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ORIGINAL RESEARCH PAPER

Evaluation of contamination and antibiotic susceptibility pattern of bacteria isolated from bottled water in Kabul, Afghanistan.

Hashmatullah Yousufi, Hafiza Hamid, Khadija Habibi khishki, Najla Haidari, Sahar Mohammadi
Kabul University

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Corresponding Author:
yousufihashmatullah@gmail.com

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A B S T R A C T

The study's objective was to assess the bacterial quality of some domestic brands of bottled water sold in Kabul, Afghanistan. A total of 60 bottled water samples from 20 different domestic brands were collected from the stores in Kabul and analyzed for TPC, TCC, and FCC. The inoculated plates were incubated at 37 °C for 24 – 48 hours. The antibiotic susceptibility tests for bacterial isolates were then performed. The result of the current study revealed that 55% of bottled water had bacterial contamination; 25% had total plate counts that were within an acceptable range, and 30% had counts that were higher than the acceptable range. 15% of the samples from three brands had coliform bacteria. All the samples were free of fecal coliforms. Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, and Staphylococcus aureus were isolated from contaminated samples. Serratia marcescens was ESBL-positive. The result of this study suggests that some of the bottled water brands sold in Kabul, Afghanistan, exceeded the limits set by the WHO. We recommend that MoPH/AFDA strictly monitor bottled water. The government authorities should visit and check these companies for QA and QC regulations on a regular basis.

Introduction

Potable water is crucial for survival, thermoregulation, metabolic processes, and the circulation-mediated delivery of nutrients to cells. Water supports the tissues and joints structurally (Mohamed, 2020). (Gurmeet Singh, 2020). Vital biological processes would cease without water (EFSA, 2011). Water serves as the solvent in the kidneys' removal of harmful metabolites. Water accounts for 50-60% of the human body (Mohamed, 2020). Access to and consumption of clean, drinkable water is important for achieving a healthy lifestyle (Mohamed, 2020). Safe potable water does not harm anyone, including those with impaired immune systems. Only 1.1 billion people have access to safe water (Shamsur Rahman, 2017). In many parts of the world, bottled water is considered safe to drink (Shams, 2019). The consumption of bottled water is increasing in most parts of the world as it is accepted as healthy drinking water. The unpleasant taste of municipal drinking water caused by chlorine is another factor (Derakhshani, 2018). According to the WHO, contaminated drinking water causes 485,000 deaths annually and approximately 80% of communicable diseases worldwide are water-borne diseases (Gurmeet Singh, 2020). The consumption of contaminated water is a risk factor for several diarrheal diseases, including Typhoid, cholera, and dysentery, which account for 2.2 million deaths annually. Roughly, 90% of children fall into this category, especially those in developing countries (Bibi Safia, 2018). It is possible to prevent 10% of disease in the world by improving water supply, hygiene and sanitation (Chauhan, 2017). The presence of different types of bacteria such as *Escherichia coli* (*E. coli*) in the water indicates fecal contamination (Chidya, 2019). Bottled water is found to be contaminated with number of bacteria, including *Vibrio cholera*, *Salmonella* species (spp), *Pseudomonas* spp, *Citrobacter* spp, *Acinetobacter* spp, and *Citrobacter violaceum* (Pant, 2016). Several pathogen strains have developed antibiotic resistance, and some of them have developed multidrug resistance (MDR) that resists several antibiotics (Hiroshi, 2009). Morbidity and mortality have increased along with the prevalence of infections caused by MDR bacteria (Yang, 2021).

According to UNICEF's Afghanistan WASH on the Brink 2022 report, around 8 out of 10 Afghans drink unsafe water. There are neither functional public water treatment plants nor water filtration systems available in Afghanistan (Hamdard, 2020). The majority of Afghans use shallow wells as a primary source of potable water, and roughly 27.5% of the Kabul population has access to a piped water supply network (Zahid, 2019). The majority of Kabul's drinking water sources, including qanats, open wells, tap water, and hand pumps, are contaminated with coliform and fecal coliform (Hamdard, 2020).That Due to the higher consumption of bottled water in Afghanistan, there are concerns about the quality of bottled water. The quality of bottled water is not subjected to strict quality control due to decades of political instability.

Material and Methods

In this cross-sectional study, 60 randomly selected samples of domestic bottled water (each bottle contains 500ml water) from 20 different brands sold in Kabul province were collected from different locations' shops in Kabul. All the brands were labeled with letters "A" to "T" in the field and transported to the microbiology lab of the Pharmacy faculty at Kabul University within three hours of being collected. The samples were kept between 1-4 °C. Samples were analyzed for total plate count, total coliform count, fecal coliform count, type of bacterial contamination, and antibiotic susceptibility pattern for the isolates. Each sample was cultured using both spreading and filtration techniques; In the spreading technique, 0.1 ml of water was inoculated at each of the Nutrient Agar, Manitol Salt Agar, and MacConky Agar (Oxoid Company), and for the filtration method, sample (for samples that did not grow in the spreading method, 100 ml of the sample was filtered directly, but for samples that did grow in the spreading method, 100 ml of the sample was filtered after dilution with sterile distilled water to dilutions of 1/100 and 1/1000.) was filtered using a cellulose nitrate filter with a of 0.45µm pore size (Sartorius Company). Thereafter, all the plates were incubated for 18 to 48 hours at 37 °C. Each plate was observed carefully. The colony morphology of various isolates was examined and recorded on the basis of size, pigmentation, form, margin, opacity, and elevation. A pure colony was chosen, and Gram staining was performed to evaluate the microscopic character of each isolate. The shape, size, arrangement, and Gram reaction properties of the isolates were carefully observed. In gram-positive cocci, the catalase test was used to differentiate *streptococci* from *staphylococci*, and the coagulase test was used to differentiate *Staphylococcus aureus* from coagulase-negative *Staphylococci*. An oxidase test was performed for gram-negative bacteria, and the biochemical characteristics of oxidase-negative bacteria were analyzed using the analytical profile index 20E (Biomerieux). The antibiotic susceptibility test was conducted using the Kirby Bauer disk diffusion method. Mueller-Hinton agar (Oxoid) was the culture medium utilized for the antibiogram. After a 24-hour incubation at 37 °C, the results were interpreted in accordance with CLSI-2020. Amoxicillin, Augmentin, Erthromycin, Gentamicin, Co-trimoxazole, Tobramycin, Ciprofloxacin, Levofloxacin, Ceftriaxone, Imipenem, Ceftazidime, Amikacin, Chloramphenicol, and Kanamycin disks from an Oxoid company were used for antibiotic susceptibility testing.

Results and Discussion

This study's objective was to evaluate the bacterial contamination and the antibiotic susceptibility profile of pathogens isolated from domestically produced bottled water brands in Afghanistan's capital city of Kabul. The price of bottled drinking water per dozen was found to be 76.3 ± 12.508 Afghani rupees. The result of the total bacterial count analysis of 60 distinct samples of bottled water from 20 different brands ranged from zero to 258,900 CFU/100 mL, with a mean ± SD 38,226.76± 70,775.01. 11 (55%) of 20 different domestic brands of bottled drinking water tested positive for bacteria. The total bacterial counts for 5 (25%) brands with a mean ± SD 88.26±38.06/100mL were under 20 CFU/mL, which is considered acceptable (European Parliament & Council of the European Union, 2009). The mean total bacterial count of 6 (30%) brands was higher than the acceptable range, the total bacterial count ranged from 12,900 to 258,900 CFU/100 mL with a mean ± SD of 89,122.2 ± 84,586.01, brands were above the permissible level (Table 1,2). Total coliform count results for 3

(15%) of the brands ranged from 3 to 160 CFU/100 mL with a mean ± SD 56.33 ± 66.55 (Table 2). None of the brands had fecal coliform contamination. The pH for 20 different brands ranged from 6.4 to 8.1, with a mean ± SD 7.4±0.7. *Staphylococcus aureus* was found in three brands (Q, R, and S), *Proteus mirabilis* was found in two brands (L, T), *Klebsiella pneumoniae* was found two brands (M, P), and *Pseudomonas aeruginosa, Enterobacter cloacae*, and *Serratia marcescens* were found in one brand (D). There was only one brand (S) where yeast contamination (*Candida* spp) was found.

Table 1. Properties of bottled water brands based on price, pH, total plate count, total colifrom count and fecal coliform count (count/100mL).

No	Company code	Price per Dozen	pH	Total Plate Count (mean±SD)	Total Coliform Count (mean±SD)	Fecal Coliform Count (mean±SD)
1	A	100	6.43±0.04	0	0	0
2	B	80	6.57±0.05	0	0	0
3	C	110	6.77±0.05	89.666±4.109	0	0
4	D	65	7.08±0.04	35,166.6 ±2,054.8	150±8.16	0
5	E	80	6.97±0.09	0	0	0
6	F	75	7.34±0.7	0	0	0
7	G	72	7.50±0.4	0	0	0
8	H	90	7.58±0.4	0	0	0
9	I	65	6.90±0.06	40,416.6±1,737.97	0	0
10	J	70	7.50±0.06	0	0	0
11	k	70	7.44±0.05	0	0	0
12	L	75	7.38±0.02	13,000±81.64	0	0
13	M	80	7.59±0.06	44,850±108.01	15±0.81	0
14	N	70	7.77±0.04	250,300±6,902.65	0	0
15	O	70	7.60±0.05	0	0	0
16	P	75	7.65±0.06	50±8.16	3±0.82	0
17	Q	80	7.70±0.02	150±8.16	0	0
18	R	65	7.70±0.06	101.66±10.27	0	0
19	S	80	8.00±0.08	50±8.16	0	0
20	T	55	7.54±0.08	151,000±5,354.12	0	0

Table 2. Total plate count, total coliform count and fecal coliform count for bottled drinking water sold in Kabul market with the range and mean ± SD.

Parameters	WHO/100mL	EPCEU[a]	Contaminated bottled water N (%)	Range per 100 mL	Mean± SD
Total plate count	< 1 CFU	< 20 CFU/mL	11 (55 %)	40-258,900	48,652.24± 76,602.86
Total coliform count	< 1 CFU	< 1 CFU/250mL	3 (15 %)	3-160	56.33± 66.55
Fecal coliform count	< 1 CFU	< 1 CFU/250mL	0 (00 %)	00	00

a. European Parliament & Council of the European Union, 2009

Table 3. The antibiotic susceptibility pattern of bacterial pathogens isolated from bottled water brands.

Pathogen	AMX	AMC	E	CN	SXT	TOB	CP	LEV	CRO	IPM	CAZ	AK	C	K	No of R
E. cloacae	S	S	R	S	S	S	S	S	S	S	S	R	S	R	3
K. pneumonia	R	R	S	R	R	S	S	S	S	R	S	R	S	S	6
K. pneumonia	S	S	R	R	S	S	R	S	S	S	S	R	S	S	4
P. mirabilis	S	S	S	S	R	S	S	R	S	S	S	R	S	S	3
P. mirabilis	R	S	S	R	S	S	S	S	S	S	S	R	S	S	3
P. aeruginosa	R	R	R	R	R	R	S	S	S	R	S	R	S	R	9
S. marcescens	R	R	R	S	S	S	S	S	S	S	S	R	S	R	5
S. aureus	S	S	S	S	S	S	S	S	S	S	S	R	S	S	1
S. aureus	S	S	R	S	S	S	S	S	S	S	S	S	S	S	1
S. aureus	S	S	R	S	S	S	S	S	S	S	S	S	S	S	1

AMX, amoxicillin; AMC, amoxicillin+ clavulanic acid; E, erythromycin, CN, Gentamicin; SXT, Sulfamthoxazole + Trimethoprime; TOB, Tobramycin; CP, Coprofloxacin; LEV, Levofloxacin; CRO, Ceftraxone; IPM, Imipenem; CAZ, Ceftazidime; AK, Amikacin; C, Chloramphenicole; K, Kanamycin; S, Sensitive; and R, Resistant.

Based on antibiotic susceptibilty testing for pathogen isolates, *P. aeruginosa* was resistant to nine antibiotics involved in this study. All the *Enterobacteriacae* isolates from water were resistant to at least three antibiotics and *S. marcescens* was ESBL. All three *S. aureus* isolated from samples were resistant to only one antibiotic (Table 2).

It has been shown in numerous studies that bottled drinking water contains microorganisms (Gurmeet Singh, 2020). Out of 20 domestic brands of bottled drinking water available in Kabul, 55% (11) brands had bacterial contamination; 25% of which was below the acceptable range (20 CFU/mL), and 30% which was beyond the acceptable limit. According to the total plate count collected in the current study. Our findings are quite similar to those of (Zeenat et al, 2009), who examined the microbiological quality of 75 samples from three brands in Taiwan and found that 28% - 68% of the samples exceeded the heterotrophic plate count standards. On the other hand another study in Taiwan evaluated 88 domestic bottled water samples, and found that 51.1% of domestic samples exceeded the heterotrophic plate count limit, which is higher than the finding of our study. A study in Hungary evaluated 492 mineral water samples; 12.4% of the samples had a heterotrophic plate count greater than 20 CFU/mL (Varga, 2011), which is lower than our findings.

As shown in Table 1, 15% of the brands were not compatible for the total coliform count, 10% contained *Klebsiella pneumoniae*, and 5% contained both Enterobacter cloacae and *Serratia marcescens*. The prevalence of coliforms in this study was higher than the 6.3% found in a study Hungary (Varga, 2011). Similar to studies done in Taiwan (Varga, 2011) and Greece (Emmanuel, 2008), none of the samples in the current investigation had *E. coli* isolated from them. Unlike to study in Hungary, where 1.4% of the samples contained *E.coli* (Varga, 2011), and Nepal, where 25% of samples contained *E. coli* (Pant, 2016), There was no fecal coliform contamination in any of the samples, which is parallel to studies in Nepal (Pant, 2016), Taiwan (Varga, 2011), and Greece (Emmanuel, 2008).

As shown in Table 3, in presentt study, different bacteria were isolated from different brands of bottled drinking water, including *E. cloacae, K. pneumonia, P. mirabilis, P. aeruginosa, S. marcescens, and S. aureus.* Other than *S. marcescens,* all other bacteria were isolated from bottled water in study carried out in Nairobi, Kenya (Safia, 2021), bacterial isolates isolated from this study are resistant to more antibiotic as those studied in Nairobi, Kenya.

Although this is the first study on the bacterial contamination of bottled drinking water in Kabul, Afghanistan, that we are aware of, our study has its limitations. We were unable to study all brands available in Afghanistan due to a lack of resources, and we only studied brands available in Kabul, the capital of Afghanistan. On the other hand, we were unable to fully identify all of the isolates due to the limited resources. The fact that the turbidity and chemical quality of the water samples were not evaluated is another drawback of this study.

Conclusion

Contrary to popular belief, bottled water is not entirely safe and bacterial-free. The results of this study indicate that some of the bottled water brands sold in Kabul, Afghanistan, exceed the limits set by the WHO, and some of them contain opportunistic bacteria that may not be safe for people with compromised immune systems. Due to the increasing use of bottled water in Kabul, we recommend that MoPH/AFDA register all the drinking water companies and strictly monitor the bottled water. The government authorities should visit and check these companies for QA and QC regulations on regular basis.

Conflict of interest

The author declares that there was no conflict of interest.

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