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BACTERIOLOGICAL STUDY OF COLIFORM BACTERIA IN TAP WATER FROM DIFFERENT AREAS OF QUETTA DISTRICT AND ITS COMPARISON WITH BOTTLED WATER

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Abstract

Quetta is a capital city of Balochistan with a population of 2.9 million. Clean water is basic need of human population. Present study was conducted to access the quality of water in targeted areas of Quetta. A total of 160 samples were collected randomly from four zones (North, South, East and West) of this city from October, 2015 to September, 2016. Lauryl Tryptose Agar was used to detect Coli form bacteria in tap water and bottle water. Among total 160 samples processed in current study, Coliform positive were (n=89) while coliform negative were (n=71). On other hand Tap water compared to bottled water was free of coliform.

Key Words: *Water, E. coli, Klebsiella, Citrobcter, Enterobacter*

INTRODUCTION

Water is essential for all cellular activities from a single cell life to multi-cellular, without water there is no life (1). It is the primary source of transmission of water borne diseases, such as typhoid fever and cholera (3). Escherichia, Enterobacter, klebsiella, Citrobacter and Serratia are the coliform or indicator bacteria (4) as their presence is indication of contaminated water. Coliforms can easily spread via water which is the cause of intestinal disturbance especially in infants, which results in diarrheal infection, dehydration, nausea, vomiting and ultimately lead to death (5).

Membrane filtration technique as well as most probable number technique can be used for checking coliform in water. In America and Europe, Lauryl Tryptose broth, a nutrient medium has been widely used for the detection of fecal contamination in water (Standard Methods for the Examination of Water and Wastewater, 1975).

Lauryl Tryptose Broth (LTB) and Brilliant Green Broth (BGB) both produce gas if the coliforms are present at 37^{0}C for 24-48 hrs, more specially the citrobacter freundii, enterobacter cloacae (7). LTB contains the high levels of succinic, acetic and propionic acids than BGB (21).

Major source of contaminated water causing diarrhea is *Escherichia Coli* (2). Even if a single colony of *Escherichia Coli* is present in the water sample then water is not beneficial for drinking. Fecal contaminants enter in the water from human and animal waste, by leakage of sewage pipes and mixing of this leakage with drinking water. On the other hand non-fecal contamination occur by connubial pets like cats, dogs, sheep, cows, goats, donkeys and horses in rural areas (11). Other sources for contaminating of water are rats, beavers, gulls, waterfowl and pigeons (12). Present study was therefore designed to assess the quality of tape water in selected area of Quetta and its comparison with bottled water.

MATERIAL AND METHODS

RESEARCH AREA

Study was performed at Quetta, which is capital city of province Balochistan which consists of 45% total land mass of Pakistan. Local inhabitants and residential of this Province relied on sustainable water sources like Karaiz, streams, waterfalls, wells and tube wells for thousands of years, present study is related to this area (14).

WATER SAMPLING

Total water samples (n=160) were collected from four zones North, South, East, and West of Quetta, (40 samples from each zone). 200ml water collected aseptically for each sample from selected area and were transported to lab and immediately processed. Similarly bottled water (n=25) were also processed for the presence of Coliform. Five available companies were selected (Five sample from each).

MEDIA AND REAGENTS

MacConkey agar, Lauryl tryptose (LTB) agar, Eosine Methyl blue agar, Simmion citrate agar, Tirple sugar iron agar, Methyl red & Voges proskauer Broth, Methyl red reagent, Kovacs reagent, Barriat's reagent, Urea broth, Crystal violet, Gram iodine, Hydrogen peroxide, Decolorizer solution were used as media and biochemical reagents.
The media and reagents were autoclaved for 15 minutes at 121^0C and then allowed to cool down up to 50^0C. Then dispensed in pre-sterile test tubes having air tight caps and agar media were poured into Petri plates, further allowed to solidify. The plates and tubes of liquid media were incubated at 37^0C for 24 to check the sterility.

MEMBRANE FILTERATION TECHNIQUE

Water samples were filtered through autoclaved/ sterilized water assembly containing 0.45 μm pore size filter membrane. Then filter paper was placed on Lauryl Tryptose agar plate.

ISOLATION AND IDENTIFICATION OF COILIFORMS

All samples were run on Lauryl Tryptose agar. Each colony was picked and inoculated on the eosin methyl blue (EMB), MacConkey agar plate for initial screening and then subjected to gram staining and Biochemical tests like Indole Production Test,

Methyl Red and Voges Proskauer Test, Citrate Utilization Test, TSI Test and Catalase Test for biochemical identification.

IDENTIFICATION OF COILIFORMSIN BOTTELED WATER

A total of twenty five (n=25) bottled water samples from five companies available in Quetta (Nestle bottled water, Aqua bottled water, Excellent bottled water, Yes bottled water, Aqua Funia bottled water). Five (n=5) samples from each company were assessed for coliform.

RESULTS

Among 160 water samples, n=89 (55.62%) were c*oliform* positive and n=71 (44.37%) were c*oliform* negative. Samples (n=40) were collected from each zone. Among c*oliform* positive North zone contain 12.49%, South zone 11.87%, East zone 18.75% and West zone contain 12.50% sample contaminated with coliform. Samples with total percentage of 29.37% found in the Domestic water, 18.12% coliforms found in the Restaurants water and 8.12% coliforms have been found in the water of Schools, colleges and universities. Detail is given in Table I.

Table I. Total percentage of contaminated water zone wise

	North No (%)	South No (%)	East No (%)	West No (%)	Total No (%)
Domestic water	12 (7.5%)	09 (5.62%)	15 (9.37%)	11 (6.87%)	47 (29.37%)
Restaurants	07 (4.37%)	07 (4.37%)	09 (5.62%)	06 (3.75%)	29 (18.12%)
School/ Colleges/ Universities	01 (0.62%)	03 (1.87%)	06 (3.75%)	03 (1.87%)	13 (8.12%)
Grand Total	20 (12.49%)	19 (11.87%)	30 (18.75%)	20 (12.50%)	89 (55.62%)

COLIFORM LOAD IN SOUTH ZONE

In south zone, n= 19 (11.87%) coliform samples were isolated. Out of which n=08 (5%) of coliform were isolated from domestic water, n=06 (3.75%) from restaurants and n= 05 (3.12 %) from School/Colleges/ Universities as shown in Table II.

Table II. Percentage and number of different *coliforms* isolated from water sample from South zone of Quetta

	E. coli (%)	*Klebsiella* (%)	*Citrobacter* (%)	*Enterobacter* (%)	Total (%)
Domestic water	02 (1.25%)	03 (1.875%)	02 (1.25%)	01 (0.625%)	08 (5%)
Restaurants	02 (1.25%)	02 (1.25%)	01 (0.625%)	01 (0.625%)	06 (3.75%)
School/Colleges/ Universities	01 (0.62%)	02 (1.25%)	01 (0.625%)	01 (0.625%)	05 (3.12%)
Grand Total	05 (3.125%)	07 (4.375%)	04 (2.5%)	03 (1.875%)	19 (11.87%)

COLIFORM LOAD IN EAST ZONE

In East zone, n= 30 (18.75%) coliform samples were isolated. Out of which n=10 (6.25%) of coliforms were isolated from domestic water, n=09 (5.62%) from restaurants and n=11 (6.87 %) from School/Colleges/ Universities (Table III).

COLIFORM LOAD IN WEST ZONE

In west zone, n= 20 (12.50%) coliform samples were isolated. Out of which n=09 (5.62%) of coliforms were isolated from domestic water, n=06 (3.75%) number of coliforms were isolated from restaurants and n=05 (3.12 %) coliforms were isolated from School/Colleges/ Universities (Table IV).

COLIFORM LOAD IN NORTH ZONE

In north zone samples n= 20 (12.50%) coliform were isolated out of which 09 (5.62%) of coliform were isolated from domestic water, 06 (3.75%) number of coliforms were isolated from restaurants and 05 (3.12 %) coliforms number from School/Colleges/ Universities (Table V).

Table III. Percentage and number of coliform isolated in from water samples collected from East zone of Quetta

	E. coli (%)	*Klebsiella* (%)	*Citrobacter* (%)	*Enterobacter* (%)	Total No (%)
Domestic water	04 (2.50%)	01 (0.625%)	03 (1.25%)	03 (1.875%)	10 (6.25%)
Restaurants	03 (1.87%)	02 (1.25%)	02 (1.25%)	02 (1.25%)	09 (5.62%)
School/Colleges/ Universities	03 (1.87%)	03 (1.875%)	02 (1.25%)	02 (1.25%)	11 (6.87%)
Grand Total	10 (6.25%)	06 (3.75%)	07 (4.37%)	07 (4.37%)	30 (18.75%)

Table IV. Percentage and number of coliform isolated from water samples collected from West zone of Quetta

	E. coli (%)	*Klebsiella* (%)	*Citrobacter* (%)	*Enterobacter* (%)	Total No (%)
Domestic water	05 (3.12%)	01 (0.625%)	02 (1.25%)	01 (0.62%)	09 (5.62%)
Restaurants	02 (1.25%)	01 (0.62%)	01 (0.62%)	02 (1.25%)	06 (3.75%)
School/Colleges/ Universities	01 (0.627%)	02 (1.25%)	01 (0.62%)	01 (0.62%)	05 (3.12%)
Grand Total	08 (5%)	04 (2.5%)	04 (2.5%)	04 (2.5%)	20 (12.50%)

Table V. Percentage and number of coliform isolated from water samples collected from North zone of Quetta

	E. coli (%)	*Klebsiella* (%)	*Citrobacter* (%)	*Enterobacter* (%)	Total No (%)
Domestic water	03 (1.87%)	04 (2.5%)	01 (0.625%)	01 (0.62%)	09 (5.62%)
Restaurants	03 (1.87%)	01 (0.62%)	01 (0.62%)	01 (0.62%)	06 (3.75%)
School/Colleges/ Universities	02 (1.25%)	02 (1.25%)	00 (0%)	01 (0.62%)	05 (3.12%)
Grand Total	08 (5%)	07 (4.37%)	02 (1.25%)	03 (1.87%)	20 (12.50%)

SELECTIVE MEDIA AND MORPHOLOGICAL CHARACTERISTICS OF *COLIFORM BACTERIA*

COLIFORMS ON MACONKEY AGAR

All 89 isolates of Coliform were streaked on MacConkey agar which gave pink color colonies due to lactose fermentation (Fig. 1a). Pink colonies on McConkey Agar were streaked on the EMB agar for further confirmation. Colonies giving Metallic sheen of *E. coli* (Fig. 1b). Circular mucoid purplish colonies of size 1-2 mm on EBM Agar were indicator of *Klebsiella* as shown in Fig. 1c. Round yellow colonies were indicator of

Citrobactor (Fig. 1d). Yellow Thick Mucoid colonies of *Citrobacter* on BGA are shown in Fig. 1e. Similarly dark purple mucoid colonies on EBM Agar were of Entrobactor (Fig. 1f).

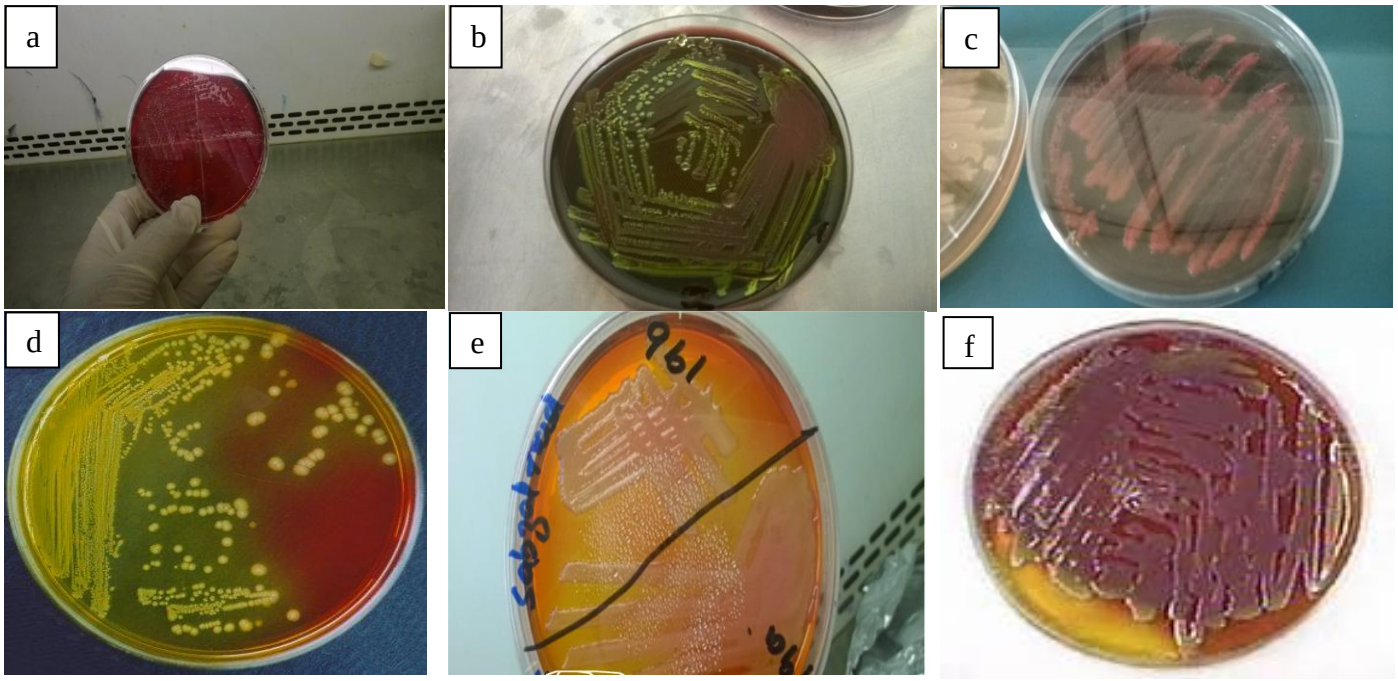

Fig.1. (a) Coliforms on MacConkey agar. (b) *Escherichia coli* showing metallic sheen on Eosin Methyl Blue agar. (c) Thick Mucoid colonies of *Klebsiella* on EMB. (d) Yellow Thick Mucoid colonies of *Citrobacter* on BGA. *(e)* Yellow Thick Mucoid colonies of *Citrobacter* on BGA. (f) Dark Purplish Mucoid colonies of *Enterobacter aerogenes* on EMB.

IMVIC TEST

For further confirmation of coliform IMVIC biochemical tests showing the positivity and negativity of *E. coli*, *Klebsiella*, *Citrobater* and *Enterobacter* isolates from water as shown in Table VI.

Table VI. Principle tests enabling differentiation of *E. coli*, *Klebsiella*, *Citrobater* and *Enterobacter* isolates from water

Tests	*E. coli*	*Klebsiella*	*Citrobacter*	*Enterobacter*
Indole Test	+	-	+	-
Methyl red Test	+	-	+	-
Voges-Proskauer Test	-	+	-	+
Citrate Test	-	+	+	+
Catalase Test	+	+	-	-

COMPARISON BETWEEN BOTTLED WATER AND TAP WATER

Similarly bottled water of the five companies Nestle Bottled water, Aqua Bottled water, Excellent Bottled water, Yes Bottled water and Aqua Funia Bottled water, and tap water from all four zones were tested. Five bottles (25 samples) from each company were tested. Each bottled water was tested on MacConkey agar and Lauryl Tryptose agar for the presence of coliform. No *Coliform* was detected in bottled water and 10 to >60% coliforms were detected in tap waters of different zones (Fig. 2).

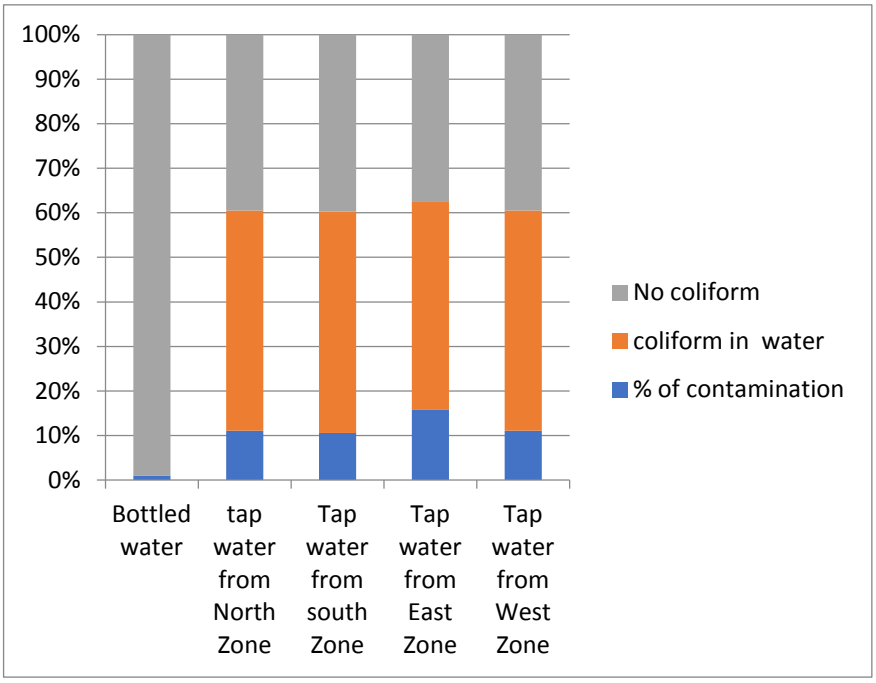

Fig. 2. Bar Chart; comparison of *coliform* between bottled and tap water

DISCUSSION

Water is essential for all cellular activities from a single cell life to multi-cellular organisms, without water there is no life without water (1). Contaminated water is source of water borne diseases and presence of *coliform* in drinking water is an indication of water contamination (3).

The most threatening coliforms are Escherichia, Enterobacter, Klebsiella, Citrobacter and Serratia (4). In underdeveloped countries, water quality via boiling can be improved to overcome the diseases many water borne diseases of infants, especially diarrhea, nausea, vomiting and dehydration (5). Water gets contaminated by different source like rats, rodents, swan, ducks and pigeons as well all other that live in water (12). Membrane filtration technique is the most probable number technique can be used for checking the presence of *coliform* in water (4).

Present study was designed to isolate coliform from tap water as well from bottled water. Water sampling from different zones of Quetta, the capital of Baluchistan were collected.

A total of 160 drinking water samples for detection of coliform were processed. Among these eighty nine (n=89) (55.62%) were *Coliform* positive while n=71 (44.37%) were *Coliform* negative. Equal number of Samples were taken from each zone (40 samples were collected from Each Zone) It was observed that out of *Coliform* positive North zone 11.25%, South zone 11.87%, East zone 18.75% and West zone contain 12.50% contaminated samples with total percentage of 29.37% found in the Domestic water, 18.12% coliforms found in the Restaurants water and 8.12% fecal coliforms have been found in the water of Schools, Colleges and Universities. East zone was highly contaminated with Fecal *Coliform* bacteria. Biochemical tests (like IMVIC) conducted for the identification of coliform along with morphological characteristics on different Media (21).

Similarly bottled water distributed by different companies was also checked for coliform contamination. Five companies were included in the study that is Nestle Bottled water, Aqua Bottled water, Excellent Bottled water, Yes Bottled water and Aqua Funia Bottled water. A total of 25 samples (5 bottles from each company were tested). None of the bottle was found contaminated. Therefore bottled water is more reliable and safe for use as shown by another study conducted by Amin & Ali 2015 (17).

Domestic water shown at the high percentage of contamination as discussed earlier secondly the high percentage of Fecal coliforms were observed at school, colleges and universities, third highly contaminated water percentage observed in restaurants because irregular monitoring of filters this makes favorable condition for bacterial growth. on the other hand underground water storage tanks mostly polluted with heavy dust which are not only responsible for bacterial growth however, also responsible for pathogenic fungal growth (Reynols *et al.,* 2005).

About 4-5 companies of bottled water were taken for checking presence of coliforms all these bottled water were tested 2-3 times for accurate and precise results, and these bottled water were mostly clear without any presence of coliforms either Nestle Pure life, Aqua bottled water so comparatively bottled water is now more reliable and hygienic for use, especially for the children from 1-5 years to avoid any severe diarrhea attack, dehydration, vomiting which leads to mostly death in children. It has been seen a great problem in children that contaminated water effects immediately and children keeps an immune system which is not strong enough to fight against these coliforms. Domestic Tap water should not be taken directly until and unless it is boiled if there is no proper filtration is present. On the other hand, many of the people as in the underdeveloped countries Pakistan can't afford the bottled water nevertheless it is recommended to use boiled water, when it is concerned with food and water drinking purposes.

It is another threatening situation that many of the filters which are used as drinking water they are mostly contaminated with coliforms as the purpose of filtration is totally failed the water sample from the filter water of University of Balochistan, CASVAB, filter water from Jinnah road bank Islami Quetta. All of the filters may have failed to filter water or there is no filtration of water was taking place at these plants, and people were using that contaminated water which was actually not properly filtered.

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