

Evaluation of Coliform Bacteria From Cattle Waste and Drinking Water of Quetta City, Balochistan

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Abstract

*All organisms, including humans, require water to survive on a global scale. Because of this, it is crucial for life to have access to adequate water supplies. Scientists and public health organizations are growing more concerned about bacterial pollution of surface waterways because pathogenic microorganisms can have detrimental impacts on human health. In this article, the regional and seasonal variability of faecal coliform bacteria (FCB) concentrations was examined. The purpose of this article was to assess the microbial quality of the drinking water supplied in Quetta city, Balochistan. The distribution of water at the surface was examined using various standard techniques, and the results indicated successful bacterial isolation. The conclusions showed that coliform group (*Klebsiella* sp., *Citrobacter* sp., *Enteriobacter* sp.), and fecal coliform (*E. coli*) were identified. Seasonal and geographic differences in the types, numbers, and quality of isolated bacteria were assessed and addressed.*

The evaluation of coliform bacteria from cow dung and drinking water in Quetta, Balochistan, has only received a little amount of attention thus far. So, while working on a new project, think about evaluating the coliform bacteria in cattle and drinking water. The number of potential pathogens is vast, and pathogen concentrations from faecal contamination are frequently low. As a result, it is impractical to analyze each water sample for pathogens. In contrast, the detection of pathogens is done indirectly by looking for "indicator" organisms like coliform bacteria. Data from one-year assessments of the microbiological quality of various faecal coliform species in drinking water and animal waste were obtained for this study from urban, rural, and private sources in Quetta. 50 samples in all, 25

from drinking water and 25 from animal waste were collected. The results indicated that faecal coliform, Escherichia coli (22,3%), and Klebsiella (2%), respectively, were the most and least common bacteria in urban water supplies. In private sources, E. coli (34%) and Klebsiella (1.3%) were the most and least prevalent, respectively, followed by E. coli (45.9%) and Enterobacter (2.6%) in rural water sources.

In conclusion, the results of this research demonstrate that all water samples were contaminated with total and faecal coliform bacteria, typically with large numbers of colonies, which poses a serious danger of waterborne illnesses for the general public, especially young children.

Key words: Biochemical analysis, Coliform bacteria, Drinking water, Animal waste, Quetta

Introduction

Water is necessary for all living things, including people, to survive globally. Because of this, having access to sufficient water supplies is essential for life. One typical explanation is that only 3 % of the land is covered by freshwater, and of that, only 0.01 % is accessible to humans and the rest is frozen in glaciers. There is not much water that is readily accessible. This response is partially accurate. Assessments should focus primarily on the flows of water resources rather than only the stocks (Liu et al., 2017; Rodell et al., 2016). Only a small amount of water is easily available. The total volume of water stored in all rivers is only 2000 km³, compared to the annual water extraction of 3800 km³/year. A more precise measure of water availability is the yearly discharge of 45,500 km³/year. The WHO estimates that 4000 children die from various water-borne diseases every day (Billa 2019). The prevalence of water-borne infections is fairly high in rural regions as a result of untreated surface water coming directly from water reservoirs used for drinking and other domestic purposes (Plaatjie 2019). In Pakistan, the similar issue occurs, with 44% of the population lacking access to clean drinking water, with rural areas having a higher percentage of this population (Ahmed, Zounemat-Kermani, & Scholz 2020). It is likely that open reservoir water contains a number of unidentified pollutants and germs, including bacteria, protozoa, viruses, and other creatures, despite the fact that it is presumed to be both safe and clean. For a healthy existence, freshwater reservoirs should be

free of certain diseases and pathogens because if the water is contaminated with heavy metals and microbes, it would be harmful to not only people but also to all other organisms (Haldar et al., 2022).

The Pakistan Council for Study in Water Resources (PCRWR) in, 2005 performed research across Pakistan, notably in major cities like Karachi, Lahore, and Islamabad, to determine the presence of bacteria, mainly coliforms. The findings revealed that almost all samples had contamination from *E. coli* and faecal coliforms. Studies show that 74 percent of Islamabad's water reservoirs contain coliform, and that 41 percent of the city's water supply is contaminated with *E. coli* (Akhtar et al., 2019; Khan 2020). The fact that water is a complex fluid in and of itself does not imply that it is the most suitable solvent for life (Stanley et al., 1999; Benner et al., 2010). Water regulation, wetting properties, transport, osmosis, and other factors are significant at scales spanning from the cellular to the organismal, and unquestionably to entire ecosystems and habitats. Coliform bacteria, and more frequently faecal coliform bacteria, have been used as indicators of faecal contamination. Other bacterial diseases include intestinal infections brought on by *Pseudomonas aeruginosa*, *Klebsiella*, and *Escherichia coli* strains, which are often only observed in sick or diseased persons. These microbes are all found in human waste and, more specifically, wastewaters, exhibit the least amount of prospective health hazards (Hsu et al., 2021).

In reference to the fact that approximately 20% of the world's population lacks access to clean water and that 0.4% pay a premium for living in conditions that are unacceptably polluted (Capodaglio 2021). Pathogens found in water cause more than 2 million deaths worldwide each year, with most of these deaths occurring in children under the age of 5 years. A major fear for public health is the significant chemical contamination of surface and groundwater, which could have detrimental long-term effects on both human and aquatic life.

In developing countries like Pakistan, diseases associated with filthy water and poor hygiene continues to be a serious public health concern. Only *E. coli* is responsible for the deaths that have occurred globally (WHO 2003). Therefore, it is essential to have efficient methods for isolating and identifying these harmful coliform bacteria. Therefore, the objective of this study is to identify and assess particular coliform bacteria in samples of the drinking water and faeces of cattle in Quetta.

According to Sartor (1972) that the main problem facing humanity in the twenty-first century, is water quality. We currently discuss the main types of marine toxins, their effects on human health, and strategies for minimising contamination of freshwater resources. The importance of chemical pollution is highlighted, particularly that caused by natural and inorganic micro pollutants, dangerous metals and metalloids (Sartor & Boyd 1972). Walker (2019) reported that Bacteria are crucial in detecting the quality of water, because particular organisms can be used as indicators of different forms of contamination. Other species are unable to mineralize things, whereas bacteria may do it through a number of metabolic processes. Examining the shortcomings of the commonly used coliform processes, new techniques for identifying organisms found in faeces are proposed (Walker et al., 2019).

Amare et al., (2019) identified that the most important bacteriological problem, is the spread of disease, which is why public health is an issue. The bacterial effluence of the soil brought on by the application of manures and sludge to the land can have an effect on the original soil populations as well as the rates of soil disintegration and nutrient recycling (Amare et al., 2019). Hagey et al., (2019) studied samples of dairy fertiliser slurry from several farms, they found a variety of unusual bacterial infections (Hagey et al., 2019).

Boccatto (2018) investigated that it was discovered that the overflow from beef feedlots contained *Salmonella infantis*. A last method being studied is the spread of disease through vegetative matter that has been watered with liquid manure or other waste effluents. Direct feeding of cattle in these locations, as well as harvested crops that are either sold for human consumption or used as trash, might result in bacterial drinking (Boccatto 2018).

Methodology

Study Design

The current study was carried out in Quetta, Balochistan, Pakistan between 2018 and 2019 (30° 10' 59.7720" N and 66° 59' 47.2272" E) (Figure 1). The region is upland in the province, 1,679 meters above sea level, with an average annual rainfall of 38 to 40 millimeters. During the average

temperature over the study years varied from 38°C in the summer to -3°C in the winter.

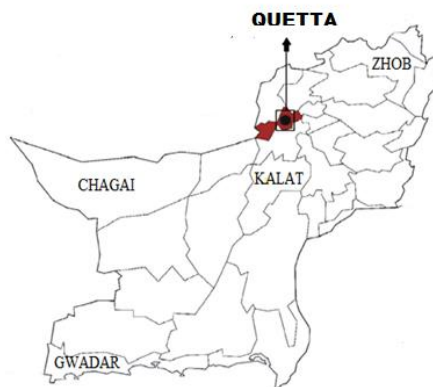


Figure 1: On the province of Baluchistan's map, the study area is shown in black, whereas Quetta District is shown in red.

Preliminary Studies

We were searched for relevant research papers on the subject of "Evaluation of coliform bacteria from cow waste and drinking water of Quetta city" using various web tools like Mendeley and Google Scholar. We'll concentrate on current works that were published between 2010 and 2020.

Sample Collection

From the city of Quetta, 50 samples total—25 from drinking water and 25 from animal waste—were taken. In accordance with the accepted procedure of collecting water for microbiological examination from various sites, samples were randomly gathered near the source of the drinking water. They were then placed in clean, leak-proof glass bottles for later analysis to count the faecal coliform bacteria.

Under well sterilized and well protected atmosphere specimens were collected randomly from drinking water, house hold taps from where water was just coming from sources and were preserved in 10ml labelled specimen bottles/tubes according to collection number. The Collected specimens were taken to the central laboratory of Microbiology CASVAB on the same for detailed description and cultured them on (VRBA) violet red bile agar. Gloves were used during sample collection and tap is first

sterilized by burning flame just before water taken from it. During sample collections it must be noted that tubes which was opened and closed and no internal surface was touched. At a time, 5 samples were collected and inoculate on media. VRBA is differential media for coliforms and was manually prepared by using proper proportion of necessary ingredients by pouring them in a conical flask dissolved in needed distilled water. boil the mixture on hot plate with magnetic stirrer while mouth of flask was covered after 2 to 3 boils then it was to cool down to room temperature and pour prepared media in sterilized media plates in laminar flow and was allowed to settle by using sterilized glass spreader sample was spread on plates. Plates were covered by lids and put upside down for incubation in incubator on 37⁰ for 24 hrs.

Identification of coliform species from water and animal waste sample

- Fecal waste samples were collected from different sites and at the same day suspended in 9% solution of NaCl which was equal to 1ml. When it was dissolved and settled at the bottom of the solution then one loop from the solution were taken and placed at the culture media for the identification of coliform bacteria.
- For drinking water 1 ml aliquot from each sample were inoculated with the spread plate method into VRBA (Violet Red Bile Agar) and incubated at 37c^o for 24 hrs.
- Next day the number of colonies were counted and noted down.
- Moreover, each colony representing different colors was subjected to gram stains and microscopic observation.
- For identification, biochemical tests such as the catalase test, indole test, MR/VP test, nitrate reduction, and sugar fermentation were carried out after gram reaction and microscopic observation.

Data Analysis

Graph Pad Prism (version 6) was used to analyze the data, and the mean and standard deviation of the results were shown. A two-way ANOVA and Turkey's post hoc analysis were conducted. When differences had a P-Value of less than 0.05, they were deemed significant.

Table 1: Location of the Stations in Quetta Included in the Study

Site number	Location of drinking water and cattle waste
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Site No.1	Brewery Road
Site No.2	Shahbaz Town
Site No.3	Sabzal Road Quetta
Site No.4	Wahdat Colony Quetta
Site No.5	Nawa Killi Quetta
Site No.6	Gulistan Road Quetta
Site No.7	Jinnah Road Quetta
Site No.8	Alamdar Road Quetta
Site No.9	TNT Colony Quetta
Site No.10	Railway Colony Quetta
Site No.11	Chaman Housing Scheme Quetta
Site No.12	Saryab Road Quetta
Site No.13	Joint Road Quetta
Site No.14	Gulshan Road Quetta

Biochemical tests

To apparently detect coliform bacteria, a variety of biochemical assays (such as urease test, oxidase test, catalase test, fermentation of sugar test, fermentation of arabinose, fermentation of fructose, fermentation of glucose, fermentation of galactose, oxidase test, MR test, VP test, oxidative/fermentative test, indole test, citrate test, H₂S test, gram staining test and nitrate reduction test) were carried out. The subsequent biochemical tests were carried out as a result.



Figure 2 (a): Biochemical test for E.coli.



Figure 2 (b): Biochemical test for Klebsiella.

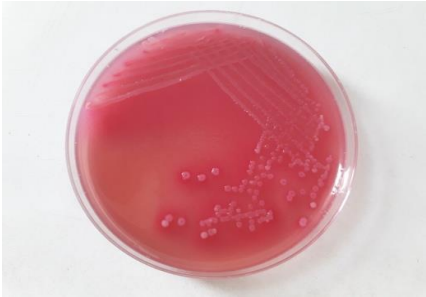


Figure 3 (a): Isolated colonies of E.coli on VRBA.

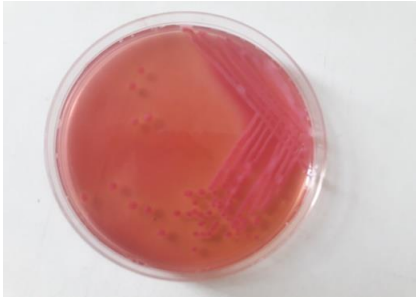


Figure 3 (b): Isolated colonies of Klebsiella on VRBA.



Figure 4: Oxidase test for colonies

Table 2: Test results of Coliform bacteria in drinking water and animals waste

(E. coli, Citrobacter, Klebsiella, Enterobacter)

S. No.	Names of Bacteria	Indole test	MR test	VP test	Citrate test	Urease test	Oxidase test	Nitrate reduction test	Catalase test	Galactose test	Glucose
1	Escherichia	+ ve	+ ve	- ve	- ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
2	Citrobacter	+ ve	+ ve	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve

3	Klebsiella	- ve	- ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
4	Enterobacter	- ve	- ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve	+ ve

Results and Discussion

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The results showed the distribution of fecal coliforms in urban, rural, and private water resources of Quetta city based on test results. The two bacteria that were most and least prevalent in urban water systems were *Escherichia coli* (22.3%) and *Klebsiella* (2%) correspondingly. *E. coli* (34%) and *Klebsiella* (1.3%) were the most and least prevalent pathogens in private sources, respectively. *E. coli* (45.9%) and *Enterobacter* (2.6%) were the next two most prevalent bacteria in rural water sources. *E. coli* was the most and least frequent bacterium discovered in private sources (34 % and 1.3 %, respectively). *E. coli* (45.9 %) and *Enterobacter* (2.6 %) were next in prevalence in rural water springs. Table 3 show the distribution of fecal coliforms by month of the year in the Quetta city's water sources. The results showed that *Klebsiella* had the lowest distribution (2.8%) and *E. coli* had the highest (an average of 38.1%). The dispersion of fecal coliforms significantly decreased, according to the data.

Table 3: Season wise distribution of faecal coliform in Quetta's drinking water of the year 2018-19

Seasons	Samples	Citrobacter	Escherichia	Enterobacter	Klebsiella	No faecal coliform
Spring	3	14.2	36.2	11	0.7	38
	2	10.8	41.7	8.8	4.3	24.3
	2	12.9	40.6	21.8	0.8	23.9
Average number of colonies	7	5.4	16.92	5.94	0.82	12.31

Summer	5	14.9	45	14.7	2.8	22.6
	3	19.7	36.1	16.5	6.2	21.5
	6	17	47.5	11.3	0.4	23.9
Average number of colonies	14	3.6	9.1	3.0	0.67	4.8
Fall	6	13.6	33.4	19.3	0.4	28.8
	4	8.9	38.6	16.1	1.7	34.7
	2	13.7	31.8	17.8	0.5	36.3
Average number of colonies	12	3.0	8.65	4.43	0.21	8.3
Winter	5	4.7	31.5	23.1	4	36.7
	4	11.7	27.4	13.8	6.4	40.6
	8	7	37.9	9.9	0.9	44.3
Average number of colonies	17	1.37	5.69	2.75	0.66	7.15
Collective average number of all colonies		12.42	38.1	184.1	2.8	29.3

The results revealed that, *Escherichia coli* is the most prevalent in water samples of urban, rural, areas however, its prevalence was found to be high in rural areas than urban. This might be because of the cattles and dairies were found to be near water bodies in rural areas. *E. coli* is the most prevalent faecal coliform species relative to other faecal coliform species in water resources, which may be due to the organism's greater resistance to environmental conditions, pH temperature, and its ability to penetrate deep in the soil (Mirzaei et al., 2015). High levels of *E. coli* can also be caused by improper hygiene practices and a lack of proper water purification in these areas (Admassu, Wubshet & Gelaw 2004). This is supported by the findings of other researchers from other parts of the world, who discovered that *E. coli* was the most prevalent coliform in the water sources they examined (Apha 2055 & Nounou et al., 2013).

Jagals *et al.*, (2013) from Australia also revealed that 46% of the samples collected from South African rural areas contained *E. coli*. *Escherichia coli* levels were observed to be 35.7, 28.6, and 50%, respectively, in protected springs, wells, and pipelines in Ethiopia (Jagals et al., 2013). The present study further showed that, faeces were present in 50% of unprotected wells and springs in the rural areas.

Citrobacte, *Escherichia*, and *Enterobacter* were found to be the most frequent in water resources during the summer months, whereas *Enterobacter*, *citrobacter*, and *Klebsiella* were found to be the most

prevalent during the winter months. It demonstrates that faecal coliform bacteria infection is a possible throughout the year, as many variables, such as rainfall, temperature, lack of continuing monitoring, a disjointed distribution system, the type of water source, and presence/grazing of animals near water bodies etc., place a pivotal role in the spread of these genera. As a result, there are some variations in the reporting of faecal coliforms at various intervals.

According to Gwimbi (2011), 71% of water sources contained *E. coli* during spring at open wells. Other researchers also demonstrated that *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter* (Gwimbi 2011). All had different distribution percentages of Surface water, groundwater, and drinking water all contain Enterobacteriaceae: 52.9, 44.1, 2.9, 62.5, 33.9, and 3.6; 45.2, 45.2, and 6.5; and 27.3, 72.7, and 0 % for other bacteria (Lösch et al., 2008). *Escherichia coli* infection rates were recorded at 10.7%. The findings of this study indicated that compared to other sources, rural water had a high prevalence of *E. coli* (%) and other faecal coliform species. This is attributed to least protection measurements, sensibility, disinfected method and more susceptible to environmental toxins in these areas. The results showed that total coliform bacteria (TCB) and fecal coliform bacteria were the only bacterial pollutants in drinking water (FCB). The water analysis quality of other researchers revealed that coliform bacteria is an important indicator to determine the drinking water quality (Szabo & Hall 2015). These bacteria increase the risk of diseases in all organisms including human. Beside these fecal Coliform this drinking water may also carry certain other infections like *Giardia*, *E. histolytica*.

Water hardness has long been considered to be a significant contributing factor to membrane filter clogging. The pH level and the total dissolved matter can affect how quickly bacteria thrive. The lack of data regarding the pH and TDM put a huge gap to guide and educate the owners properly regarding the quality of water (Pratum & Khananthai 2017). This gap should be filled by having an accurate, up-to-date record, change the filter twice a year, and test the water quality every three months.

Microbial contamination has been identified as one of the major issues in both rural and urban areas of Pakistan resulting pipe leaks, sewage line contamination getting into drinking water supplies, and other factors. Approximately 100 million instances of diarrhoea are reported in Pakistan each year, because water becomes more susceptible for disease producing organisms due to presence of coliform bacteria (Daud et al., 2017).

Conclusion

In conclusion, the region of Quetta has inadequate sanitary standards, a normal percentage of literacy among the general population, poor health management, and a risk factor for coliform bacteria infection that is easily disseminated, especially among youngsters. In Conclusion, Water samples from Quetta had a microbiological load that was higher above the Pakistan EPA's allowed limits. The transfer of organic waste from animal barns to water sources via runoff of rainwater into wells and sewage seepage are three potential sources of the microbial contamination. All samples contained tolerable levels of harmful metal concentrations, which may not pose a threat to consumers. To stop further contaminating the drinking water, it is advised that the area's drinking water sources be carefully monitored and safeguarded.

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