MICROBIOLOGICAL AND CHEMICAL QUALITY ASSESSMENT OF RESTAURANTS' DRINKING WATER SUPPLIES USING MPN INDEX AND PH VALUE IN PESHAWAR CITY, PAKISTAN

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Abstract

Background: Most of water-related illnesses and acute diarrheal cases can be traced back to poor microbiological water quality and unhygienic sanitary practices. The widely accepted standard in literature for Microbiological quality of drinking water is that an "Ideal' drinking water sample should have a Most Probable Number / 100 ml value of 0. But samples having value of 1-10 can be labelled as "Acceptable" given that they are subjected to regular water quality analysis.

Objective: To assess the chemical and biological properties of drinking water supplies in local restaurants of Peshawar city, Pakistan.

Methods: A cross-sectional study in which 50 drinking water samples collected from local restaurants of Peshawar city using non-probability convenient sampling technique were assessed for "Total Coliform Organisms" by using "Multiple Fermentation Tube" or "Most Probable Number" technique using MacConkey's broth and pH was measured by using Digital pH meter.

Results: pH values of water samples were within the World Health Organization's standard permissible limits. 64% of water samples had MPN /100 ml value of >10 while 36% had value in the range of 1-10 with no water sample having a value of 0.

Conclusions: The results draw attention to a considerable environmental and human health dilemma that is the contamination of drinking water. Suggestions include treating the water via a filtration and chlorination unit before distributing it, carrying out regular assessment of water quality and enhancing water quality safety measures in such places as restaurants.

Keywords*:* Biological & Chemical Assessment, pH, Presumptive Coliform Test, Pakistan, Water Quality

Introduction

Water plays a pivotal part in every field of life and human physiological processes including growth and development (1). A thorough review of literature reveals that many of the water-related and water borne illnesses that have been known to claim many lives in some of the most populous portions of the world occur because of poor drinking water quality (2). It has been estimated that water related diseases and disasters account for death of almost 6-8 million people yearly around the world (3). In the developing countries, 2.4 billion people live without an access to adequate sanitation. Thus it is not surprising when studies show that 80 percent of all diseases and deaths in this portion of the world are mainly caused by lack of adequate sanitation, safe drinking water and hygienic sanitary practices thereby resulting in the death of a child in every 8 seconds (4).

Technological developments which on one hand have led to advancements in health care sector, on the other hand are also associated with physical, biological and chemical contamination of drinking water resources, the most important of which is contamination of biological origin causing acute and chronic diseases or even death (5). Contamination of water by human and animal fecal

matter is indicated by the presence of coliform bacteria in water samples (6).

The sum of the physical, chemical, biological, and radiological characteristics of water constitutes Water Quality (7) as per World Health Organization (WHO) Guidelines for Drinking Water Quality (GDWQ) 4th edition (8). For the biological parameters "Indicator organisms" are detected and measured. An appropriate indicator of microbiological quality of drinking water is "Coliform organisms", which are gram-negative rod-shaped bacteria, because they are easy to detect and enumerate in water (8).

In previous literatures, coliform bacteria were classified in the genera *Citrobacter*, *Klebsiella*, *Escherichia*, and *Enterobacter*. However, the revised and modern taxonomical methods include both lactose fermenting bacteria (*Citrobacter freundii, Enterobacter cloacae* etc.), which grow in a nutrient rich environment be it polluted drinking water, soil, feces or decaying plant material as well as species that multiply in relatively clean drinking-water and are seldom detected in feces e.g. *Rabnella aquatilis*, *Buttiauxella agrestis* and *Serratia fonticola* (9). The Total coliform test thus serves as an indicator of water treatment efficacy and integrity of the water distribution system (9). Despite having minimal direct effect on health, pH of drinking water is also an important characteristic because indirectly it can provide a favorable environment for growth of pathogens. When water pH increases and moves into alkaline range, it causes water to become bitter in taste (10).

In Pakistan, distribution lines of drinking water and drainage system lines are placed side by side, causing water quality to deteriorate as a result of leakage and intermixing (11). Ground water which can contain many microbes like viruses, bacteria and protozoa and which is the main source of water supply for most cities of Pakistan has been studied to cause 2.5 million endemic diarrheal deaths each year (12).

Poor water quality accounts for 30% and 40% of all diseases and all deaths respectively in Pakistan. Studies reveal that a leading cause of infant and child death in Pakistan is diarrhea (13). Amongst 450 children under the age of 5 years admitted to 3 teaching hospitals of Peshawar, frequency of diarrhea was found to be 59% i.e. 264 children (14). A study on 288 children from rural areas of Peshawar reported an overall 41% prevalence (n=17) of parasitic infestation (15). Of the 100 patients of Pyrexia of Unknown origin admitted to Department of Medicine of Khyber teaching Hospital Peshawar from March 2008 to March 2009, 7% of cases were found to be due to typhoid (16).

A study from Sylhet City, Bangladesh discussed that water served by restaurants is a major source of drinking water for travelers and working community but its quality is not according to recommendations (17). Similar findings were reported by Vollaard et al. (18), Farooq et al. (19) and Abebe et al. (20). The restaurants of Peshawar City see a huge influx of diners and hence if the water served in the restaurants is not at par with the National and WHO standards of drinking water quality, it can lead to new outbreaks of enteric ailments in the community but not much has been done to investigate/study water quality in these restaurants. Thus this study was conducted to assess chemical and biological quality of drinking water supplies in the restaurants of Peshawar City using Most Probable Number (MPN) index and pH value. Study was aimed to provide insight needed to dig out the main source of already existing and exponentially increasing gastrointestinal system-related cases presented to the hospitals on a regular basis and necessary steps required to improve the existing condition. The objective of the present study is to assess the chemical and biological properties of drinking water supplies using MPN index and pH value in local restaurants of Peshawar City, Pakistan.

Methods

Ethical approval

Ethical approval for the study was issued by the Institution Research and Ethical Review Board (IREB) of Khyber Medical College, Peshawar (No.709/ADR/KMC).

Study areas

This cross-sectional descriptive study was conducted in the Peshawar city, the capital city of Khyber Pakhtunkhwa Province of Pakistan from December 2019 - March 2020. "Most Probable Number" index (MPN index) was selected as an assessment tool of bacteriological quality analysis as this technique detects "Total coliform organisms" (MPN value) per 100 ml of water sample which serve as "Indicator organisms" of bacteriological water quality (9). Amongst many parameters that indicate Chemical quality of water sample (9), pH was included in our study. Additional parameters, to provide a full picture of quality of drinking water samples, couldn't be assessed because of lack of resources and funding/financial support. Data was collected after obtaining ethical approval from the Institution Research and Ethical Review Board (IREB) of Khyber Medical College Peshawar. A total of 10 study areas (SA) including Hayatabad (SA1), Shaheen Town (SA2), Gulbahar Town (SA3), Tehkal Bala (SA4), Saddar Bazaar (SA5), Cantonment Area (SA6), University Town (SA7), Wahidabad (SA8), Haji Camp bus stop (SA9), Namak Mandi (SA10) areas were selected from different union councils of two towns i.e. Town 1 and Town 2 of Peshawar District as shown in Figure 1. These areas were selected in our study based upon the fact that they are some of Peshawar City's most populous portions hosting a broad range of activities from business to education and tourism/travel and do not differ significantly from each other in terms of mentioned activities.

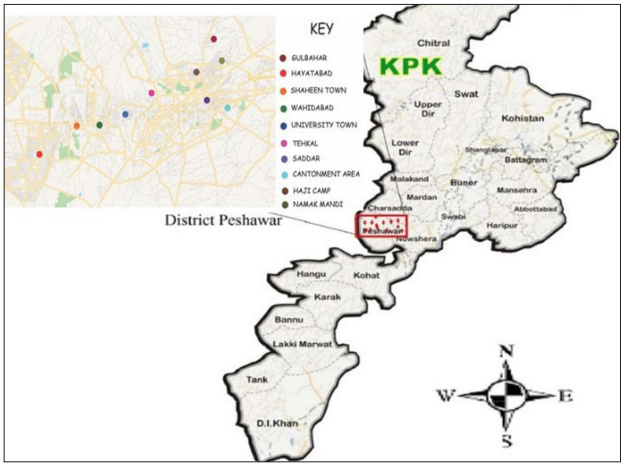

Figure 1: Map of Peshawar showing water sampling sites

Using the non-probability convenient sampling technique, 5 restaurants were selected from each of the 10 study areas thus giving a total sample size of 50 water samples. The restaurants selected were those that are known to serve a large number of customers daily and thereby could serve as a representative of restaurant facilities in their area. Only those restaurants were included in study that were willing to allow us to collect water sample from their restaurant for quality analysis and take field observation notes. The inclusion criteria included water samples utilized for drinking and cooking purposes and mineral water samples were placed in the exclusion criteria of this study.

Water quality sampling and analysis

In accordance with WHO guidelines for water sampling for bacteriological and physicochemical analysis (9), Water samples were collected in clean sterilized bottles made up of neutral glass that could hold at least 500 ml of water sample. Insulated boxes that were lightproof and equipped with melting ice were used to immediately store samples. Central water taps that were regularly used for obtaining water for cooking purposes or for serving to customers were selected as sample collection points. After the sample was collected in a clean sterilized glass bottle, it was properly sealed and labeled. Afterwards a "Sample collection form" was filled at the sampling site and both the sample bottle and that restaurant was allocated a reference number to keep the data analysis organized and accurate. The sample was delivered to the Public Health Laboratory, Khyber Medical College (KMC) Peshawar as soon as possible keeping a time frame of less than 2 hours.

Descriptive Field observation data was collected in the "Sample collection form" (Annexed) and it included data on suspected source of pollution in vicinity, method of purification (if used) along with date and time of collection and area of sampling plus the required investigations. "Recent rainfall" and "Proximity to an open garbage dump (either of hotel itself or a public one)" were included under the heading of "Source of pollution in vicinity". In addition to "Sample collection form", Field observation diary was maintained by sample collectors to observe hygienic practices including cleanliness of place, staff and utensils and method of water storage before serving to customers. "Multiple Fermentation Tube" or "Most Probable Number" technique was used to determine presence of "Total coliforms" in water sample. According to the multiple fermentation tube technique discussed by Bartram and Pedley (21), we used MacConkey's Broth as a growth medium and the prepared test tubes were incubated at 37 ± 0.5°C for 48 hours. The prepared test tubes were equipped with inverted Durham tubes to record gas formation. MPN/100 ml values were calculated using "MPN Index Table", a sample of which is given by Bartram and Pedley (21). The pH was recorded in laboratory using a Digital pH meter (HI9124, HANNA instruments, USA). Three solutions of pH 4.0, 7.0 and 10.0 were used to calibrate pH meter before taking measurements (using pH combination buffer solution kit, HANNA instruments, USA). The pH meter probe was placed in water samples for a couple of minutes until a stable reading was obtained while analysis. Pakistan's National Standards for Drinking Water Quality 2008 (22) and WHO's global overview of National Regulations and Standards for Drinking-Water Quality 2018 (23) were used to define standard pH and MPN/100 ml values of drinking water samples for this study. pH value of 6.5-8.5 was considered as "Acceptable" (22, 23). Water samples having an MPN/100 ml value of 0 were defined as "Ideal" in accordance with guidelines (22, 23). However, drinking water samples with MPN/100 ml values up to 10 were labelled "Acceptable" and any sample having a value of >10 was labelled as "Not acceptable" in accordance with national regulations and standards for drinking-water quality of some countries (23). Data was processed and analyzed using Microsoft Word 2013, Microsoft Excel 2013 and IBM SPSS Statistics 23. All the data is presented in the form of tables and charts. The following operational definition was used by this study:

Restaurant – A business place where meals are cooked and prepared on the premises and served to the customers.

Results

The dataset included the pH, MPN/100 ml values and descriptive field observation data reporting source of pollution in vicinity and method of purification (if used) for each of the water sample that were collected and then subsequently tested by the Public Health Laboratory, KMC. The descriptive statistics including mean pH and MPN/100 ml values of drinking water samples (n=50) from each of 10 study areas are shown in Table 1.

Table 1: Mean concentrations (±Standard Deviation) of the selected parameters

Parameters / Areas	pH	MPN/100 ml Drinking water sample
Hayatabad	7.35 (±0.09039)	24.8 (±6.535)
Shaheen Town	7.32 (±0.04301)	27.8 (±9.445)
Gulbahar Town	7.66 (±0.00837)	17.4 (±9.555)
Tehkal Bala	7.62 (±0.07328)	20.4 (±10.407)
Saddar Bazaar	7.50 (±0.12700)	7.8 (±5.718)
Cantonment Area	7.55 (±0.18472)	8.0 (±7.141)
University Town	7.21 (±0.15502)	11.8 (±4.970)
Wahidabad	7.17 (±0.14775)	15.2 (±9.338)
Hajji Camp	7.49 (±0.23266)	12.8 (±11.862)
Namak Mandi	7.65 (±0.09460)	21.4 (±16.319)

The main result findings were: Mean pH values of water samples for each of the study area falls within standard pH value for drinking water i.e. 6.5-8.5 (22, 23). The maximum pH value recorded was 7.84 in Cantonment area (SA6) and minimum pH value recorded was 7.07 in Wahidabad (SA8) as shown in following Figure 2.

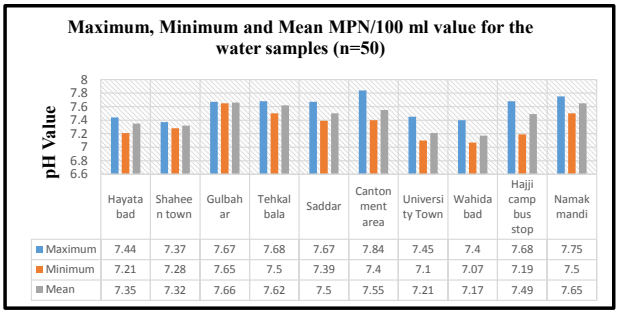

Figure 2: Maximum, minimum and mean pH values for the water samples (n=50)

The findings of significant importance were that the Mean MPN/100 ml values of water samples (total coliform indicator) for each of the study area were observed to be very high than the recommended standard value which states that in any 100 ml drinking water sample, total coliform bacteria count must be 0 i.e. not detectable (22, 23). 64% of water samples had MPN /100 ml value of greater than 10 thus were labelled "Not acceptable" while 36% had value in the range of 1-10 thus were labelled "Acceptable" (23) with no water sample having a value of 0.

The maximum MPN/100 ml value recorded was 50 in a water sample from Namak mandi (SA10) and minimum MPN/100 ml value recorded was 3 in water samples from Saddar (SA5), Cantonment area (SA6) and Haji Camp bus stop (SA9). Taking MPN/100 ml value as a reference, Saddar (SA5) and Cantonment areas (SA6) had a relatively better quality of their water samples as the Mean MPN values for these two areas were lowest amongst all study areas i.e. 7.8 and 8 respectively as shown in Figure 3.

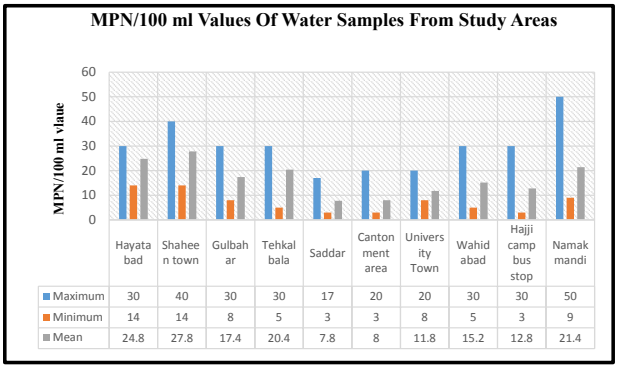

Figure 3: Maximum, minimum and mean MPN/100 ml value for the water samples (n=50)

Descriptive field observation data from "Sample collection forms" reported that none of the collected water samples were subjected to a method of purification by restaurants before providing it to its customers. Observatory data of water samples from Shaheen Town (SA2) and Namak Mandi (SA10) were found to record "Recent rainfall" and "Proximity to an open garbage dump". Data from Haji Camp bus stop (SA9) reported "Recent rainfall" only.

Field observation diary provided an insight into hygiene practices of selected restaurants. Hygiene practices in all of the selected restaurants except for some restaurants in Cantonment area (SA6) were not satisfactory. Staff workers had little knowledge of water safety guidelines and importance of water quality. Regular handwashing practice was not observed in most places. Sanitation of places where food was served to the customers was also not maintained. Water used for cooking and for other purposes was kept stored in open hard-plastic containers that were not regularly washed from inside. However, water being served to customers was usually filled directly from central tap into serving utensils when needed and was not kept stored for long period.

Discussion

In Pakistan, community health studies have demonstrated that poor quality of drinking water leads to about 50% of diseases and 40% of deaths (10). More than 80% population of Khyber Pakhtunkhwa (KP) province uses surface water and groundwater as drinking water the quality and quantity of which is below the acceptable standards due to the lack of proper water and sanitation management services and water treatment facilities in urban areas (10). This study focused exclusively on the chemical and biological properties of drinking water in restaurants of Peshawar City. Our target areas for water quality assessment have

been restaurants. The restaurants of Peshawar City are known for their hustle and bustle. Every day a huge influx of diners is seen in these places and hence if the water served in the restaurants is not at par with the national and WHO standards of drinking water quality, it can lead to new outbreaks of enteric ailments in the community.

Our study utilized MPN index and pH to study biological and chemical characteristics of drinking water supplies of restaurants of Peshawar City. Results of both of these variables could have been affected by laboratory equipment, glassware and plastic ware, how they are maintained and the frequency with which they are cleaned/sterilized (21). The quality of growth media and the expertise with which it is prepared, and the precision control measures could also possible had an effect on laboratory results (21).

Our study showed that almost all samples collected from restaurants located in selected study areas of Peshawar City had pH values within recommended standard range of 6.5-8. With the highest value being 7.84 (as shown in Figure 2) recorded in Cantonment area (SA6) with a mean pH value for all samples being 7.455. It is very important to ensure that drinking water pH levels fall within the recommended standard limits because too high or too low pH can be indicative of heavy metal and chemical contamination of the water reservoir. Additionally, water with unsafe pH can be unpleasant to drink and smell and might as well corrode metal pipes and pose serious health hazards. Our study finding of water pH correlates with another study that was conducted in new urban Peshawar to assess physical and biological parameters of water quality that indicated that the pH was within permissible limits (24).

The Mean MPN/100 ml values of water samples (total coliform indicator) for each of the study area were observed to be very high than the recommended standard value. During assessment of biological contents in tube wells, supply channels, and storage tank samples in Peshawar, biological contamination of two third of samples particularly with *E. coli* were also reported by Ali et al. (24). It is no news that coliform bacteria, which are gram-negative rods, have been known to cause potentially life-threatening diseases like diarrhea, dysentery, cholera and typhoid both in the form of individual cases and as epidemic or endemic spread, as was indicated in contrast study of 2 villages in Pakistan (25) where difference in coliform concentration was found proportional to difference in number of childhood diarrhea cases.

Similarly the study conducted in Karachi (26) has also successfully established the association between unclean drinking water and occurrence of typhoid fever. Hence, measuring MPN/100 ml of water at restaurants and reporting the places with unsatisfactory hygiene measures can serve as an important step in preventing coliform-related diseases within the general population.

Meteorological/climate factors and human activities can impact drinking water quality (27). In our study, 2 study areas, namely, Shaheen Town (SA2) and Namak Mandi (SA10) had their water samples associated with "Recent rainfall" and "Proximity to an open garbage dump" and field observational data from Haji Camp bus stop (SA9) recorded "Recent rainfall" only . This correlates with the finding that mean MPN/100 ml values of water samples from SA2 and SA10 were also quite high amongst our data set (27.8 and 21.4 respectively). Similar findings were reported in a study from Madagascar (28) where rainfall was associated with increased level of contamination of drinking water with intestinal enterococci*, E.coli*, Total coliform and Sulfite-reducing clostridia. Cavuşlu stream in Giresun, Turkey, which serves as the main source of drinking water to inhabitants of this Giresun City was found to have its water adversely affected by a garbage disposal facility near the stream in a health risk assessment study (29). Field observation notes also revealed that none of the restaurants practiced water treatment before its utilization or serving to customers. This correlates with our findings that Mean MPN/100 ml values of water samples for each of the study area were observed to be very high than the recommended standard value. Water treatment has been studied to effectively reduce the risk of water-related health hazards (30) and treatment of water supplies via disinfection carries a significant importance for public health (31).

Field observation diary provided us with the insight those hygienic practices in selected study areas. The poor knowledge, attitude and practice standards with respect to water safety and hygiene that were observed in restaurants selected in our study might as well contribute to the poor water quality of these restaurants. It is a well-established fact that poor hygienic practices (both personal and environmental) can lead to water resource contamination and outbreaks of food-borne illnesses (32, 33).

Supply of poor quality drinking water poses a serious health risk to the community. In Pakistan, progressively increasing water demand along with lack of awareness, lack of technology and skill for effective water treatment, and lack of quality supervision has kept the water and sanitation agency focused more on water quantity with water quality being seriously neglected (10).

Limitations

1. Our study assessed MPN index and pH of drinking water samples to indicate their microbiological and chemical quality respectively. Additional important parameters (to provide a full picture of quality of drinking water samples) could not be assessed because of lack of resources and funding/financial support.

2. Sample size for this study was 50 drinking water samples. Larger studies are required to develop a more complete understanding of situation.

3. This study assessed the quality of drinking water supplies in restaurants at a single point in time.

Follow-up collection of water samples and their analysis over an extended period is required to observe significant variations in water quality and factors associated with these variations.

4. Samples were taken only from restaurants and not from their water supply centers (tube wells etc.) due to lack of resources and funding/financial support. Sampling needs to include source/storage point sampling, distribution line sampling and collection/ consumer point sampling to possibly indicate where the problem originates from.

Conclusion

The results concluded by drawing attention to one of the most considerable environmental and human health dilemma that is the sewage contamination of drinking water. The study recommends that in order to reduce the health risk, government and concerned authorities will have to take strong initiatives which include regular monitoring and maintenance of water supply system, sewage drainage system, immediately stopping the supply of water from contaminated sources and setting up water supply lines far away from solid waste dumps or sewage lines system so that clean drinking water can be provided to the citizens. For sustainable improvement of the quality of drinking water supplies, restaurant staff needs to be properly trained via training programs to maintain quality at the point of consumption of clean water being supplied and to educate the masses about water quality, its importance and the sources of its contamination via education programs and awareness campaigns.

To develop a more complete understanding of drinking water contamination picture, such researches should be conducted that study a wide variety of water quality indicator parameters, include source/storage point sampling, distribution line sampling and collection/ consumer point sampling to determine the major contaminants (physical, chemical, biological or radiological) and their source of origin. Follow-up collection and analysis of water samples over an extended period is required to observe any significant variations in water quality and factors associated with those variations. This data can then be shared with respective policy makers and stake holders (including government officials and departments, public health departments, hospital administrations and restaurant associations) to ensure to put in place adequate measures for controlling contamination from where it is originating. Government should take special interest in this regard so that policies implemented can see the path of completion and water quality can be improved. This will be beneficial for health of general public reducing a substantial burden (both physical and economical) from health sector.

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Competing Interest

The authors declare no conflict of interest.

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