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# ISOLATION AND IDENTIFICATION OF COLIFORM BACTERIA FROM DRINKING WATER OF QUETTA BALOCHISTAN

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## ABSTRACT
Quetta is the capital of Balochistan and has approximately 2.8 million populations. Clean water is a basic need but unfortunately, water is one of the major problems in Quetta. This study was conducted to examine the water quality of target areas. A total of 240 samples were collected randomly from each zone (north, south, east, and west) of Quetta, during the periods of September (2022) to September (2023). Out of the total samples, 68.3% were coliform positive, 31.3% negative. Among coliform positive north zone was 18.3 %, the south zone was 15.8 %, the east zone was 14.12 % and the west zone was 19.96 %, contaminated with coliform bacteria (P= 0.13). Each zone was further divided into parts such as schools, hotels, and homes. The result revealed that schools, hotels and homes drinking water contains coliform bacteria with different percentages i.e. 17.49 % in schools, 24.16 % in hotels, and 26.65 % in homes. The total positive samples contain E. coli at 29.14%, Klebsiella at 21.21%, Citrobacter at 11.21%, and Enterobacter at 6.61 % in drinking water of Quetta. Isolated genera of coliform bacteria were grown on the selective media and confirmed through different biochemical tests (IMVIC and different sugar fermentation tests). Under the electron microscope, E. coli is 1.61 µm, Klebsiella 12.9 nm, Citrobacter 3.0 µm, and Enterobacter 0.4 µm in size.

**Key Words:** Water, Coliform, Pollution, Quetta, Balochistan

## INTRODUCTION
In the 21st century, approaching clean drinking water is the greatest challenge in worldwide. Water plays a paramount role in sound health. Water is a fundamental need for all living forms. About 75% of the earth's planet is covered with water of which only 2% is potable. A sufficient amount of water is needed for the survival of life but the human gets impended for this basic need (Bichi et al., 2002).
Different microorganisms enter into body through potable water and cause different diseases (Khan et al., 2013). Waterborne diseases are classified into main three groups: viruses, parasites, and microbes (Jabeen et al., 2011). These are the major sources to infect the water itself (Mehmood et al., 2013). The drinking water quality varies from time to time and place to place because of local

factors like irregular water supply, pipe leakage, irregular or backflow of water supply sewerage system run along the water supply line, distribution system, and different human activities (kanwal et al. 2015) superfluous monsoon rains, natural disasters like flood, human unawareness related to water quality, crude municipal water, different construction are the sources which contaminate the water biologically and give the drastic effect on the human health and responsible to spread the diseases (Anonymous 2009).

People of Pakistan are not well aware of the water quality and myths in their minds the water is clean through the natural purification process (Achakzai et al., 2014). Intestinal worms, diarrhea, typhoid, different strains of hepatitis, cryptosporidium, different types of infection, and gastroenteritis are the diseases that are caused due to the utilization of biologically contaminated drinking water (Haleem, 1996).

Coliform bacteria are gram-negative, rod-shaped, non-spore-forming, oxidase-negative, and lactose fermenters. They belong to the family Enterobacteriaceae and the main genera including Escherichia, Enterobacter, Klebsiella, and Citrobacter, are found in environmental water (Omari and Manu, 2012).

In this study coliform is the target organism from the drinking water of Quetta, as well as to investigate the quality of drinking water from Quetta schools, homes, and hotels and also to check the antibiotic effects against the isolated coliform bacteria.

## METHODOLOGY

### Study area

This study was conducted in the Quetta and the main targeted areas were schools, hotels, and homes were chosen for study to check the quality of the drinking water. This study was conducted in Quetta covering a 2656 $km^2$ area with an elevation of 1679m above sea level.

### Sampling size and sampling procedure

Samples were collected from each zone (north, south, east, and west) of Quetta, Balochistan. The samples were collected and examined from September 2022 to September 2023 to analyze their bacteriological characteristics all of the four seasons were passed during the sampling. A total of 240 random water samples were collected from the schools, hotels, and homes drinking water.100 ml of drinking sample was collected aseptically in the sterilized 200ml caped glass bottles.100ml of water sample were passed aseptically in BSL-II cabinet through mile pore assembly containing 0.45µm pore size cellulose nitrate sterile membrane filter (MF). The cellulose nitrate membrane filter containing the microorganism was transferred aseptically to the MacConkey agar plate and then incubated (facultative condition) for 24 hours at 37℃ to obtain maximum bacterial colonies.

### Isolation and identification of Coliform bacteria

Colonies were confirmed on the selective media, gram staining, and different biochemical tests.

### Antibiograms test

The antibiotic sensitivity test was done through the disc diffusion method. Turbidity of the test inoculums matched with the McFarland 0.5 standard (Baur et al., 1966) following clinical and laboratory standards institute (CLSI, 2008) protocol. Isolates organisms were considered as sensitive, and resistant to a particular antimicrobial agent based on inhibitory zone.

### Scanning electron microscopy

Scanning electron microscopy was performed to check the actual size and shape of the isolated genera of the coliform bacteria by taking the cultured colony and suspended into BPS (bathophenanthroline disulfonic acid) solution after the centrifugation shake with the 1.5% Glutaraldehyde and allowing for fixation then dehydrate the free cell with acetone. Take the drop on silver tape and allow drying in hot air. Dried-free cells are seen under the scanning electron microscope.

## RESULT

Among 240 drinking water samples, n=164 were coliform positive while n=76 negative. It was observed that out of coliform positive north zone was 18.32%, the south zone was 15.82%, the east zone was 14.16 and the west zone had 20% contaminated samples. The West zone was highly contaminated with coliform bacteria. Specially homes were highly affected as compared to hotels and schools as shown in Table 1. This data was statistically analyzed by using the chi-square test. The analysis suggests that there is no significant difference (P= 0.13) between locations.

**Table-1:** Total percentage of contaminated water zone-wise**.**

| | North | South | East | West | Total |
|---|---|---|---|---|---|
| **Homes** | 6.66% | 8.33% | 4.16% | 7.5% | **26.65%** |
| **Hotels** | 7.5% | 4.16% | 5% | 7.5% | **24.16%** |
| **School** | 4.16% | 3.33% | 5% | 5% | **17.49%** |
| **Total** | **18.32%** | **15.82%** | **14.16%** | **20%** | **68.3%** |

Chi- square = 19.35, P = 0.13

### North zone pathogens load

It was found that the presence of coliform bacteria in the north zone was 18.29%. Out of which E. coli was 10.41%, Klebsiella was 4.99%, Citrobacter was 2.07% and Enterobacter was 0.82% (Table-2). Homes contained coliform positive 6.65%, hotels 7.48%, and schools 4.16%. Hotels of the north zone were highly contaminated with coliform bacteria. High percentage of E. coli was observed in the north zone as shown in Table 2. For analyzing the data a chi-square test was used. The analysis suggests that there is no significant difference (P= 0.08).

**Table-2:** Pathogenic load in north zone of Quetta

| | E. coli | Klebsiella | Citrobacter | Enterobacter | Total |
|---|---|---|---|---|---|
| **Homes** | 4.16% | 1.25% | 0.83% | 0.41% | **6.65%** |
| **Hotels** | 5% | 1.66% | 0.41% | 0.41% | **7.48%** |
| **Schools** | 1.25% | 2.08% | 0.83% | 0% | **4.16%** |
| **Total** | **10.41%** | **4.99%** | **2.07%** | **0.82%** | **18.29%** |

Chi- square = 13.76, P= 0.08

### South zone pathogens load

It was found that the presence of coliform bacteria in the south zone was 15.8%. Out of which E. coli was 5.83%, Klebsiella was 6.24%, Citrobacter was 2.91% and Enterobacter was 0.82% (Table-3). Homes contained coliform-positive samples at 8.32%, hotels 4.17%, and schools at 3.32%. Homes in the north zone were highly contaminated with coliform bacteria and a high percentage of Klebsiella was observed as shown in Table-3. After statistical data was analyzed P= 0.869.

**Table-3:** Pathogenic load in the South zone of Quetta

| | E. coli | Klebsiella | Citrobacter | Enterobacter | Total |
|---|---|---|---|---|---|
| **Homes** | 3.33% | 3.33% | 1.25% | 0.41% | **8.32%** |
| **Hotels** | 1.25% | 1.25% | 1.25% | 0.41% | **4.16%** |
| **Schools** | 1.25% | 1.66% | 0.41% | 0% | **3.32%** |
| **Total** | **5.83%** | **6.24%** | **2.91%** | **0.82%** | **15.8%** |

Chi- square = 2.493, P= 0.869.

### East zone pathogens load

It was found that the presence of coliform bacteria in the east zone was 14.12%. Out of which E. coli was 5.41%, Klebsiella was 4.15%, Citrobacter was 2.91% and Enterobacter was 1.65% as shown in Table-4. Homes contained coliform-positive samples at 4.15%, hotels at 4.98%, and schools at 4.99%. Schools and hotels were more contaminated with coliform bacteria as compared

to home, with a high percentage of E. coli observed as shown in Table-4. After the static analysis P= 0.881.

**Table-4:** Pathogenic load in the east zone of the Quetta

| | **E. coli** | **Klebsiella** | **Citrobacter** | **Enterobacter** | **Total** |
|---|---|---|---|---|---|
| **Homes** | 1.25% | 1.66% | 0.83% | 0.41% | **4.15%** |
| **Hotels** | 1.66% | 1.66% | 1.25% | 0.41% | **4.98%** |
| **Schools** | 2.5% | 0.83% | 0.83% | 0.83% | **4.99%** |
| **Total** | **5.41%** | **4.15%** | **2.91%** | **1.65%** | **14.12%** |

Chi- square = 2.390, P= 0.881

**West zone pathogens load**

It was found that presence of coliform bacteria in west zone was 19.96%. Out of which E. coli was 7.49%, Klebsiella was 5.83%, Citrobacter was 3.32% and Enterobacter was 3.32% (Table-5). Homes contained coliform positive samples 7.49%, hotels 7.49% and schools 4.98%. Homes and hotels were more contaminated with coliform bacteria as compared to schools. A high percentage of E. coli was observed in west zone water samples as shown in Table-5. After the statistical calculation P= 0.25.

**Table-5:** Pathogenic load in the west zone of Quetta

| | **E. coli** | **Klebsiella** | **Citrobacter** | **Enterobacter** | **Total** |
|---|---|---|---|---|---|
| **Homes** | 1.25% | 3.33% | 1.66% | 1.25% | **7.49%** |
| **Hotels** | 3.33% | 1.25% | 1.25% | 1.66% | **7.49%** |
| **Schools** | 2.91% | 1.25% | 0.41% | 0.41% | **4.98%** |
| **Total** | **7.49%** | **5.83%** | **3.32%** | **3.32%** | **19.96%** |

Chi- square = 7.847, P= 0.25

**Identification of coliform bacteria through biochemical tests**

Coliform bacteria were identified through different biochemical tests, checked growth on selective media, and gram staining as shown in Table-6.

**Table-6:** Biochemical tests used for the identification of coliform bacteria

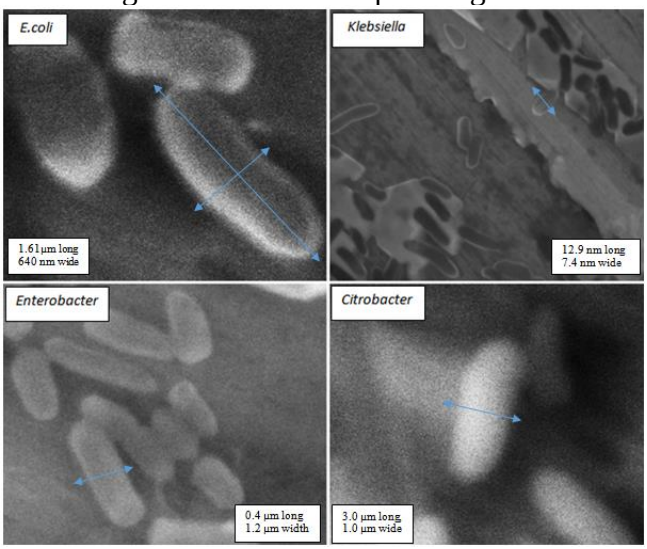
| **Biochemical test properties** | | **E. coli** | **Klebsiella** | **Enterobacter** | **Citrobacter** |
|---|---|---|---|---|---|
| Gram staining | | G- pink rod | G- pink rod | G- pink rod | G- pink rod |
| Motility | | + | - | + | + |
| Citrate test | | - | + | + | + |
| Indole test | | + | - | - | + |
| Methyl red test | | + | - | - | + |
| Voges- Proskauer | | - | + | + | - |
| Triple Sugar iron | Glucose | + | + | + | + |
| | Sucrose | + | + | + | + |
| | Lactose | + | + | + | + |
| Catalase test | | + | + | - | - |
| Urea hydrolysis test | | - | + | - | + |
| Gelatin hydrolysis | | - | - | - | - |
| Casein hydrolysis | | - | - | + | + |
| Ornithine decarboxylase | | + | - | + | + |
| Lysine decarboxylase | | + | + | + | - |
| Sorbitol fermentation | | + | + | + | + |
| Mannitol test | | + | + | + | + |

+ 100% positive, (+) 10-80% partially positive, - negative.

**Scanning electron microscope**

After the confirmation of the biochemical test, all the isolated microorganisms were seen under the scanning electron microscope. The isolated organisms were rod-shaped but different in size. Escherichia coli was 1.61µm long and 640 nm wide, Klebsiella was 12.9 nm long and 7.4nm wide, Citrobacter was 3.0 µm long and 1.0 µm wide while Enterobacter was 0.4 µm long and 1.2 µm in width as shown in the Figure-1.

**Figure-1:** Scanning electron microscopic image of coliform bacteria




**Antibiotic sensitivity test**

The antibiogram result revealed that Ciprofloxacin, Streptomycin, Chloramphenicol, and antibiotics were highly sensitivity against E. coli, Klebsiella, Citrobacter, and Enterobacter while Vancomycine, Lincomycine, Cefixime and Erythromycin showed complete resistance towards the E. coli, Klebsiella, Citrobacter and Enterobacter. Ampicillin and Tetracycline showed sensitivity towards Citrobacter while showing resistance towards E. coli, Klebsiella, and Enterobacter. Sulfamethoxazole showed sensitivity towards E. coli, Citrobacter, and Enterobacter while showing resistance towards Klebsiella. Colistin sulphate and Enrofloxacin showed sensitivity towards E. coli, Citrobacter, and Enterobacter while resistance showed towards Klebsiella as shown in Table-7.

**Table-7:** Antibiotic resistance and sensitivity test against coliform bacteria

| Class | | Antibiotics name | Pathogens | | | |
|---|---|---|---|---|---|---|
| | | | **E. coli** | **Klebsiella** | **Citrobacter** | **Enterobacter** |
| 1 | Quinolones | Ciprofloxacin | 30mm | 20mm | 20mm | 10mm |
| 2 | Aminoglycosides | Streptomycin | 15mm | 2mm | 20mm | 20mm |
| 3 | Pencillins | Ampicillin | Resistant | Resistant | 15mm | Resistant |
| 4 | Tetracyclines | Tetracycline | Resistant | Resistant | 10mm | Resistant |
| 5 | Glycopeptides | Vancomycine | Resistant | Resistant | Resistant | Resistant |
| 6 | Lincosamides | Lincomycine | Resistant | Resistant | Resistant | Resistant |
| 7 | Other | Chloramphenicol | 30mm | 25mm | 20mm | 17mm |
| 8 | Cephalosporins | Cefixime | Resistant | Resistant | Resistant | Resistant |
| 9 | Sulfonamides | Sulfamethoxazo | 25mm | Resistant | 11mm | 10mm |
| 10 | Macrolides | Erthromycine | Resistant | Resistant | Resistant | Resistant |
| 11 | Polypeptides | Colistin sulphate | 15mm | Resistant | 10mm | 7mm |
| 12 | Flouroquinoline | Enrofloxacin | 35mm | Resistant | 19mm | 13mm |

## DISCUSSION

This study was designed to isolate the coliform bacteria from the drinking water of Quetta (Hotel, Home, and Schools). Water samples were collected from different sources which Quetta people

utilized for drinking purposes. A total of 240 samples were collected out of them 68.33% were coliform positive, and 31% were coliform negative. Among the positive samples, E. coli was 42.68%, Klebsiella was 31.09%, Enterobacter was 9.75% and Citrobacter was 16.46%. The result also revealed that schools, hotels and homes drinking water contains coliform bacteria with different percentages i.e. 17.49 % in schools, 24.16 % in hotels, and 26.65 % in homes. Among coliform positive north zone was 18.3 %, the south zone 15.8 %, the east zone was 14.12 % and the west zone was 19.96 %, highly contaminated with coliform bacteria. Different biochemical test was performed for the conformation of coliform bacteria from the drinking water of Quetta, All the morphological characteristics were as same as described by (Mukhtar et al., 2001, Sharma et al., 2006).

The antibiogram result revealed that Ciprofloxacin, Streptomycine, Chloramphenicol, and antibiotics were highly sensitivity against E. coli, Klebsiella, Citrobacter, and Enterobacter while Vancomycine, Lincomycine, Cefixime and Erythromycin showed complete resistance towards the E. coli, Klebsiella, Citrobacter, and Enterobacter. Ampicillin and Tetracycline showed sensitivity towards Citrobacter while showing resistance towards E. coli, Klebsiella, and Enterobacter. Sulfamethoxazole showed sensitivity towards E. coli Citrobacter, and Enterobacter while showing resistance towards Klebsiella. Colistin sulphate and Enrofloxacin showed sensitivity towards E. coli, Citrobacter, and Enterobacter while resistance showed towards Klebsiella as same as described by (Din et al., 2014).

Quetta home water contamination percentage was high as compared to schools and hotels because of irregular monitoring of filtrates this situation makes the favorable condition for bacterial growth. Another source of infecting the house water is the underground water storage tanks mostly which are polluted with heavy dust which not only gives coliform bacterial growth but also supports other pathogenic fungal growth as described by (Borchardt et al., 2004).

Hotel water contains a moderate number of coliform bacteria, most of the restaurants utilize dig well water which quality is better as compared to that hotels utilize tanker water, during transportation the water quality degrades as well and the crockery that is used in the hotels is not as clean as's the reason to contaminate the water are responsible to spread the diseases.

In our collection that was seen most of the schools have their dig wells and filters, regular monitoring was done by the school's administration while during collection few schools utilized tanker water which is contaminated.

## CONCLUSION

The main issue is to contamination of the drinking water in Quetta, is water pipeline runs along with the drainage pipeline, rusted pipelines, irregular or backflow of water supply, and mishandling during transportation are the sources of contaminating the water.

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