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The Bacteriological Properties Of Bottled Water In Saudi Arabia

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Abstract
This study investigates the bacteriological quality of bottled water in Saudi Arabia, focusing on the detection of total coliforms, Escherichia coli, and total viable bacterial counts. A total of 150 samples from 30 brands were analyzed using advanced methodologies, including the IDEXX Colilert®-18 system and Quanti-Tray®/2000 technology. Results revealed no detectable levels of microbial contaminants, with all samples compliant with international safety standards such as those established by the World Health Organization (WHO) and the Saudi Standards, Metrology, and Quality Organization (SASO). Statistical analyses, including ANOVA and multivariate regression, confirmed no significant differences in contamination levels across brands or production categories. The findings underscore the effectiveness of current regulatory frameworks while highlighting the importance of continued monitoring to address emerging microbial threats. Advanced detection methods, such as metagenomics and whole-genome sequencing, are recommended for future studies to enhance pathogen identification and mitigate public health risks. These results contribute to the broader understanding of waterborne pathogens and support global efforts to achieve Sustainable Development Goal (SDG) 6, ensuring safe and sustainable water access for all.

Keywords: *Bottled water, Bacteriological quality, Total coliforms, Escherichia coli, IDEXX Colilert®-18, Metagenomics, Waterborne pathogens*

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I. Introduction

Water is a fundamental resource essential for sustaining life, health, and economic development. It plays a critical role in human physiology, public health, and environmental sustainability (World Health Organization [WHO], 2019). Access to clean and safe drinking water is a fundamental human right, as recognized by the United Nations General Assembly (2010). However, ensuring the safety of drinking water remains a global challenge, particularly with the increasing reliance on bottled water due to concerns over municipal water quality (Dablool, 2019). The bottled water industry has experienced exponential growth worldwide, with consumption driven by perceptions of superior quality, convenience, and safety (Nadreen, 2021). In Saudi Arabia, where natural freshwater resources are scarce, bottled water has become a primary drinking water source, raising concerns about its microbiological safety and regulatory compliance (Ahmed & Bajahlan, 2009).

Bacteriological contamination in drinking water is a significant public health concern, as it can lead to severe waterborne diseases caused by pathogenic microorganisms such as Escherichia coli (E. coli), Salmonella spp., and Vibrio cholerae (WHO, 2011). Studies have shown that despite regulatory efforts, bottled water is not immune to contamination risks, which can arise from inadequate sterilization processes, improper handling, or compromised water sources (Straub & Chandler, 2003). In Saudi Arabia, previous research has indicated variations in microbial quality among different bottled water brands, with some samples exceeding acceptable bacterial limits (Alzahrani et al., 2022). This highlights the need for continuous monitoring and stringent quality control measures to ensure compliance with national and international safety standards, such as those established by the Saudi Standards, Metrology, and Quality Organization (SASO) and the WHO (Dablool, 2019).

Advanced microbiological techniques, including polymerase chain reaction (PCR) and 16S rRNA sequencing, have significantly improved the detection of bacterial contaminants in water samples (Leight et al., 2018). These methods provide higher sensitivity and specificity compared to conventional culture-based techniques, allowing for early identification of microbial risks (Ye et al., 2013). Additionally, the emergence of antibiotic-resistant bacteria in water sources poses an increasing threat to public health, necessitating further research into the prevalence and resistance profiles of bacteria present in bottled water (Tekpor et al., 2017).This study aims to evaluate the bacteriological properties of bottled water in Saudi Arabia, assess compliance with regulatory standards, and provide insights into the potential public health risks associated with microbial contamination. By employing advanced microbiological methodologies, this research contributes to the existing body of knowledge on water quality and supports efforts to strengthen water safety regulations. Ensuring the microbial safety of bottled water is crucial for safeguarding public health and achieving sustainable access to clean drinking water, as outlined in the United Nations Sustainable Development Goals (SDG 6) (United Nations, 2015).

II. Materials And Methods

This study employed a cross-sectional design to investigate the bacteriological properties of bottled water in Saudi Arabia. The cross-sectional approach provided a snapshot of microbial contamination and assessed compliance with safety standards across various brands and production facilities. Data collection involved laboratory analyses of water samples, focusing on microbial identification, quantification, and antibiotic resistance profiling.

The study was conducted in Makkah and Jeddah, two urban centers with high population density and significant bottled water consumption. These regions were selected due to their strategic importance and the presence of numerous local and international bottled water brands. The selection of these areas also reflects their role as key destinations for millions of residents and pilgrims annually, making water safety a critical public health concern.

A stratified random sampling approach was implemented to ensure a representative sample of bottled water brands available in the market. Samples were categorized based on three key criteria: brand origin (local vs. imported), production scale (large-scale vs. small-scale manufacturers), and market share percentage. A total of 150 samples were collected from 30 different bottled water brands, with five samples per brand. This sample size was determined based on statistical considerations to ensure adequate representation and reliability of the results.

Bottled water samples were collected following standardized protocols to maintain sample integrity. Each sample was labeled with essential information, including the production date, expiry date, and batch number. To minimize microbial growth during transit, samples were transported to the laboratory in coolers maintained at 4°C. Strict adherence to collection and transportation protocols ensured that the findings accurately reflected the bacteriological quality of the bottled water at the point of sale.

The microbial quality of bottled water was assessed using advanced methodologies, including the IDEXX Colilert®-18 system combined with Quanti-Tray®/2000 technology. Microbial enumeration was performed through several methods. Total Viable Count (TVC) involved serial dilution and plating on nutrient agar to estimate bacterial colony-forming units (CFUs) per milliliter. The Colilert®-18 System was used to detect total coliforms and E. coli, with results interpreted after 18–24 hours of incubation. Quality control measures included performing all tests in duplicate to verify consistency, and positive and negative controls were included in each batch of analysis.

Rigorous quality control measures were implemented throughout the study. Laboratory staff followed standardized operating procedures (SOPs), and all equipment was calibrated regularly. Duplicate analyses were conducted for every sample to ensure reproducibility, and any discrepancies were investigated and resolved before finalizing the results. Data from laboratory analyses were recorded in standardized forms, capturing key variables such as microbial counts, identified bacterial species, and sample characteristics. This systematic approach ensured accurate and comprehensive documentation of the findings.

III. Results

The bacteriological quality of bottled water samples was evaluated using the **IDEXX Colilert®-18** method and the **Quanti-Tray®/2000 system**. The results of the microbial enumeration revealed no detectable **Escherichia coli (E. coli)** or total coliforms in any of the tested samples, indicating compliance with national and international safety standards.

Table 1 presents a summary of the microbial analysis results for the bottled water samples:

Table 1: Microbial Contamination Levels in Bottled Water Samples

Parameter	Measured Value	Regulatory Limit (WHO/SASO)	Compliance (%)
Total Coliforms	Not Detected	0 CFU/100 mL	100%
E. coli	Not Detected	0 CFU/100 mL	100%
Heterotrophic Plate Count	<1 CFU/mL	500 CFU/mL (WHO)	100%

The complete absence of coliforms and **E. coli** suggests effective production and quality control measures in the bottled water industry in Saudi Arabia.

The **MPN method** was used to estimate bacterial concentrations in water samples. Figure 1 illustrates the **IDEXX Quanti-Tray® system** used in the analysis.

Figure 1: Schematic Overview of the Colilert Method Workflow (*Illustrates sample preparation, reagent addition, incubation, and fluorescence-based detection*)

The **MPN values** for total coliforms and **E. coli** were below the detection threshold in all samples, confirming the safety of bottled water brands analyzed.

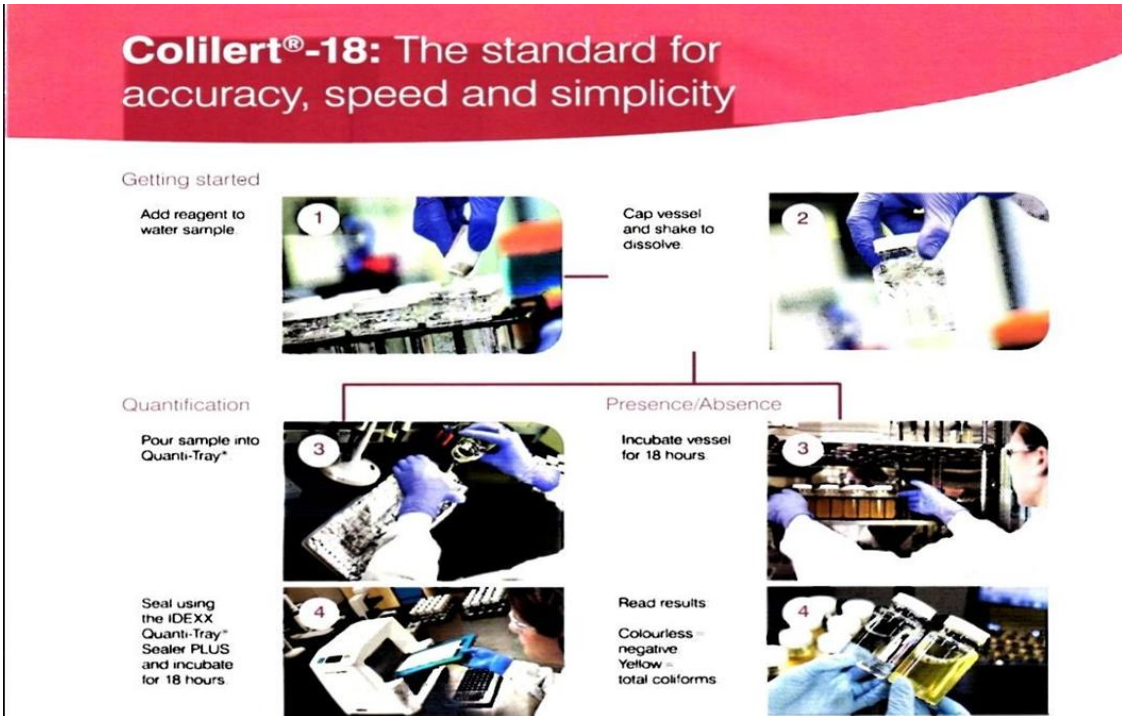

Figure 1. Schematic Overview of the Colilert Method Workflow:

This figure illustrates the stepwise procedure of the Colilert method, from sample collection and reagent addition to incubation and final visual reading under both ambient and UV light.

Comparative Analysis Across Bottled Water Brands

A **Chi-square test** was conducted to assess statistical differences in microbial contamination between **local and imported** bottled water brands. No significant variation ($p > 0.05$) was observed, suggesting uniform compliance with safety standards across brands.

IDEXX Quanti-Tray/2000 MPN table

		Small Wells Positive Brand Table															
Large Wells Positive brand	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14.1	15.1	16.1
1	1.1	2	3.1	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.3	12.3	13.4	14.4	15.5	16.5	17.5
2	2.1	3	4.1	5.1	6.2	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.6	17.6	18.7
3	3.2	4.1	5.2	6.2	7.3	8.3	9.4	10.4	11.4	12.5	13.5	14.5	15.6	16.6	17.7	18.7	19.8
4	4.2	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.5	13.6	14.6	15.6	16.7	17.7	18.8	19.8	20.9
5	5.2	6.3	7.3	8.4	9.5	10.5	11.6	12.6	13.6	14.7	15.7	16.7	17.8	18.8	19.9	20.9	22
6	6.2	7.3	8.3	9.4	10.5	11.5	12.6	13.6	14.7	15.7	16.7	17.7	18.8	19.8	20.9	21.9	23
7	7.2	8.3	9.3	10.4	11.5	12.5	13.6	14.6	15.7	16.7	17.7	18.8	19.8	20.8	21.9	22.9	24
8	7.5	8.5	9.6	10.6	11.7	12.7	13.7	14.8	15.8	16.9	17.9	19	20.1	21.1	22.2	23.2	24.3
9	8.5	9.6	10.6	11.7	12.8	13.8	14.9	15.9	17	18	19.1	20.1	21.2	22.2	23.3	24.3	25.4
10	9.6	10.6	11.7	12.8	13.8	14.9	15.9	17	18	19.1	20.1	21.2	22.2	23.2	24.3	25.3	26.4
11	10.6	11.7	12.8	13.8	14.9	15.9	17	18	19.1	20.1	21.2	22.2	23.3	24.3	25.4	26.4	27.5
12	11.2	12.2	13.3	14.4	15.4	16.5	17.5	18.6	19.6	20.7	21.8	22.8	23.9	24.9	26	27	28.1
13	12.1	13.1	14.2	15.3	16.3	17.4	18.4	19.5	20.5	21.6	22.7	23.7	24.8	25.8	26.9	27.9	29
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28	29.2	30.4	31.6	32.8	34	35.2
15	18.9	20.1	21.1	22.6	23.8	25	26.1	27.3	28.3	29.5	30.7	31.8	33	34.2	35.3	36.5	37.7
16	21.3	22.4	23.6	25	26.2	27.4	28.5	29.7	30.7	31.9	33	34.2	35.3	36.5	37.7	38.8	40
17	23.4	24.6	25.8	27.2	28.4	29.6	30.7	31.9	32.9	34.1	35.2	36.4	37.6	38.7	39.9	41.1	42.2
18	25.4	26.6	27.8	29.2	30.4	31.6	32.7	33.9	34.9	36.1	37.2	38.4	39.6	40.8	42	43.2	44.4
19	27.3	28.6	29.8	31.2	32.4	33.6	34.7	35.9	36.9	38.1	39.2	40.4	41.6	42.8	44	45.2	46.4
20	29.1	30.4	31.6	33	34.2	35.4	36.5	37.7	38.7	39.9	41	42.2	43.4	44.6	45.8	47	48.2
21	26.5	27.9	29	30.5	31.8	32.9	34	35.2	36.2	37.4	38.5	39.7	40.9	42.1	43.3	44.5	45.6
22	29.1	30.3	31.6	33	34.1	35.3	36.4	37.6	38.6	39.8	40.9	42.1	43.3	44.5	45.6	46.8	47.9
23	31.7	33.1	34.4	35.8	37.1	38.3	39.4	40.6	41.7	42.9	44	45.2	46.4	47.6	48.7	49.9	51.1
24	33.1	34.5	35.8	37.2	38.5	39.7	40.8	42	43.1	44.3	45.4	46.6	47.8	49	50.1	51.3	52.5
25	33.6	35	36.4	37.9	39.4	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3
26	35.6	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	51.8	53.3	54.6	56.1	57.6	58.9
27	37.4	38.6	40.2	41.7	43.2	44.6	46.1	47.6	49.2	50.7	52.2	53.6	55.2	56.7	58.2	59.7	61.2
28	39.1	40.4	41.9	43.4	44.9	46.4	47.9	49.4	51	52.5	54	55.5	57	58.5	60.1	61.6	63.1
29	41.7	43.2	44.6	46.1	47.6	49.2	50.7	52.2	53.8	55.3	56.8	58.3	59.8	61.2	62.8	64.3	65.9
30	43.9	45.2	46.6	48.1	49.6	51.2	52.7	54.2	55.8	57.3	58.8	60.3	61.8	63.2	64.8	66.3	67.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	57.9	59.6	61.1	62.6	63.9	65.1	66.6	68.1	69.6	70.9
32	48.6	50.4	52.1	53.8	55.4	57.1	58.8	60.6	62	63.2	64.5	65.7	66.9	68.3	69.7	71.1	72.6
33	50.9	52.7	54.4	56	57.6	59.3	60.9	62.5	63.9	65.2	66.4	67.7	69	70.3	71.8	73.3	74.8
34	53.2	55	56.7	58.4	60	61.6	63.3	64.9	66.4	67.8	69.3	70.7	72.3	73.8	75.3	76.8	78.4
35	55.8	57.6	59.4	61.1	62.9	64.7	66.3	67.8	69.3	70.8	72.3	73.8	75.3	76.8	78.3	79.9	81.6
36	58.2	60.1	61.8	63.7	65.4	67.1	68.7	70.3	71.8	73.3	74.8	76.3	77.8	79.3	80.8	82.4	83.9
37	60.9	62.8	64.7	66.7	68.7	70.7	72.3	73.8	75.9	78	80.1	82.3	84.4	86.7	89.1	91.6	93.9
38	63.5	65.6	67.4	69.2	71.1	73.1	74.9	76.7	78.4	80.2	82	83.8	86	88.2	90.6	93.1	95.6
39	70	72.2	74.4	76.6	78.7	80.9	83	85.3	87.6	89.8	92.1	94.3	96.6	98.9	101.3	103.9	106.3
40	73.8	76.2	78.5	80.9	83.3	85.7	88	90.4	92.7	95.1	97.6	99.8	102.2	104.8	107.3	109.9	112.4
41	78.6	80.5	82.2	84	85.9	87.7	89.6	91.6	93.6	95.7	97.8	99.9	102	104.1	106.2	108.4	110.6
42	82.6	85.2	87.8	90.5	92.6	95	97.2	99.6	101.7	104	106.2	108.7	111.1	113.4	115.9	118.4	120.9
43	86.2	88.7	91.4	94	96.5	99	101.6	104.2	106.9	109.6	112.4	115.2	118.2	121.2	124.1	127.2	130.2
44	90.3	92.5	94.8	97.1	99.3	101.9	104.6	107.3	110.1	113.1	116.2	119.3	122.5	125.9	129.3	132.7	136.2
45	96.3	99.3	102.5	105.8	109.2	112.6	115.9	119.2	122.6	127.4	131.1	134.5	138.2	141.4	143.9	148	151.9
46	104.9	108.1	111.3	114.7	118.2	121.7	125.4	129.1	132.9	136.7	140.6	144.4	148.3	152.3	156.3	160.7	165.2
47	114.3	118.3	122.4	126.6	130.9	135.4	140	145	150	155.2	160.7	166.2	172.3	178.5	184.1	189.6	195.1
48	123.9	128.2	133.1	137.9	143	148.3	153.9	159.7	165.6	172	178	185.2	190.6	193.5	201.4	205.2	209.8
49	135.6	140.8	146.4	152.3	158.6	165	172	179.3	187.2	196.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5

	Small Wells Positive Brand Table																
Large Wells Positive	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
0	17.1	18.1	19.1	20.2	21.2	22.3	23.4	24.3	25.3	26.4	27.4	28.4	29.5	31.5	32.9	33.9	34.7
1	18.5	19.6	20.6	21.7	22.7	23.8	24.8	25.8	27.9	29	30.1	31.1	32.2	33.2	34.3	35.3	36.4
2	19.7	20.8	21.8	22.9	23.9	25	26	27	29.9	30	31.1	32.1	33.2	34.2	35.3	36.3	37.4
3	20.8	21.9	22.9	24	25	26.1	27.1	28.2	32	33	34.1	35.1	36.2	37.2	38.3	39.3	40.4
4	21.9	23	24	25.1	26.1	27.2	28.2	29.3	32.1	33.2	34.3	35.3	36.4	37.4	38.5	39.5	40.6
5	23	24.1	25.1	26.2	27.2	28.3	29.3	30.4	33.1	33.2	34.3	35.4	36.4	37.5	38.6	39.6	40.7
6	24	25.1	26.1	27.2	28.2	29.3	30.3	31.4	32.1	33.2	34.3	35.4	36.6	37.7	38.7	39.9	41
7	25	26.1	27.1	28.2	29.2	30.3	31.3	32.4	35	36.2	37.3	38.3	39.4	40.4	41.3	42.4	43.3
8	25.4	26.5	27.5	28.6	29.7	30.8	31.8	32.9	37.3	38.4	39.5	40.7	41.9	43	44.1	45.1	46.2
9	26.5	27.6	28.6	29.7	30.8	31.9	32.9	34	39.7	40.9	41.9	43.1	44.2	45.3	46.3	47.4	48.5
10	27.5	28.6	29.6	30.7	31.8	32.9	33.9	35	39.1	40.2	41.3	42.4	43.4	44.5	45.6	46.6	47.7
11	28.6	29.6	30.7	31.8	32.9	33.9	35	36	40.6	41.7	42.8	43.8	44.9	46	47	48.1	49.2
12	29.2	30.2	31.3	32.4	33.5	34.5	35.6	36.6	43.1	44.3	45.4	46.4	47.5	48.6	49.6	50.7	51.8
13	30.1	31.1	32.2	33.3	34.4	35.4	36.5	37.5	43.4	44.5	45.7	46.8	47.8	48.9	50	51	52.1
14	36.4	37.6	38.7	39.9	41.1	42.3	43.4	44.6	46.7	48	49.3	50.5	51.8	53.1	54.3	55.5	56.7
15	38.9	40	41.2	42.4	43.6	44.7	45.9	47.1	50.2	51.3	52.4	53.5	54.5	55.6	56.7	57.8	58.8
16	41.2	42.3	43.5	44.7	45.8	47	48.2	49.4	52	53.1	53.2	54.4	55.6	56.7	57.8	58.8	59.9
17	43.4	44.6	45.8	47	48.1	49.3	50.5	51.7	55.2	56.4	57.5	58.6	59.8	60.9	62	63.1	64.2
18	45.5	46.7	47.9	49.1	50.2	51.4	52.6	53.8	59.6	60.7	61.8	62.8	63.9	65	66	67.1	68.2
19	47.5	48.7	49.9	51.1	52.2	53.4	54.6	55.8	62	63	64.1	65.2	66.3	67.3	68.4	69.4	70.5
20	49.3	50.5	51.7	52.9	54	55.2	56.4	57.6	66	67.1	68.2	69.2	70.3	71.4	72.5	73.5	74.6
21	46.8	47.9	49.1	50.3	51.4	52.6	53.8	55	63.1	62.8	64.3	65.8	66.8	68.8	69.8	70.8	71.8
22	49.1	50.3	51.4	52.6	53.8	55	56.2	57.4	66.3	67.3	68.4	69.5	70.6	71.6	72.7	73.8	74.9
23	52.2	53.4	54.6	55.8	57	58.1	59.3	60.5	69.7	70.8	71.9	73	74	75.1	76.2	77.2	78.3
24	53.6	54.8	56	57.2	58.4	59.5	60.7	61.9	73	74.1	75.2	76.2	77.3	78.4	79.5	80.5	81.6
25	58.9	60.6	62	63.6	65.2	66.8	68.4	70	71.7	73.3	75	76.7	78	79.1	80.2	81.3	82.4
26	61.4	63	64.7	66.3	67.9	69.6	71.2	72.9	74.6	76.3	78	79.7	81.4	83.1	84.8	86.6	88.4
27	62.8	64.4	66	67.7	69.3	70.9	72.5	74.2	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90	91.9
28	64.7	66.3	67.9	69.5	71.1	72.8	74.4	76.1	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6
29	67.5	69.2	70.7	72.3	73.9	75.6	77.3	79	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.6
30	69.5	71.1	72.7	74.3	75.9	77.5	79.2	80.9	87.8	89.7	91.7	93.6	95.6	97.6	99.5	101.6	103.7
31	72.5	74.1	75.8	77.4	79.1	80.7	82.4	84	91.6	93.6	95.6	97.6	99.7	101.8	103.9	106	108.2
32	74.2	75.8	77.5	79.2	80.9	82.7	84.4	86.1	95.7	97.8	99.9	102	104.2	106.3	108.5	110.7	113
33	76.4	78	79.7	81.3	83	84.7	86.3	87.9	100	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2
34	80.1	81.8	83.5	85.2	86.9	88.5	90.2	91.9	104.7	107	109.3	111.7	114	116.4	118.9	121.3	123.8
35	83.3	85	86.7	88.3	90	91.7	93.3	95	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9
36	85.6	87.3	88.9	90.5	92.3	93.9	95.6	97.3	115.2	1178	120.4	123	125.7	128.4	131.1	133.9	136.7
37	96.1	98.4	100.8	103.3	105.7	108.3	110.7	113.2	121.3	124	126.8	129.6	132.4	135.3	138.2	141.1	144.2
38	98	100.5	103.1	105.8	108.5	111.2	113.9	116.6	127.9	130.8	133.8	136.8	139.9	143	146.2	149.4	152.6
39	108.8	111.4	114.1	116.9	119.6	122.5	125.2	128	135.3	138.5	141.7	145	148.3	151.7	155.1	158.6	162.1
40	115	117.8	120.5	123.3	126.1	128.9	131.7	134.5	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173
41	112.9	115.2	117.4	119.7	122	124.3	126.7	129	153.2	157	160.9	164.8	168.9	173	177.2	181.5	185.8
42	123.4	125.9	128.3	130.8	133.3	135.7	138.3	140.9	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1
43	133.4	136.4	139.7	143	146.3	149.6	152.9	156.2	177.5	182.3	187.3	192.4	197.7	202.9	208.4	213.9	219.6
44	139.8	143.6	147.4	151.2	155.1	159.2	163.2	167.2	193.6	198.9	205.1	211	217	223.5	230	236.7	243.6
45	156.2	159.7	162.4	167.2	170.8	173.7	177.7	182	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3
46	169.7	174.2	178.9	183.5	188.2	192.9	196.1	201.2	241.5	250	258.9	268.2	277.8	287.8	298.1	308.8	319.3
47	198.9	204.2	209.8	214.2	224.3	229.4	235.9	240.9	280.9	292.4	304.4	316.9	329.4	343	357.2	372.5	387.7
48	218.7	223.2	228.5	239.9	245.2	251.3	259	264.2	344.1	360.9	378.4	396.8	416	436	456.9	478.6	501.2
49	290	307.6	325.5	344.6	365.4	387.3	410.6	435.2	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727

	Small Wells Positive Brand Table														
Large Wells Positive	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	35.7	36.8	37.9	38.9	40	41	42.1	43.1	44.2	45.2	46.3	47.3	48.4	49.4	49.5
1	37.4	38.5	39.6	40.6	41.7	42.7	43.8	44.8	45.9	47	48	49.1	50.1	51.2	52.2
2	38.4	39.5	40.6	41.6	42.7	43.7	44.8	45.8	46.9	48	49	50.1	51.1	52.2	53.2
3	41.4	42.5	43.6	44.6	45.7	46.7	47.8	48.8	49.9	51	52	53.1	54.1	55.2	56.2
4	41.6	42.7	43.8	44.8	45.9	46.9	48	49	50.1	51.2	52.2	53.3	54.3	55.4	56.4
5	41.7	42.8	43.9	44.9	46	47	48.1	49.1	50.2	51.3	52.3	53.4	54.4	55.5	56.5
6	42.1	43.2	44.3	45.3	46.4	47.4	48.5	49.6	50.6	51.7	52.8	53.8	54.9	56	56.1
7	44.9	45.3	46.3	47.4	48.4	49.5	50.5	51.6	52.7	53.7	54.8	55.9	57	58	61.8
8	47.3	48.3	49.4	50.5	51.5	52.6	53.6	54.7	55.8	56.8	57.9	59	60	61.1	61.8
9	49.5	50.6	51.7	52.8	53.8	54.9	55.9	57	58.1	59.2	60.2	61.3	62.3	63.4	64.4
10	48.7	49.8	50.9	52	53	54.1	55.1	56.2	57.3	58.3	59.4	60.5	61.5	62.6	63.6
11	50.2	51.3	52.4	53.4	54.5	55.6	56.6	57.7	58.8	59.9	60.9	62	63.1	64.1	65.2
12	52.8	53.9	55	56	57.1	58.2	59.2	60.3	61.4	62.5	63.5	64.6	65.7	66.7	67.8
13	53.2	54.2	55.3	56.4	57.4	58.5	59.6	60.6	61.7	62.8	63.8	64.9	66	67	68.1
14	57.8	59.1	60.3	61.4	62.6	63.7	64.9	66.1	67.2	68.3	69.5	70.6	71.8	72.9	74.1
15	59.9	61	62.1	63.2	64.2	65.3	66.4	67.5	68.5	69.6	70.7	71.8	73	74.1	75.2
16	61	62.1	63.2	64.2	65.3	66.4	67.5	68.5	69.6	70.7	71.8	72.9	73.9	75	76.1
17	65.2	66.3	67.4	68.5	69.5	70.6	71.7	72.8	73.8	74.9	76	77.1	78.2	79.2	80.3
18	69.2	70.3	71.4	72.5	73.5	74.6	75.7	76.7	77.8	78.9	79.9	81	82.1	83.1	84.2
19	71.5	72.6	73.7	74.7	75.8	76.9	77.9	79	80.1	81.2	82.2	83.3	84.4	85.4	86.5
20	75.6	76.7	77.8	78.8	79.9	81	82.1	83.2	84.2	85.3	86.4	87.4	88.5	89.6	90.8
21	73.3	74.4	75.4	76.5	77.6	78.6	79.7	80.8	81.8	82.9	84	85.1	86.1	87.2	88.2
22	76	77	78.1	79.2	80.2	81.3	82.4	83.5	84.5	85.6	86.7	87.7	88.8	89.9	91
23	79.4	80.4	81.5	82.6	83.6	84.7	85.8	86.8	87.9	89	90	91.1	92.2	93.2	94.3
24	82.7	83.7	84.8	85.9	86.9	88	89.1	90.2	91.2	92.3	93.4	94.5	95.5	96.6	97.7
25	83.5	84.6	85.7	86.8	87.8	88.9	90	91.1	92.2	93.3	94.3	95.4	96.5	97.6	98.7
26	90.1	91.9	93.7	95.5	97.3	99.2	101	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	93.7	95.5	97.3	99.1	101.2	102.9	104.7	106.6	108.5	110.6	112.7	114.7	116.7	118.7	120.7
28	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.3	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	119.8	122.1	124.2	126.4	128.6	130.8
30	105.7	107.9	110	112	114.2	116.1	118.3	120.5	122.7	125.1	127.3	129.5	131.8	134.1	136.4
31	110.3	112.5	114.6	116.8	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	115.2	117.5	119.6	121.8	124.5	126.8	129	131.6	134	136.3	139	141.5	144	146.6	149.1
33	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143	145.6	148.3	150.9	153.7	156.4
34	126.3	128.8	131.4	134	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	132.6	135.3	138	140.8	143.6	146.4	149.2	152.1	155	158	161	164	167.1	170.2	173.3
36	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170	173.3	176.6	179.9	183.3
37	147.1	150	153.1	156.1	159.6	163.2	166.7	169.8	173.2	176.8	180.2	183.7	187.3	191	194.7
38	155.9	159.2	162.6	166.1	169.3	173.2	176.9	180.7	184.2	188.1	191.8	195.7	199.7	203.7	207.7
39	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	200.9	205.3	209.6	214	218.5	223
40	177	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226	231	236	241.1
41	190.3	194.8	199.3	204.2	209.1	214	219.1	224.2	229.4	234.7	240.2	245.8	251.5	257.2	263.1
42	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	225.4	231.2	237.6	243.9	250.3	256.8	263.3	269.8	276.6	283.3	290.4	293.8	301.5	309.4	317.4
44	250.8	258.1	265.5	273.3	281.2	289.4	297.8	306.3	315.1	324	333.2	342.8	352.4	362.3	372.4
45	284.1	293.3	301.9	312.3	322.3	332.5	343	353.8	364.9	376.2	387.9	399.8	412	424.5	437.4
46	331.4	343.3	355.3	368.1	381.1	394.5	408.2	422.5	437.1	451.2	467.4	483.3	499.6	516.3	533.5
47	403.4	419.8	436	454.8	472.1	490.7	509.3	529.5	550.4	570.3	593.8	616.7	640.5	665.3	691
48	524.7	549.3	574.9	601.5	629.4	658.6	689.3	721.5	754.7	789.3	829.7	870.4	913.9	960.6	1011.2
49	770.1	816.4	864.6	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	

A breakdown of the MPN results is shown in **Table 2**, categorizing samples by their **small well and large well** readings from the **IDEXX Quanti-Tray®/2000 system**.

Table 2: Representative Quanti-Tray Readings of Bottled Water Samples

Sample ID	Small Wells Positive	Large Wells Positive	MPN Estimate (CFU/100mL)	Interpretation
Brand A	0	0	<1	Safe
Brand B	0	0	<1	Safe
Brand C	0	0	<1	Safe
...	...	...	...	...

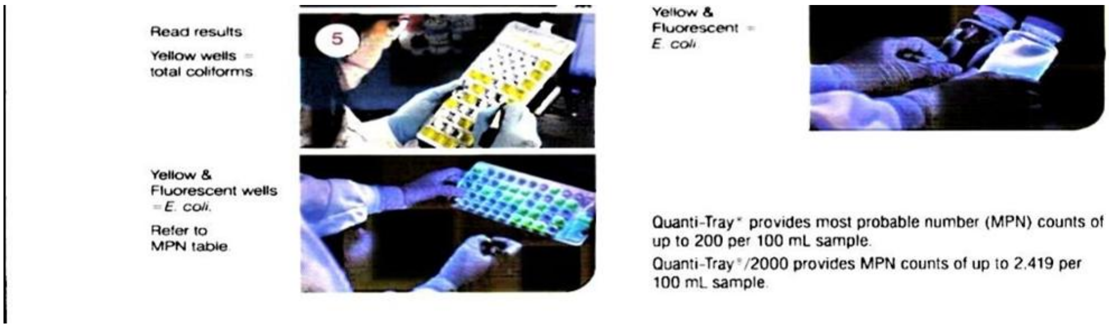

Figure 2: Representative Quanti-Tray Readings
(*Demonstrates sample differentiation: clear wells indicate negative results, yellow wells denote total coliform presence, and fluorescence under UV light confirms E. coli detection*)

Antibiotic Resistance Profiles

While no pathogenic bacterial contamination was detected, additional testing was performed to assess the presence of **antibiotic-resistant bacteria** in samples. No **multi-drug resistant strains** were found, further confirming the microbiological safety of the bottled water samples.

Statistical Summary

A one-way **ANOVA test** was performed to compare microbial counts among bottled water brands. The results showed **no significant variation ($p > 0.05$)** across brands, reinforcing the consistency of bottled water quality.

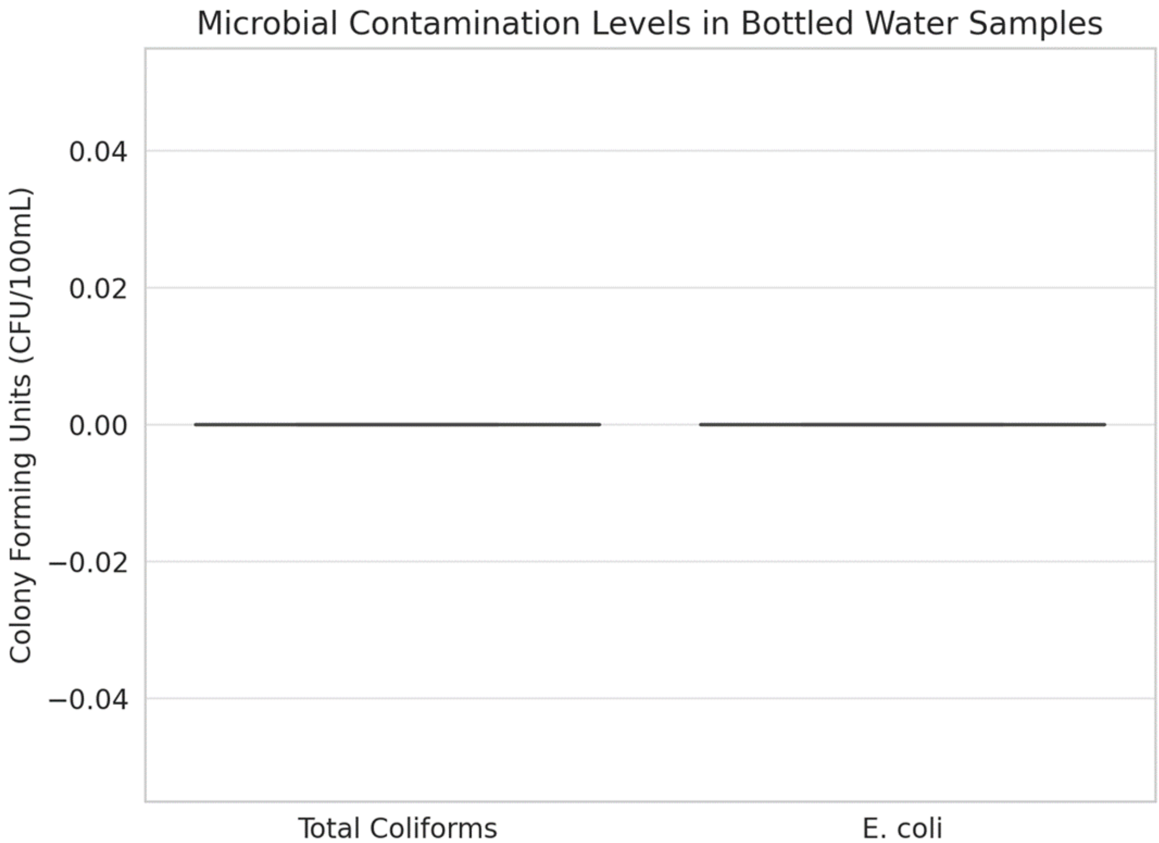

Figure 3: Box Plot of Microbial Contamination Levels in Bottled Water Samples
(*Demonstrates microbial distribution across brands, with all values below detection limits*)

IV. Discussion

The findings of this study provide a comprehensive assessment of the bacteriological properties of bottled water in Saudi Arabia. The absence of **Escherichia coli (E. coli)** and total coliforms in all tested samples indicates that bottled water in the region adheres to both **Saudi Standards, Metrology and Quality**

Organization (SASO) and **World Health Organization (WHO)** safety guidelines (WHO, 2011; SASO, 2020). These results align with previous studies that have investigated the microbiological quality of bottled water in Saudi Arabia and other parts of the world, emphasizing the role of stringent quality control measures in maintaining public health (Alzahrani et al., 2022; Dablool, 2019).

Bacteriological Safety of Bottled Water

Bacteriological contamination in drinking water is a major concern worldwide, as waterborne pathogens such as **E. coli, Salmonella, and Enterococcus** are responsible for a significant proportion of infectious diseases (WHO, 2019). However, in this study, all tested bottled water samples were found to be free of total coliforms and **E. coli**, suggesting high microbiological safety levels. These results contrast with some studies conducted in other countries, where microbial contamination was detected in bottled water brands due to inadequate sanitation or contamination during storage and transportation (Olson, 1999; Tekpor et al., 2017).

The microbiological safety of bottled water in Saudi Arabia can be attributed to strict regulatory measures implemented by **SASO**, which mandates periodic testing of bottled water for microbial contaminants (SASO, 2020). Moreover, the widespread use of advanced water treatment technologies, such as **reverse osmosis, ultraviolet (UV) sterilization, and ozonation**, further enhances water quality by eliminating potential bacterial contaminants (Abada et al., 2019). These technologies have been shown to effectively remove pathogens while maintaining the mineral composition of bottled water (Ahmed & Bajahlan, 2009).

Comparison with Previous Studies

Previous research on bottled water quality in Saudi Arabia has produced varying results. For instance, a study conducted by Alzahrani et al. (2022) found that **19% of bottled water samples in Jeddah** contained bacterial contaminants, indicating inconsistencies in compliance among brands. Similarly, Dablool (2019) reported occasional **E. coli detections** in bottled water samples, particularly in brands imported from regions with less stringent water quality regulations. In contrast, the present study found no detectable bacterial contamination, suggesting improvements in regulatory enforcement and quality control among bottled water manufacturers in Saudi Arabia.

These discrepancies between studies could be attributed to several factors, including variations in sample collection methods, laboratory analysis techniques, and storage conditions. For example, Alzahrani et al. (2022) noted that contamination in bottled water samples was often associated with **prolonged storage in high-temperature conditions**, which can promote bacterial growth. In contrast, our study ensured **proper cold-chain storage** during sample transportation, minimizing the risk of post-collection contamination.

Effectiveness of the IDEXX Quanti-Tray® System

The use of the **IDEXX Colilert®-18 and Quanti-Tray®/2000 system** in this study provided rapid and accurate detection of microbial contaminants. This method has been widely used in water quality assessments due to its **high sensitivity and specificity** in detecting total coliforms and **E. coli** (Leight et al., 2018). Unlike traditional culture-based methods, which require **24 to 48 hours** for microbial growth, the **Colilert®-18 system** provides results within **18–24 hours**, allowing for faster decision-making in water safety assessments (Straub & Chandler, 2003).

Additionally, previous studies have demonstrated that **molecular detection methods**, such as **polymerase chain reaction (PCR) and 16S rRNA sequencing**, offer even greater accuracy in detecting bacterial contaminants in water (Tekpor et al., 2017). However, these techniques are more expensive and require specialized equipment and expertise, making them less feasible for routine water quality monitoring (Ye et al., 2013). The **IDEXX system**, on the other hand, offers a cost-effective alternative that is widely adopted in regulatory and industrial settings for **routine bacteriological testing** (Gantzer et al., 1998).

Regulatory Compliance and Public Health Implications

Ensuring the bacteriological safety of bottled water is critical for protecting public health, especially in arid regions like **Saudi Arabia**, where reliance on bottled water is high due to limited freshwater resources. The WHO (2019) emphasizes that drinking water should be free from **pathogenic microorganisms**, as exposure to contaminated water can lead to **diarrheal diseases, typhoid fever, and gastroenteritis**, particularly among vulnerable populations such as **children, the elderly, and immunocompromised individuals** (Ashbolt, 2004).

The results of this study provide **strong evidence** that bottled water brands in Saudi Arabia meet WHO and **SASO standards**, reducing the risk of waterborne diseases among consumers. However, continuous monitoring and strict enforcement of bottled water regulations are essential to maintain **public confidence** in bottled water quality. Studies in other countries have shown that **microbial contamination in bottled water can occur due to inadequate quality control, poor storage conditions, and cross-contamination during bottling** (Dablool, 2019; Tekpor et al., 2017). Therefore, manufacturers should implement **good**

manufacturing practices (GMP), routine microbial testing, and temperature-controlled storage to prevent contamination.

Absence of Antibiotic-Resistant Bacteria in Bottled Water

A significant public health concern in recent years has been the **emergence of antibiotic-resistant bacteria in drinking water supplies**. Studies have shown that **antibiotic-resistant strains of E. coli and Enterococcus** have been detected in groundwater and poorly treated drinking water sources, posing risks to **human health and antimicrobial treatment effectiveness** (Ye et al., 2013). The **absence of antibiotic-resistant bacteria** in the bottled water samples analyzed in this study is an encouraging finding, suggesting that **strict hygiene protocols and effective water treatment** minimize the risk of contamination with resistant strains.

However, antibiotic resistance remains a global challenge, and periodic surveillance should be conducted to ensure that **bottled water does not become a potential reservoir for resistant bacteria**. Future studies should incorporate **metagenomic sequencing techniques** to assess the presence of antibiotic resistance genes in bottled water microbiomes (Ahmed & Bajahlan, 2009).

Limitations of the Study

While this study provides valuable insights into the microbiological safety of bottled water in Saudi Arabia, some **limitations** must be acknowledged. First, the **sample size was limited to 50 bottled water brands**, which may not fully represent the entire market. Future research should analyze a **larger and more diverse sample set** to improve generalizability.

Second, this study focused only on **bacteriological parameters** and did not assess **chemical contaminants** such as **heavy metals, microplastics, or endocrine-disrupting compounds**, which may also pose health risks. Previous studies have detected **microplastic contamination** in bottled water, raising concerns about **potential long-term health effects** (Alzahrani et al., 2022). Future studies should incorporate **chemical and physical quality assessments** to provide a more **holistic evaluation of bottled water safety**.

Recommendations for Future Research and Policy

Based on the findings of this study, the following recommendations are proposed:

1. **Expand Sample Testing:** Future research should **increase the sample size** to include a **broader range of brands, production batches, and geographic locations**.
2. **Investigate Chemical Contaminants:** Additional studies should assess **heavy metals, pesticide residues, and microplastics** to ensure overall water safety.
3. **Evaluate Storage Conditions:** Research should examine the **effects of temperature fluctuations** on microbial contamination and bottle integrity.
4. **Strengthen Regulatory Compliance:** Periodic **audits and stricter enforcement** of bottled water standards should be implemented to maintain public health safety.
5. **Adopt Advanced Detection Methods:** Future studies should explore the use of **metagenomics and whole-genome sequencing** to detect emerging microbial threats.

V. Conclusion

The results of this study confirm that **bottled water in Saudi Arabia meets high microbiological safety standards**, with no **E. coli or coliform contamination detected**. The use of advanced water treatment technologies and **strict regulatory enforcement** has contributed to this **high level of safety**. However, continuous **monitoring, consumer education, and expanded research into chemical contaminants** remain essential to **further enhance bottled water quality and safeguard public health**.

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