms, which may pose a high ecological risk to the waters. ***doi**: 10.5829/ijee.2021.12.01.10*

INTRODUCTION

Water is essential to mankind's life, so an adequate, safe, and accessible water supply must be available to all [1-3]. Access to safe drinking water has been recognized as a basic human right; nevertheless, many people particularly in developing countries struggle to get access to safe water [4, 5]. In developing countries, the residents of rural and urban areas depend on both surface water and groundwater as drinking water sources without any treatment. Therefore, the use of these sources has been associated with outbreaks of lethal diarrheal diseases [6-8]. Frequently, the ability to make comprehensive decisions regarding water and sanitation is limited by data scarcity, particularly concerning the effects of environmental conditions on water contamination.

Therefore, it is imperative to investigate the microbial contamination of water sources in terms of occurrence and causes [8]. Also, many rural and urban communities have forced to use untreated water sources as alternative supplies such as springs, dams, and rainwater or water tankers due to the failure to meet the increasing demand for drinking water services [9-11]. Usually, the microbial water quality of these sources is significantly influenced by a microbial load of atmosphere washing, surface runoff, and adjacent agricultural activities [4, 12-15].

Water sources contaminated by human and animal excreta is a vehicle for the transmission of microorganisms responsible for several diseases, particularly in rural and urban areas where inhabitants entirely depend on untreated drinking water [2, 7, 16-18]. From the pathogens present in water sources, enteric

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pathogens such as *E. coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae* that are the most spreading and frequently transmitted through the ingestion of contaminated water and food to human [14, 19, 20]. The risks associated with these pathogens increase if were resistant to the antibiotic. Commonly, acute diarrheal diseases remain a major public health problem associated with the consumption of untreated water, poor hygiene facilities, and low financial resources [3, 21]. The mortality of diseases associated with water exceeded five million people per year, 50% related to microbial intestinal infection such as *Vibrio cholerae* [22].

Indeed, the microbial quality of drinking water was the major focus of the water treatment plants; therefore, the occurrence of antibiotic resistant bacteria in drinking water may significantly affect public health and cause an emerging problem of the industry of drinking water [1, 23, 24]. Prevalently, microbial indicators particularly *E. coli* has been used worldwide as a tool to assess the microbial quality of drinking water, the occurrence of *E. coli* in drinking water indicates fecal contamination that may be accompanied by waterborne diseases which cause harms to human health [25-27]. Currently, the concern is growing respecting the water quality influenced by antimicrobials agents due to they may accelerate the selection for antibiotic-resistant bacteria [28, 29]. In recent decades, the antibiotic resistance among enteric bacteria has increased since the escalating usage of antibiotics; therefore, bacteria with antibiotic resistance are isolated from various water sources such as surface water and groundwater [25, 30, 31]. Additionally, the microbiological contamination of water is the primary cause of waterborne diseases [32], and the ingestion of water contaminated with excreta causes most of the hazardous health risks [18, 27].

The number of suspected cholera cases reported from October 2016 to December 2018 were 1417156 including 2870 associated deaths with a case fatality rate of 0.20%. Besides, in 2019, the suspected cholera cases started increasing and reached more than 29500 cases during the first three months. The trend of suspected cases then fluctuated over the following months until June when it stabilized. Only in January 2019, 4429 samples of 90552 samples have been confirmed as cholera-positive by the culture at the central public health laboratories [33].

Dhamar city is one of the Yemeni cities that lack access to clean water and sanitation, as reported by the world health organization [33]. Dhamar has recently confronted rapid population growth associated mainly with the conflict in Yemen that lead to the arrival of large numbers of national refugees from struggle areas to the city. The WHO stated that most of the population in Dhamar city relies essentially on drilled wells water transported by water tankers as sources of drinking water [33]. The deterioration of the water distribution system due to the war events make the water tankers are the alternative source of drinking water, although its

consideration one of contamination source associated with the spreading of various diseases by various microorganisms such as cholera which become a widespread epidemic in Dhamar city. The microbial contamination of drinking water at Dhamar city is a public health problem. Therefore, for the best understanding of the potential risks posed by the microbial contamination of this water, this study aimed to precisely determine the microbial indicators as evidence on the fecal contamination as well as its focus on the occurrence of several antibiotic-resistant *E. coli* in drinking water sources to assess the potential risk using multiple antibiotic resistance index (MARindex).

MATERIAL AND METHODS

Study area

Dhamar city is located at 14°33'08" N. and 44 023'50" E., in the middle of the northern portion of Yemen and far from the capital of Yemen (Sana'a city) with around 100 km to south direction (Figure 1). In Dhamar city, the unique source of drinking water is groundwater. According to the Census of 2004, the population of Dhamar city is 175159 residents [34]. The annual average of rainfall at Dhamar city ranges from 200 to 400 mm/y [34-36]. The highest rainfall occurs in July and August as well as lower rainfall amounts in March, April, and May with an annual average of temperature around 24°C which was measured during 2019 [34, 35].

Sampling sites and samples collection

In this study, sampling was selected during the period of cholera outbreaks (July 2019) as well as sampling sites were selected to include entirely Dhamar city, the selection of sampling sites was depended on study area survey to pick out the sites considered as main sources of drinking water at Thamar city, including drilled wells, water tankers, water tanks (stabilized tanks distributed in the city) and domestic plastic bottles. Each sample was comprised of consecutive sample collection (Sequential sample), started with drilled wells and completed with domestic plastic bottles (Figure 2). Permanently drilled wells are utilized to fill water tankers by which water transport into the water tanks distributed in the city squares to be utilized by a human. From water tanks, people use plastic bottles (20 L) to obtain water for drinking and other domestic purposes. Five drilled wells, 10 water tankers, 20 water tanks, and more than 100 plastic bottles were including in sampling. Each water sample was collected in triplicate in sterile glass (500ml) according to literature [37, 38].

Counting, isolation, and identification of coliform bacteria

Coliforms bacteria were counted and isolated from water samples using the procedure of membrane filtration
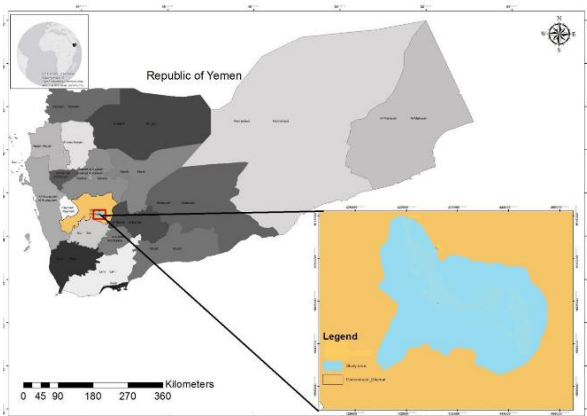
Figure 1.- Map showing location of Dhamar city (study area). The source of map [34]

Figure 2. Diagram shows the categorizations of samples in each sequential sample

technique recommended by the Standard Methods for the Examination of Water and Wastewater [37]. Six water samples from each collection point were filtered by a sterile membrane with pore size 0.45±0.02 μm and 47-mm-diameter [37]. After filtration, Petri dishes with eosin methylene blue agar (EMB) (Merck) and chromocult coliform agar (CCA) (Oxoid) were utilized as selective and differential media. Three filtrated membranes comprising bacteria placed on EMB, while the three remaining filters placed on CCA. Then, Petri dishes containing the filtrated membranes were incubated at 37°C for 24 h [37, 38]. After incubation and based on colonial morphology, coliform bacteria colonies were distinctly counted into *E. coli* and total coliform [12, 37]. Then, three *E. coli* strains from each plate were transferred into the nutrient broth (Oxoid) to confirm the identification of *E. coli* and incubated at 35°C for 24 h. Bacteria from the broth were identified by biochemical tests (Biomerieux, Marcy l'Etoile-France). Based on the biochemical results, the bacterial isolates confirmed as *E. coli* were chosen for the antibiotic susceptibility testing. Finally, the confirmed bacterial isolates were resuspended in normal saline until the turbidity of suspension equaled 0.5 of McFarland standards.

Antibiotic susceptibility testing
The bacterial isolates of *E. coli* from plastic water bottles (20 L) were used in the antibiotic resistance test due to people uses bottles to obtain water from the water tank for drinking and other domestic purposes. The antibiotic resistance pattern of *E. coli* isolates was performed using the Kirby-Bauer method [38, 39]. The pure colonies that transferred from nutrient broth into normal saline until the turbidity of solution equaled McFarland 0.5 standards were utilized in the antibiotic resistance test. The bacterial suspensions were entirely streaked onto Muller Hinton agar (MHA) (Oxoid) using a sterile cotton swab. Then, the antibiotic discs were placed on MHA 30 mm apart and 10 mm away from the plate edge and incubated at 37°C
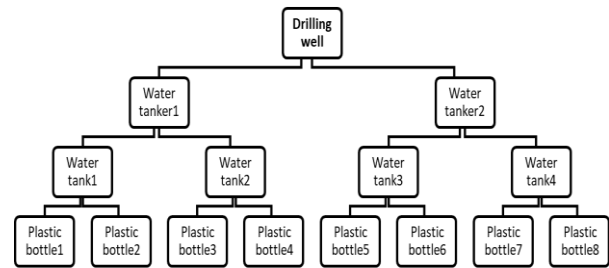
for 18-24 hours under aerobic conditions. After incubation, the inhibition zone of each antibiotic disk was measured and recorded. Based on the measured inhibition diameter, the bacterial isolates were categorized into resistant and sensitive as described in the guidelines of the National Committee for Clinical and Laboratory Standards [40]. The tested antibiotics were including, Ampicillin (10 μg), Gentamicin (10 μg), Ceftriaxone (30 μ g), Amoxiclav (20/10 μg), Ciprofloxacin (5 μg), and Tetracycline (30 μ g). The antibiotic discs were obtained from Oxoid, UK. In addition, the common antibiotic in the treatment of bacterial infections of humans was selected in this study to provide data about the multiple antibiotic resistance by which the potential risk can be assessed [12, 21, 30, 32].

Multiple Antibiotic Resistance index (MAR index)
Multiple antibiotic resistance index is executed to evaluate the potential risk of the environments contaminated by the antibiotic-resistant bacteria [29, 32, 41]. The bacteria are termed multiple antibiotic resistant if it is found to be resistant to three or more antibiotic [42]. Multiple antibiotic resistance index of 0.2 is used as threshold limit for differentiating between low and high-risk contamination with multiple antibiotic resistant bacteria, the values greater than 0.2 are indicating of a high risk of contamination, while the values less than 0.2 are indicating to low risk of contamination [25, 26, 32]. Generally, the multiple antibiotic resistance index of the water samples is calculated by the Equation (1) [43, 44]:

$$\text{MAR Index} = \frac{y}{nx} \tag{1}$$

where, y= Total number of resistance scored; n = number of isolates; x = Total number of antibiotics tested.

Statistical analysis
Statistical software (SPSS, v 20) was used to count and calculate the mean values of microbial from triplicate samples obtained from each sequential sample. In addition, regression analysis was carried out to determine the impact of microbial indicators on the prevalence of antibiotic resistance of *E. coli*.

Table 1. The mean values of microbial indicators (*E.coli*)

	Sequential sample 1		Sequential sample 2		Sequential sample 3		Sequential sample 4		Sequential sample 5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DW	170	1.0	175	5.0	00	00	00	00	00	00
WT 1	175	4.0	184	4.0	140	5.0	65	4.0	55	3.0
WT 2	185	0.6	172	8.0	135	4.0	80	4.0	100	4.0
SWT 1	335	2.9	325	5.0	145	6.0	35	3.0	140	8.0
SWT 2	325	2.0	335	8.9	240	7.0	60	6.0	125	6.0
SWT 3	375	4.0	350	6.0	195	5.0	40	5.0	130	5.0
SWT 4	280	2.5	310	5.0	165	5.0	50	4.0	185	9.0
PB 1	1630	15.1	1230	10.0	240	10.6	145	8.0	750	8.5
PB 2	1305	13.8	1275	15.0	225	5.0	160	5.0	420	7.0
PB 3	1375	22.6	1315	8.0	225	5.0	35	3.0	265	7.0
PB 4	1055	17.7	1285	5.0	195	6.0	100	6.0	315	5.0
PB 5	1050	15.3	1270	16.5	245	5.0	105	7.0	180	5.0
PB 6	1275	25.0	1320	10.1	165	7.0	85	5.0	235	7.0
PB 7	1250	15.0	1235	20.0	155	5.0	40	4.0	135	5.0
PB 8	1210	10.1	1260	10.0	160	8.6	60	5.5	175	3.0

Note: Drilled well (DW), Water tanker (WT), Stabilized water tank (SWT), Plastic bottle (PB)

RESULTS AND DISCUSSION

Microbial indicators (*E. coli* and total coliform)
The detection of microbial indicators in water used for drinking is of particular concern since they have been related to gastrointestinal infections such as diarrhea and dysentery, shigellosis, and other diseases [2, 21]. These diseases remain a heavy burden in developing countries and have been particularly complicated due to the occurrence of antibiotic resistance, particularly used in the treatment of these bacterial infections [26]. In this study, the *E.coli* values of water samples ranged between a minimum 0.00 cfu/100ml at drilled wells of sequential samples 4 and 5 and a maximum of 375 cfu/100ml in bottle 3 of sequential sample 1 (Table 1). For total coliform, the values ranged from 0.00 to 3135 TC/ 100ml. The highest value was recorded at bottle 6 of sequential sample 2, whereas the lowest value was at the drilled well of sequential sample 3 (Table 2). The highest values of *E. coli* and total coliform were recorded in plastic bottles of all sequential samples by which water transport from stabilized water tanks to households. Water samples of bottles recorded high levels of microbial contamination compared to the other water samples. The values of *E. coli* and total coliform of bottles for all samples exceeded the acceptable level for WHO [45]. According to the WHO [45], *E. coli* and total coliform must not be detectable in any 100-ml sample, also U.S. Environmental Protection Agency [47] reported that *E.*

coli and total coliform must be zero/100ml in drinking water.

For samples collected from water tankers, the highest contamination by *E. coli* was recorded at water tanker 2 of sequential sample 1 (185 cfu/100ml), while the lowest contamination was recorded at water tanker 1 of sequential sample 5 (55 cfu/100ml). Furthermore, the total coliform showed high contamination at water tanker 1 of sequential sample 2 (735 cfu/100ml), while showed low contamination at water tanker 2 of sequential sample 4 (125 cfu/100ml). The contamination of water tankers was attributed to the lacking of cleanliness and chlorination of the water tankers, also the lacking of good coverage of tanks that result in the entry of dust loaded by bacteria. In samples collected from stabilized water tanks in the city, total coliforms ranged from 120 cfu/100 ml at stabilized water tanks 3 of sequential sample 4 to 2450 cfu/100 ml at stabilized water tanks 1 of sequential sample 2. *E. coli* population ranged from 35 to 375 cfu/100ml, the lowest value was at stabilized water tanks 1 of sequential sample 4, whereas the highest value was at stabilized water tanks 3 of sequential sample 1. For the samples from plastic bottles utilized to transport water to households, the maximum value of *E. coli* was recorded in plastic bottle 3 (1375 cfu/100 ml) of sequential sample 1, whereas the minimum value was recorded in plastic bottle 7 (40 cfu/100 ml) of sequential sample 4. Conversely, the maximum value of total coliform was recorded in plastic bottle 6 (3135 cfu/100 ml) of

sequential sample 2, whereas the minimum value was recorded in plastic bottles 3 and 8 (110 cfu/100 ml) of sequential sample 4. Samples from plastic bottles recorded the highest levels of contamination by coliform bacteria compared to the other samples. The contamination of plastic bottles is affected by the poor personal hygiene of persons (generally children) who fill bottles with water from stabilized water tanks as well as the contamination during the transportation of water bottles for long- distances from stabilized water tanks to households that make plastic bottle exposed to contamination due to pollutants such as small landfills that are virtually dispersed around the stabilized water tanks.

The Occurrence of coliform in drinking water is adequate evidence of the fecal contamination associated with microbial pathogens. Among the coliform bacteria, *E. coli* is of importance as a microbial indicator of drinking water so its presence is usually correlated to gastrointestinal pathogens [18]. In this study, the results of *E. coli* and total coliform showed that the drilled wells of sequential samples 3, 4, and 5 were free of *E. coli*, therefore, these results were consistent with those obtained by Johnson et al. [48]. In the study of drinking water quality in Lalu commune (Benin), they found that the drinking water was uncontaminated by *E. coli* due to the lack of human and animal excreta. On the other hand, *E. coli* and total coliform population recorded high concentrations at all sequential samples. Consequently, these results were higher than the results obtained by Gwimbi [49], in the study of the microbial quality of

drinking water in Manonyane community: Maseru District (Lesotho), due to the high contamination by coliform bacteria in this study is largely attributed to several contamination sources, including the existence of the small landfills close to the stabilized water tanks utilized to distribute water to citizens, the suspended particles in the air that contain bacteria, the wind carrying microbes from these landfills and insects that may carry microbes to water sources. Besides, the utilization of deep holes to collect wastewater at each house due to the lacking of good sanitation is also a major cause of contamination of wells water in the sequential samples 1 and 2 because these wells are located in urban frequently is an indication of fecal contamination and can represent a risk of water-borne diseases [3, 7, 45, 50].

Antibiotic resistance of *E. coli* bacteria
In developing countries such as Yemen, where the people is exposed to various diseases (cholera epidemic, malaria, malnutrition, and other diseases), people only use contaminated water sources as a source of drinking water, the exposure to antibiotic-resistant bacteria can increase exacerbation of health risk, mostly to the children, elderly, immunocompromised [26, 44, 51-53]. In this study, a total of 240 isolates of *E. coli* were obtained from drinking water in plastic bottles used to transport water to households. From eight plastic bottles of each sequential sample, six bacterial isolates were tested against the antibiotic. The antibiotic resistance pattern (Table 3) of *E. coli* strains for sequential sample 1 (bottles) was ampicillin (100%), gentamycin (79.2%), ceftriaxone

Table 2. The mean values of microbial indicators (Total coliform)

Sites	Sequential sample 1		Sequential sample 2		Sequential sample 3		Sequential sample 4		Sequential sample 5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DW	565	5.0	615	15.0	00	00	00	00	00	00
WT1	645	7.5	735	12.0	150	4.7	142	8.0	370	7.0
WT2	565	10.0	705	5.0	175	5.5	125	5.0	295	7.0
SWT1	735	6.5	2450	27.5	225	5.0	167	7.0	1205	10.0
SWT 2	805	10.0	1570	20.0	535	9.0	130	6.0	1235	13.0
SWT 3	714	6.0	2245	22.6	425	10.0	125	5.0	1260	10.0
SWT4	680	11.0	1385	15.0	435	5.0	120	4.0	1230	6.0
PB1	4135	31.0	2530	30.0	615	20.0	450	11.0	1500	9.0
PB 2	3025	25.0	2570	25.0	580	10.0	485	5.0	1115	10.0
PB 3	3110	10.0	2565	22.6	560	16.0	110	10.0	655	8.0
PB4	2810	10.8	2115	15.0	545	11.0	245	9.0	670	10.0
PB5	2405	11.2	2650	20.0	575	15.0	240	6.0	420	9.0
PB6	2920	20.0	3135	35.0	510	10.0	170	6.0	315	9.0
PB7	3005	23.2	2610	10.0	525	4.9	120	5.0	305	6.0
PB8	2040	20.2	2215	12.0	510	10.0	110	5.0	290	5.0

(45.8%), ciprofloxacin (33.3%), amoxiclav (33.3%) and tetracycline (45.8%). In addition, *E. coli* strains for sequential sample 2 (bottles) were resistant to ampicillin (91.7%), gentamycin (83.3%), ceftriaxone (66.7%), ciprofloxacin (25%), amoxiclav (16.7%) and tetracycline (41.7%). The extreme prevalence of *E. coli* resistant to antibiotics at sequential samples 1 and 2 was ascribed to small rubbish dumps and untreated sewage holes located beside the stabilized water tanks. According to Kümmerer [30], the resistance of several antibiotics can be preserved in bacteria populations over time, despite selection pressure, which can cause a comprehensive increase in antibiotic resistance over time. Therefore, the bacterial population exposed to one or more antibiotics may induce resistance to other antibiotics without any previous exposure. Furthermore, the resistance of *E. coli* isolated from sequential sample 3 (bottles) was recorded as follows: ampicillin 70.8%, gentamycin 83.3%, ceftriaxone 62.5%, ciprofloxacin 8.3%, amoxiclav 8.3% and tetracycline 16.5%, while the *E. coli* isolates of sequential sample 4 (bottles) were 58.3 % resistant to ampicillin, 58.3% to gentamycin, 70.8 % to ceftriaxone, 8.3 to ciprofloxacin, 8.3 to amoxiclav and 16.7 % to tetracycline. Lastly, *E. coli* isolates of sequential sample 5 (bottles) were resistance to ampicillin(83.3%), gentamycin (87.5%), ceftriaxone (66.7%), ciprofloxacin (25%), amoxiclav (25%) and tetracycline (45.8%). Generally, *E. coli* bacteria isolated from sequential samples 3 and 4 (bottles) showed low resistance to antibiotics than other samples mainly regarding amoxiclav, tetracycline, ampicillin, and ciprofloxacin due to these samples exposed to small amounts of contaminants compared to the other samples. Berto et al. [54] stated that bacteria resistant to antibiotics were found in drinking water affected by contaminants around the primary water source such as wells water. Vanneste et al. [55] also found that pathogenic bacteria isolated from various watercourses were resistant to antibiotics even in regions where no apparent pollutants, the results indicated that natural watercourse could be sources of antibiotic resistance. *E. coli* isolated from the water samples in plastic bottles showed high levels of resistance to several antibiotics used. Ordinarily, antibiotics resistance among bacteria will not only complicate future antibiotics therapy but can also potentially stimulate the transmission of resistance genes to other bacteria [1, 43].

Previously, several studies emphasized the prevalence of antibiotic-resistant bacteria in drinking water, a potential risk to human and animals [1, 21, 24, 26, 32, 52, 56-59]. Frequently, the direct infections of human by antibiotic-resistant pathogens may provoke the transmission of antibiotic resistance genes to opportunist bacteria and decrease the efficiency of the antibiotic therapy, drinking water considers an important source of antibiotic resistance particularly in developing countries where access to drinking water is limited to contaminated environmental sources which are utilized without any treatment [24, 26, 31, 44].

Regression analysis
The impact of microbial indicators on the antibiotic-resistant *E. coli* occurrence by the linear regression analyses have been carried out for the microbial indicators as independent variables and the *E. coli* bacteria resistant to the different antibiotics as dependent variables. The different dependent characteristics of antibiotic-resistant *E. coli* bacteria were calculated using the regression equation and by substituting the values for the independent variables in the equations. From the regression analysis (Table 4), the P values between the microbial indicators and antibiotic-resistant *E. coli* bacteria including ampicillin, gentamycin, and ceftriaxone showed statistical significance (P≤0.05). This provides evidence of the existence of a linear relationship between the predictor (microbial indicators) and response (antibiotic-resistant *E. coli* bacteria). This means that, the regression analysis we have executed is well determined by the factors.

The regression analysis also revealed that coliform bacteria (*E. coli* and total coliform) showed statistical significance with antibiotic-resistant *E. coli* bacteria (gentamycin, tetracycline, ciprofloxacin, and chloramphenicol) (P < 0.05) because antibiotic-resistant *E. coli* bacteria is part of coliform bacteria. Generally, the

Table 3. Total of antibiotic-resistant *E.coli* isolated from drinking water in plastic bottles utilized in water transport to households

Sequential sample (bottles)	Isolates	Ampicillin resistance		Gentamycin resistance		Ceftriaxone resistance		Ciprofloxacin resistance		Amoxiclav resistance		Tetracycline resistance	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	48	48	100	38	79.2	34	70.8	16	33.3	16	33.3	22	45.8
2	48	44	91.7	40	83.3	32	66.7	12	25	8	16.7	20	41.7
3	48	34	70.8	40	83.3	30	62.5	4	8.3	4	8.3	8	16.7
4	48	28	58.3	28	58.3	34	70.8	4	8.3	4	8.3	8	16.7
5	48	40	83.3	42	87.5	32	66.7	12	25	12	25	22	45.8

Table 4. Summary of linear regression analysis (P values) between microbial indicators and antibiotic resistant *E. coli*

Variables	Amp.R	Gen.R	Ceft.R	Cep.R	AmoC.R	Tet.R
E. coli	0.04	0.05	0.04	0.10	0.27	0.13
TC	0.03	0.04	0.05	0.08	0.24	0.14

Note: (Amp.r) Ampicillin resistance, (Gen. R) Gentamycin resistance, (Ceft. R) Ceftriaxone resistance, (Cip. R) Ciprofloxacin resistance, (AmoC. R) Amoxiclav resistance, (Tet.R) Tetracycline resistance

Table 5. The regression equations of the linear regression analysis

Microbial indicator*	Antibiotic resistant *E.coli***	Regression equations
E.coli	Amp.R	Amp.R = 63.79 + 0.0413 *E.coli*
	Gen. R	Gen.R = 73.41 + 0.0119 *E.coli*
	Ceft. R	Ceft.R = 66.55 + 0.00230 *E.coli*
	Cip. R	Cip.R = 9.66 + 0.0250 *E.coli*
	AmoC. R	AmoC.R = 10.59 + 0.0187 *E.coli*
	Tet.R	Tet.R = 21.12 + 0.0296 *E.coli*
T. Coliform	Amp.R	Amp.R= 62.05 + 0.01800 T.coliform
	Gen. R	Gen.R = 72.19 + 0.00588 T.coliform
	Ceft. R	Ceft.R = 66.59 + 0.00087 T.coliform
	Cip. R	Cip.R = 8.40 + 0.01110 T.coliform
	AmoC. R	AmoC.R=9.67+ 0.00829 T.coliform
	Tet.R	Tet.R = 19.03+ 0.01372 T.coliform

*Independent variables
**Dependent variables
Note: (Amp.r) Ampicillin resistance, (Gen. R) Gentamycin resistance, (Ceft. R) Ceftriaxone resistance, (Cip. R) Ciprofloxacin resistance, (AmoC. R) Amoxiclav resistance, (Tet.R) Tetracycline resistance

environmental conditions which are suitable for coliform bacteria growth are also suitable for antibiotic-resistant *E. coli* bacteria; hence the linear relationship between those bacteria was nearly perfect. Commonly, the antibiotic-resistant bacteria from various sources such as sewage, animal farms, domestic wastewater, and agricultural runoff (contain non-antibiotic resistant and antibiotic-resistant bacteria) can transmit their resistance to another bacteria through lateral transfer contributed to the spread of antibiotic-resistant bacteria, increasing selective pressure on the bacteria, and thus favoring resistance [60]. This was observed in this study in plastic bottles that recorded high concentrations of coliform bacteria related to high concentrations of antibiotic-resistant *E. coli* bacteria due to the accumulation of pollutants from the different sources that contain them together. Generally, ampicillin, gentamycin, and ceftriaxone were from the antibiotic used in the treatment of humans and animals as well as their use in agricultural activity since the last

century, therefore, throughout the years, the bacteria acquired resistance against these antibiotics [61].

On the other hand, the regression analysis (Table 4), between the microbial indicators as independent variables and the *E .coli* bacteria resistant to ciprofloxacin, amoxiclav, and tetracycline were insignificant at $P \geq 0.05$. In this study, the coliform bacteria showed high resistance to ampicillin affected by the preservation of resistance due to the selection pressure, which causes an increase in antibiotic resistance over time. Thus, this verified the statistical significance of regression between the microbial indicators and ampicillin-resistant *E. coli* bacteria. According to Eregno [60], the use of antibiotics for a long time, even when used appropriately, creates selective pressure for resistant microorganisms. This result was similar to those reported by many research around the world in their studies regions [62]. From these relationships, it is concluded that the regression analysis has led to the formulation of equations of the linear regression for each antibiotic-resistant *E. coli* bacteria in the drinking water (Table 5). Based on this table, the regression equation can be used to predict the dependent variables. For example, from a Table 5, it is seen from the equation that for every unit increase in total coliform, a 73.41 unit increase in the gentamycin resistant *E. coli* bacteria is predicted.

MAR index in drinking water
In this study, The multiple antibiotic resistance index of *E. coli* bacteria resistant to three antibiotics of plastic bottles samples of sequential sample 4 (0.19) was found in a low range indicating a low risk of contamination, whereas the high values of multiple antibiotic resistance index were observed to be exceeded the threshold limit of plastic bottles samples of sequential sample 1, 2, 3 and 5 with value exceeded 0.21 at these stations (Table 6). The main source of water of all sequential samples particularly 1, 2, 3, and 5 were situated in the urban area impacted by various anthropogenic activities. Moreover, plastic bottle samples of sequential samples 1, 2, 3, and 5 showed multiple antibiotic resistance index above 0.2, indicating high-risk contamination of drinking water. The difference in multiple antibiotic resistance index among samples indicated the effect of anthropogenic activities related to urbanization on antibiotic resistance levels. Commonly, the high values of multiple antibiotic resistance index reveal misuse and greater exposure to antibiotics in humans and poultry farms, which may pose a high ecological risk to the waters [41]. This was observed at sequential sample 1, which is considered a high-risk sample of contamination. The high value of this sample was attributed to the small landfills and domestic wastewater (situated nearby the source) that cause contamination of the main source of water (drilled well) due to the high concentration of detergents containing antibacterial components as well as the landfills

Table 6. The multiple antibiotic resistant index (MAR index) for *E. coli* isolated from drinking water in plastic bottles utilized in water transport to households

Sequential sample (bottles)	No. of *E.coli* isolates	*E.coli* resistant to three antibiotics	MAR index	*E.coli* resistant to four antibiotics	MAR index	*E.coli* resistant to five antibiotics	MAR index
1	48	34	0.24	22	0.12	16	0.07
2	48	30	0.21	20	0.10	12	0.05
3	48	30	0.21	12	0.07	8	0.03
4	48	28	0.19	16	0.08	12	0.05
5	48	32	0.22	22	0.12	12	0.05

containing poultry dumps, which may previously this poultry use the antibiotic for disease treatment and growth promotion, all these factors resulted in the high-risk contamination sample with antibiotic-resistant bacteria. According to Bohm and Gozalan [63], the observation on the high multiple antibiotic resistance index points to that isolates originated from high risks sources of contamination where antibiotics are frequently used and high levels of antibiotics usage and resistance are related to poultry farms and domestic wastewater. In addition, in the plastic bottles samples of sequential sample 2, 3, and 5, the multiple antibiotic resistance index was exceeded the high-risk level (0.2), signifying these samples were considered as high-risk sources of contamination, affected by the accumulated microbial contamination during the various transferring processes of water from the water source into households. According to Berendonk et al. [64], antibiotic resistance hotspots are present not only in medical settings but also in environmental systems, which are subjected to precipitation of polluted air and anthropogenic activities, including municipal wastewater, agricultural activities, pharmaceutical manufacturing, and animal husbandry farms. These locations are characterized by tremendously high bacterial loads joined with subtherapeutic concentrations of antibiotics, and they contribute to the release of antibiotic-resistant bacteria into the aquatic environment. The findings of this research are in agreement with those reported by Odonkor and Addo [26], for the drinking water sources in Accra, Ghana. They found that the multiple antibiotic resistance index was above 0.2 associated with the act that the water sources may be extremely contaminated with antibiotics caused by the massive usage of these chemicals in the adjacent areas of the various sources of water. Besides, the results of this study are consistent with the results reported by Chen et al. [32] for the drinking water sources in Hangzhou City, China, which showed a high risk of contamination with multiple antibiotic resistance index exceeded 0.2 for 25% of deliberated samples, influenced by the several sources of domestic and industrial sewage that discharge into the drinking water sources. Additionally, the multiple antibiotic resistance index is lower than the range of 0.11 to 0.55, reported by Varghese and Roymon [31], for the *E .coli* bacteria from different water sources in India, they showed that water sources were contaminated with antibiotic-resistant *E. coli* arising from high-risk sources of contamination due to human and non-human fecal contamination of surface water and groundwater. Furthermore, the results were lower than those observed by Titilawo et al. [41] for ten rivers utilized for drinking and domestic purposes in Osun State, South-western Nigeria, in which multiple antibiotic resistance index in *E.c oli* bacteria was significantly greater than 0.2 resulted in high-risk contamination in the area associated with the misuse and overuse of antibiotic in animal husbandry farms. A similar study was carried out by Chitanand et al. [25] who concluded that Godavari River in India is a high risk of the contaminated environment due to high values of multiple antibiotic resistance index (ranged from 0.15-0.48) related to the domestic waste and urban runoff, which impacted on the antibiotic resistance levels in the river. This indicates that the phenomenon of multiple antibiotic-resistant bacteria in the aquatic environment used as drinking water is of worldwide concern since it is an international rather than national problem [4, 28, 32].

Generally, bacteria that have resistance to antibiotics can cause direct or indirect risks to human health due to the use and exposure to contaminated water by these bacteria. The direct risks are related to exposure to harmful bacteria, which are resistant to antibiotics relevant for the treatment of infection caused by these harmful bacteria, as this can result in diseases that are difficult to treat [61, 65, 66]. The indirect risks are related to exposure to harmless bacteria, which carry antibiotic resistance. These bacteria can colonize skin or intestines without resulting in disease, and transfer their resistance to other bacteria that inhabit these tissues [61, 67]. The resistance gene transfer occurs through a horizontal gene transfer process between the same or different species of bacteria [68, 69]. Thus, the resistance genes

can be transferred to pathogenic bacteria (non-resistant) to become resistant, resulting in difficulty to treat the Infection caused as well as most bacteria such as *E. coli* are called opportunistic bacteria, which can result in disease in people who are more vulnerable to infection.

CONCLUSION

The study concluded that the contamination level of drinking water at Dhamar city by *E. coli* and total coliform of all the five sequential samples, particularly related to plastic bottles samples, exceeded the acceptable level for WHO concerning the quality of drinking water affected by the accumulated contaminants of sequential samples, the lacking chlorination of the water, the poor personal hygiene of persons (generally children) who fill bottles with water from stabilized water tanks as well as the contamination during the transportation of water bottles for long-distances from stabilized water tanks to households that make plastic bottles exposed to contamination by bacteria in the air and small landfills that are virtually dispersed around the stabilized water. Also, the antibiotic resistance of *E. coli* bacteria isolated from plastic bottle samples was widespread principally for the sequential samples 1, 2, and 5. The *E. coli* isolates demonstrated high resistance to ampicillin and gentamicin as well as moderate resistance to ceftriaxone and tetracycline, whereas the lowest resistance was recorded against ciprofloxacin and amoxiclav. Moreover, the multiple antibiotic resistance index of *E. coli* bacteria resistant to antibiotics of plastic bottles samples of sequential samples were found in a high range indicating a high risk of contamination impacted by various anthropogenic activities associated with urbanization. The risks are related to the exposure to harmful bacteria that are resistant to antibiotics relevant for the treatment of infection caused by these harmful bacteria, as this can result in diseases that are difficult to treat, as well as the exposure to harmless bacteria that carry antibiotic resistance and colonize skin or intestines without resulting in disease can transfer their resistance to other bacteria that inhabit these tissues.

ACKNOWLEDGEMENT

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Persian Abstract

چکیده

در این مطالعه آلودگی میکروبی و خطر بالقوه آلودگی با استفاده از شاخص‌های میکروبی و شاخص مقاومت آنتی‌بیوتیکی بر روی منابع آب آشامیدنی شامل چاه‌های حفر شده(DW)، تانکرهای آب(WT)، مخازن آب تثبیت شده (SWT) و بطری‌های پلاستیکی (PB) به عنوان نمونه متوالی در شهر ضرار یمن مورد بررسی قرار گرفت. نمونه متوالی شامل ۵ حلقه چاه حفر شده، ۱۰ تانکر آب، ۲۰ مخزن آب و ۱۰۰ بطری پلاستیکی بوده است. این مطالعه شامل پنج نمونه پی در پی بود. از هر نمونه آب، سه نمونه جمع‌آوری شد و از نظر ای. کولای به عنوان شاخص میکروبی و تست حساسیت به آنتی‌بیوتیک مورد تجزیه و تحلیل قرار گرفت. نتایج نشان داد که تمام نمونه‌های آب توسط ای. کولای آلوده شده و کل کلی‌فرم از سطح قابل قبول توصیه شده توسط سازمان بهداشت جهانی با کیفیت میکروبی آب آشامیدنی فراتر رفته است. ایزوله‌های ای. کولای (۲۴۰) مقاومت بالایی در برابر آنتی بیوتیک‌های آزمایش شده داشت که ۷۹/۸۲ درصد به آمپی‌سیلین، ۷۸/۳۲ درصد به جنتامایسین، ۶۷/۵ درصد به سفتریاکسون، ۱۹/۹۸ درصد به سیپروفلوکساسین، ۱۸/۳۲ درصد به آموکسی‌کلاو، و ۳۴/۳۳ درصد به تتراسایکلین مقاوم بودند. شاخص مقاومت آنتی‌بیوتیکی متعدد ای. کولای که نسبت به سه آنتی‌بیوتیک مقاومت نشان داد از ۰/۱۹ تا ۰/۲۴ برای همه نمونه‌های آزمایش شده بود و برای همه نمونه‌ها به جز نمونه متوالی ۴ از حد آستانه ۰/۲ فراتر رفت. این مقدار نشان‌دهنده خطر بالای آلودگی برای آب آشامیدنی است که به دلیل فعالیت‌های انسانی مربوط به شهرنشینی، تجمع آلودگی میکروبی در طی فرآیندهای مختلف انتقال آب از منبع به مناطق مسکونی و همچنین سوء‌مصرف و قرار گرفتن بیش از اندازه انسان‌ها، مراتع مرغداری‌ها در معرض انواع آنتی‌بیوتیک از حد مجاز فراتر رفته است. این مسأله ممکن است به خطر اکولوژیکی بالا برای آب‌ها تبدیل شود.