Drinking water quality, water distribution systems and human health: a microbial evaluation of drinking water sources in salt range

Abstract

In developing countries such as Pakistan, waterborne infections are more common among infectious diseases due to unsafe supply of drinking water and inadequate sanitation systems. In the salt range of Pakistan, sources of drinking water in rural and suburban areas are not protected against microbial contamination resulting from poor sanitation systems and saline water seepage. To assess the bacterial contamination levels and their impact on public health, drinking water samples were collected from various sources in Khewra and Pind Dadan Khan. Total Coliforms including faecal Coliforms, E. coli, Staphylococcus aureus and faecal Streptococcus were determined using the Most Probable Number (MPN) method to quantify the bacterial contamination. The data collected were subjected to statistical analysis to establish the potential relationship between water contamination levels among different sources and health risks posed by different bacterial species. The results revealed the presence of different bacterial species and there was a significant difference in average microbial concentrations among different water supply systems and natural sources. Almost all of the sources were infected either with Coliform group, Staphylococcus aureus or with faecal Streptococcus. An interview-based survey was also conducted to gather information regarding disease incidence rate and results showed a strong relation in the contamination levels and disease prevalence rate.

Keywords: drinking water, human health, microbial assessment, salt range, Pakistan

Introduction

In developing countries, most of the population is exposed to a wide range of enteric pathogens and suffers numerous episodes of gastrointestinal illnesses as a result. Microbiologically contaminated drinking water, unsafe supplies of water for personal hygiene and underprivileged sanitation, are the main contributors to an estimated 4 billion cases of diarrhea each year causing 2.2 million deaths, mostly among infants. Waterborne infectious diseases are caused by certain types of microbes present in drinking water, which is related to inadequate water supply, and/or contamination of water sources. The most familiar pathogens found in water are total coliforms, E. coli, Staphylococcus, Salmonella spp., Shigella spp., Cryptosporidium, Legionella spp., Vibrio cholerae and some viruses like Hepatitis virus. By 2001, a total of 1415 species of infectious microbes known to be pathogenic to humans had been identified. Diseases like diarrhea, cholera, typhoid and sometimes hepatitis are also referred with water as a medium to cause and spread of such diseases because of their presence in water. Poor sanitation system is considered as a major cause of such diseases. The extent of bacterial contamination in drinking water is strongly related to seasonal trends as well as geographical variations. The capacity of bacteria to survive and grow at alkaline pH values is of widespread importance in the epidemiology of pathogenic bacteria present in saline waters either used for drinking or any other purpose. E. coli when grown in a buffer medium at pH 7.0, E. coli cells exhibit a generation time of 18 minutes, whereas at a pH level of 8.7 the cells exhibit a generation time of 25 minutes.

The drinking water supply sector has mostly targeted the waterborne spread of pathogens. The most common method in use is the chlorination of drinking water at treatment plants and in the supply systems. Chlorination is considered vital to make it safe for drinking, especially when water is obtained from surface water sources. Although there are worldwide acceptance and use of drinking water chlorination in epidemic outbreak situations, there is an ongoing discussion on whether the interruption of waterborne transmission of pathogens reduces the occurrence of endemic diarrheal diseases in rural areas in developing countries. However, in the case of Pakistan, most water-supply systems are not working according to design, and many are completely dysfunctional. This raises the question whether improvements in these schemes and provision of microbiologically safe drinking-water would have an impact on the incidence of diarrheal disease. The available water in the salt deposited areas contains high amounts of sodium, chlorides, nitrates, magnesium and fluoride etc. In the salt range of Pakistan, drinking water resources are not protected from chemical and microbial contamination resulting from poor sanitation systems and rainwater runoff. Health complaints by residents of different locations of the salt range include hypertension, stone formation in kidneys, dental fluorosis, limb deformities, skin problems, nausea and abdominal diseases are very common. The microbial contamination of drinking water is also very common due to leakage in pipelines and cross connections with draining lines and also due to unsafe water storage practices. In the current perspective, it is presumed that use of contaminated water for drinking purposes pose varying degree of health problems to the local residents. The objectives of this study were to generate a comprehensive baseline data on the microbial status of drinking water in different residential areas and to identify the strength of association between water contamination level and overall disease incidence and morbidity rate in salt range.

Material and methods

Major localities

The salt range is a strip of hill system in Punjab Province deriving its name from extensive deposits of rock salt. The range extends...
from Jhelum River to Indus across the northern portion of Punjab Province. The most populated areas in the salt concentrated strip are Khewra, Pind Dadan Khan and Choa Saiden Shah (Figure 1). Khewra is a crowded town located close to Khewra Salt Mines and Pind Dadan Khan is an administrative subdivision of Jhelum District with a settlement of over 0.7 million people. The area considered as the primary target was Khewra and Pind Dadan Khan because of the maximum concentration of salt in drinking water owing to salt mining activities in Khewra and rainwater runoff from mines towards Pind Dadan Khan.

Microbial Evaluation of Drinking Water Sources in Salt Range

Fermentation Technique (MTFT). Water samples were inoculated into (MPN) method. This method was performed using the Multiple Tube Fermentation Technique (MTFT). Water samples were inoculated into tubes and incubated at 35°C for 48 hours. The tubes were then examined for the presence of gas production. The MPN method was used to estimate the number of colonies of total coliforms, which were detected as gas production in the tubes.

Sampling and transportation

A total of 51 water samples were collected from different water sources in Khewra and Pind Dadan Khan. Pre-sterilized glass bottles were used as sample containers. A solution of 0.3% sodium thiosulphate was added to store the samples in a ratio of 0.1ml in 120ml of the sample; a temperature of 4°C was maintained during transportation, and the samples were subjected to microbial analysis within 6 hours after collection of samples. All the samples were collected according to the protocol set by FAO (Food and Agriculture Organization) and SMWW (Standard Methods for the Examination of Water and Wastewater). The most commonly found indicator species of microorganisms. All the methods followed for the analysis of different types of bacteria were according to SMWW and FAO.13,14

Survey of disease incident rate

A survey was conducted to collect monthly data from hospitals, dispensaries and local clinics regarding the prevalence of different waterborne diseases for duration of one year. In this study, the waterborne diseases were focused in order to understand the relationship between the contamination levels of water and the rate of disease occurrence related to that specific disease.

Statistical analysis

ANOVA was performed to obtain data to investigate the difference between contamination levels of various types of water sources. Disease incidence rate was also assessed to understand its relationship with the level of microbial contamination, and the vulnerability of people exposed to saline water from a health perspective in salt the range of Pakistan.

Results and discussion

The average value for Most Probable Number Index per 100ml (MPN/100ml) of total Coliforms group was 54.88 MPN/100ml which was relatively higher than Staphylococcus aureus and faecal Streptococcus having average counts of 38.25 MPN/100ml and 10.68 MPN/100ml respectively (Figure 2). Faecal Streptococcus was observed with the lowest average MPN Index among all other microbial parameters. Microbial counts in different water sources (TMA Supply, ICI outlets and Natural Springs) were also determined which were found to be different in concentrations from each other. Analysis of drinking water quality samples clearly indicated that the contamination level of water was increasing at an alarming rate that may pose a serious threat to human health and the environment. Microbial contamination of drinking water supplies, as well as other natural sources, may be either due to the failure of the disinfection of the raw water at the distribution systems or to the infiltration of infected water (sewage) through cross connections or leakage points. In piped supplies, the likelihood of microbial contamination increases with discontinuity of supply as well as the increased pressure of the surrounding medium i.e. soil or water which may contain infected effluent from leaked sewers. Municipal sewage contains human faeces and water contaminated with these effluents may contain disease-causing organisms and consequently, may be harmful to human wellbeing if used as drinking water or in food preparation.14

Comparative significance of microbial counts in drinking water samples

ANOVA was performed to compare the average MPN counts among three microbial parameters (total Coliforms, Staphylococcus aureus and faecal Streptococcus) which clearly indicated a significant difference between all parameters (Figure 2). The average microbial counts of total Coliforms were found to be significantly higher (p = 0.0001) than faecal Streptococcus. Similarly, average counts of Staphylococcus aureus in water samples were found significantly higher (p = 0.0059) than faecal Streptococcus. Although there was no significant difference observed among microbial counts of total Coliforms group and Staphylococcus aureus, but both species were found abundant in samples analyzed. Total coliforms group was found to be present in 71% of the total water samples. However,
Staphylococcus aureus and faecal Streptococcus were not very much common in samples (55% and 28% respectively). Statistical analysis has shown significant difference among average microbial counts of different microbial species. The microbial standards by WHO suggest a complete absence of total coliforms, Staphylococcus aureus and faecal Streptococcus from drinking water. The maximum microbial counts in drinking water samples were of total coliforms. The contamination of drinking water with total coliforms, E. coli and faecal coliforms is suggestive to much more related with the incidence of diarrhea in children under the age of 5 years and adults.\(^\text{17}\)

**Microbial counts in different types of water sources**

All the water sources were categorized to obtain an overview on contamination intensity in terms of microbial counts. Analyses results of water samples from hospitals, public water taps, schools, households, natural water springs and ICI plant water outlets were combined to form a comprehensive data list indicating contamination level (Table 1). The difference in average microbial counts found in local water supply systems prominently indicated their quality. Among different water supply networks, the TMA supply was found to be responsible for posing serious threats to human health and the environment. The maximum average counts of faecal Coliforms were found in samples taken from TMA supply channels or houses. The TMA network for supplying drinking water was found to be highly contaminated with total Coliforms group, Staphylococcus aureus as well as faecal Streptococcus as compared to natural springs and ICI water outlets.

**Hospitals:** The drinking water samples from hospitals were found free from total Coliforms contamination. However, contamination with Staphylococcus aureus was at its peak in water from hospitals (average counts=93.5 MPN/100ml). On the other hand, only one water sample from THQ Hospital was found contaminated with faecal Streptococcus having a concentration of 1 MPN/100ml (Table 1). Water samples taken from hospitals found free from total Coliforms. This is probably because of installation of water filters equipped with UV radiations that are capable enough to disinfect a few types of bacteria. However, microbial counts of Staphylococcus aureus and faecal Streptococcus were determined in samples taken from hospitals probably due to development of resistance against various water treatment technologies.\(^\text{18}\)

**TMA public water taps:** A total of 5 water samples taken from public taps at different points in the city, which clearly indicated the presence of total Coliform and Staphylococcus aureus with average counts of 96.2 MPN/100ml and 43.8 MPN/100ml respectively. Faecal Streptococcus were not detected in samples taken from public taps built by TMA (Table 1).

**ICI water outlets:** Water samples from outlets of ICI Chemical Plant were contaminated with average counts of 7.6 MPN/100ml and 9.6 MPN/100ml for total Coliforms including E. coli and faecal Coliform; and Staphylococcus aureus, respectively. Faecal Streptococcus was not detected in water samples taken from ICI outlets posing this supply as a better option (Table 1).

**Natural springs:** Samples from the natural water springs present in the outskirts of Khewra town were also highly contaminated with total Coliforms (106.83 MPN/100ml). The average microbial counts for Staphylococcus aureus and faecal Streptococcus were 8 MPN/100ml and 6 MPN/100ml, respectively (Table 1). Maximum microbial counts of Coliforms were determined in water samples taken from natural springs present in the surroundings of Khewra town. The water from Mitha Patan spring is being supplied to two-third population of Khewra town by PMDC Water Supply System (Pakistan Mineral Development Corporation). The microbial analyses of water samples taken from these natural springs were not satisfactory because all the samples were contaminated with high concentrations of Staphylococcus aureus and faecal Streptococcus as well. Water for drinking purposes from Mitha Patan spring is supplied by PMDC to all facilities within the premises of Khewra Salt Mines.

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**Table 1 Average microbial counts in water samples taken from different sources**

<table>
<thead>
<tr>
<th>Sources</th>
<th>No. of Samples</th>
<th>Total coliforms</th>
<th>Staphylococcus aureus</th>
<th>Faecal streptococcus</th>
<th>WHO standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average Counts (MPN/100ml)</td>
<td></td>
<td></td>
<td>MPN/100ml</td>
</tr>
<tr>
<td>Hospitals</td>
<td>4</td>
<td>0</td>
<td>93.5</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>TMA Public Taps</td>
<td>5</td>
<td>96.2</td>
<td>43.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICI Public Taps</td>
<td>5</td>
<td>7.6</td>
<td>9.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Natural Springs</td>
<td>6</td>
<td>106.83</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Households</td>
<td>22</td>
<td>65.9</td>
<td>39.5</td>
<td>23.36</td>
<td>0</td>
</tr>
<tr>
<td>Schools</td>
<td>5</td>
<td>3.16</td>
<td>57.5</td>
<td>0.16</td>
<td>0</td>
</tr>
<tr>
<td>Hotels &amp; Restaurants</td>
<td>3</td>
<td>57.67</td>
<td>16</td>
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</tr>
</tbody>
</table>

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Growth and high counts of Coliform bacteria depend upon high temperature and rainwater runoff which carries animal and human wastes in major water reservoirs used as sources of drinking water for many communities. The study also revealed that the presence of faecal Coliform showed the contamination of water with animal and human faecal wastes, while the total Coliform counts indicate the presence of bacteria from other resources like soil.

**Households:** A total of 22 water samples were taken from different houses from Khewra and Pind Dadan Khan City. Analysis results showed average counts of 65.9 MPN/100ml, 39.5 MPN/100ml and 23.36 MPN/100ml for total Coliforms, Staphylococcus aureus and faecal Streptococcus, respectively. Figure 3 clearly indicates the contamination level of total Coliforms in different households.

**Schools:** The highest average microbial counts calculated in water samples taken from schools was of Staphylococcus aureus (57.5 MPN/100ml). The average microbial counts for total Coliforms and faecal Streptococcus were 3.16 MPN/100ml and 0.16 MPN/100ml, respectively (Table 1). Samples taken from several educational institutes from Khewra and Pind Dadan Khan were also not safe from contamination with Coliforms and Staphylococcus aureus. Faecal Streptococcus was also determined from these samples but with very lower counts.

**Hotels and restaurants:** Samples taken from different hotels and restaurants were also free from faecal Streptococcus contamination. But a higher microbial count was determined for the presence of Coliforms and Staphylococcus aureus. The microbial counts of total Coliforms and Staphylococcus aureus in water samples taken from hotels and restaurants were found to be 57.67 MPN/100ml and 16 MPN/100ml respectively (Table 1).

The significance of microbial counts in different water supply systems

ANOVA was performed to assess the significance of microbial concentrations in different water supply systems and resources. Three major water sources (TMA Supply, ICI outlets and natural springs) were subjected to statistical analysis i.e. TMA supply, natural water springs and private boreholes. According to the data obtained, there was no significant difference between the three water resources (Natural Springs, TMA supply, ICI outlets & Boreholes) regarding contamination of drinking water with total Coliforms group (Figure 4). The p-value for analysis between springs and TMA supply was 0.32, between TMA Supply and Boreholes was 0.17, and between springs and Boreholes was 0.3 (Figure 4). However, a significant difference was observed between springs and TMA supply regarding microbial counts of Staphylococcus aureus (p=0.038). The counts of Staphylococcus aureus were also found significantly different in TMA supply and boreholes (p=0.0074). However, no significant difference was observed in the mean counts of springs and boreholes (p=0.3089) (Figure 5). On the other hand, there was no significant difference observed among counts of faecal Streptococcus in samples from springs and TMA supply line (p=0.055). However, there was a significant difference among the samples from TMA supply and private boreholes (p= 0.03). No such difference was found in the microbial concentrations in samples of springs and boreholes (p=0.059) (Figure 6).
According to WHO guidelines all the positive water samples with microbial counts more than 1MPN/100ml exceeded the permissible limits for drinking water. Water used for drinking purposes must be free from the presence of any kind of bacteria including indicator species. Considering microbial standards devised by WHO, no *Coliforms* should be present in drinking water, however, contamination of water samples with *Coliforms* should not exceed 5% of 40 samples collected for monthly assessment of water quality. Similarly, *Staphylococcus aureus* and faecal *Streptococcus* are also set compulsory not be present in drinking water as they can cause skin diseases and abdominal disorders in humans. Intestinal *enterococci* are typically excreted in the faeces of humans and other warm-blooded animals. Intestinal *enterococci* are present in large numbers in sewage and water environments polluted with sewage or wastes from humans and animals (Junko, 2001). Presence of any member of this group indicates the water as not suitable for drinking purposes. In this study, it became clear that the situation of drinking water quality from a microbial perspective in the Salt Range of Pakistan is not merely close to the requirements devised by WHO.

### Survey of disease incidence rate in Khewra and Pind Dadan Khan

The survey results of disease incidence rate in the population living in premises of Khewra and Pind Dadan Khan indicated that contaminated drinking water is a serious threat to public health in the region (Table 2). In Tehsil Headquarters Hospital (THQ) Pind Dadan Khan, visits for diarrheal patients under the age of 5 years were recorded as an average of 152 patients per month and 108 patients per month above the age of 5 years. The greater number of diarrheal patients visits in hospital proves the strong relationship between poor water quality and disease incidence rate in developing countries.

In THQ Hospital, a daily report is prepared for the prevalence of gastroenteritis, according to which about 25% of outdoor patients belong to diarrhea, and child mortality rate is increasing due to gastrointestinal diseases. According to data obtained by interviewing physicians practicing in Khewra and Pind Dadan Khan, about 80% of diseases prevailing in the Salt Range are waterborne. Skin diseases become more common in winter due to the presence of *Staphylococcus aureus* in water. Females and children are more vulnerable to waterborne diseases because of unhygienic practices, poor economic conditions and lack of awareness.

**Table 2** Monthly data of patients visits to hospitals and clinics in Khewra and Pind Dadan Khan for the duration of one year

<table>
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<tr>
<th>Diseases</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
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<tbody>
<tr>
<td>Acute Respiratory Infections</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
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<tr>
<td>Pneumonia &lt;5yrs</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>3</td>
<td>13</td>
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<td>5</td>
</tr>
<tr>
<td>Pneumonia &gt;5yrs</td>
<td>4</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>22</td>
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<td>TB Suspects</td>
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<td>23</td>
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<td>110</td>
<td>6</td>
<td>16</td>
<td>31</td>
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<td>78</td>
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<td>82</td>
<td>64</td>
<td>57</td>
<td>76</td>
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<td>11</td>
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<td>Diarrhea &lt;5 yrs</td>
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<td>85</td>
<td>71</td>
<td>53</td>
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<td>59</td>
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<td>158</td>
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<td>165</td>
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<td>78</td>
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<td>65</td>
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<td>123</td>
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<td>15</td>
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Conclusions and suggestions

In the salt range of Pakistan, drinking water resources in the rural areas are not protected from chemical and microbial contamination resulting from poor sanitation systems and rainwater runoff. The microbial contamination of drinking water is also very common due to leaking pipelines and cross connections with draining lines. The outcome of the study showed that almost all of the water resources were contaminated either with Coliform group, Staphylococcus aureus or with faecal Streptococcus. It is concluded from the study that the availability and supply of safe drinking water in Salt Range of Pakistan was inadequate. People were using contaminated water provided either by different authorities or from general water sources available. Lack of awareness was also a vital factor that was playing an important role in unhygienic conditions to promote the incidence of waterborne diseases in the study area. However, health status can be improved by some initiatives that could be taken at the administrative level. Following are some recommendations that can be helpful to improve health status in the region:

a. Creating awareness about hygiene and sanitation practices, incidence and transmission of waterborne diseases.
b. Installation of drinking water treatment plants to improve the quality of drinking water in the research area.
c. Installation of pipelines to transport water from springs/rivers to decrease the threat of faecal contamination of water.
d. Up gradation of water supplying infrastructure in order to maintain a safe infrastructure for safe water distribution.
e. Creation of better economic conditions can also help the residents to resist against common waterborne diseases.

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Conflict of interest

The author declares there is no conflict of interest.

References