MICROBIAL EVALUATION OF DRINKING WATER AND FREQUENCY OF BACTERIAL ISOLATES FROM RAWALPINDI PAKISTAN

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ABSTRACT

Objective: To find the frequency and identification of different bacterial isolates from drinking water samples.

Study Design: Descriptive


Patients and Methods: Total 521 water samples were studied. Microbiological testing was performed using the membrane filter technique. Different biochemical tests were applied to differentiate the organisms e.g. Oxidase Test, Indole test, Triple Sugar Iron (TSI) test. Frequencies and percentages of data were calculated and presented in form of graphs and tables and analyzed using SPSS version 17.

Results: Out of 521 water samples, 353 (67.8%) water samples were unsatisfactory and the other 168 (32.2%) water samples were satisfactory for drinking purpose. In the present study, Coliform was the most common organisms isolated.

Conclusion: The study indicates that there are 2 main microorganisms that are responsible for water-borne disease. These include Pseudomonas and more importantly Total Coliforms because it is a major fecal contamination indicator.

Keywords: Coliforms, Escherichia coli, Membrane filtration Technique, Microbiological Analysis, NIH Islamabad.


INTRODUCTION

The most common and widespread risk associated with drinking water is bacterial contamination that mostly are coliforms especially fecal coliforms, Pseudomonas spp, Mesophiles and fecal Streptococci. They make up around 10 % of the intestinal flora of the human and animal intestine. Coliforms belong to the family Enterobacteriaceae and include Escherichia, Enterobacter, Klebsiella and Citrobacter spp [1]. Fecal Coliforms are subset of total coliforms, comprising not a single bacterium but a group of species. These coliforms can utilize the lactose at optimum temperature to produce acid, CO2 and aldehyde within 1-2 days. Community and ecological health safety requires consuming nontoxic water. Water bases have greatly been polluted by agricultural and biochemical waste removals from industries [2].

One of the paramount concerns of drinking safe water is that 75% of all diseases in developing countries arise from polluted drinking water. The diversity and severity of waterborne diseases are high in under-developed countries like Pakistan due to contaminated drinking water. Pathogens present in drinking water that can cause serious water borne diseases includes Salmonella, Shigella, E. coli, Vibrio cholera, Yersinia, enterocolitica, Campylobacter jejuni. Other bacteria that opportunistically may cause diseases in humans includes Pseudomonas aeruginosa, Klebsiella spp, Aeromonas spp, and certain slow-growing Mycobacteria opportunistic pathogens. A large number of diseases are caused by polluted drinking water such as typhoid, dysentery, diarrhoea, cholera, and infectious hepatitis. Estimated water-related mortalities reported per year were more than 5 million people [3].

World Health Organization (WHO) in 2000 estimated that 2.2 million people died per year due to Diarrhea only. Approximately 200,000 children die each year with diarrheal diseases in Pakistan (UNSP 2003). An estimated 250,000 child deaths occur each year in Pakistan due to waterborne diseases...
(USAID). Fourteen to thirty thousand individuals, commonly young children die each day due to water associated infections globally. According to WHO, 1.1 billion individuals don’t have approach to safe and clean drinking water. In Pakistan, the availability of drinking water has greatly been reduced due to the increased urbanization. According to WHO, 80% of the total human diseases in under-devolving countries are due to contamination of drinking water. A huge number of pathogens can be transferred to humans through fecal contamination of water. By using “Indicator organisms” bacteriological superiority of water can be checked. Coliforms chiefly *E. coli* is the suggested indicator organism for manageable water and indicator of fecal contamination which is also the intestinal normal flora in humans [4].

Majority of the microorganisms are microscopic having diameter less than 0.1mm. They are classified into different groups like total coliform, fecal coliform, *Mesophile* and *Pseudomonas spp* depending on their structure and metabolic capabilities [5]. *Pseudomonas aeruginosa* is normally present in fresh water, sewage and soil and in fecal materials from animals and human beings. It can breed in low nutrient moist environments and can live for several months in water. It is the prominent source of nosocomial infections. Community acquired infections due to *Pseudomonas aeruginosa* are frequently confined and are related with contamination of drinking water. They are not able to cause infection without allowing them to multiply [6].

*E. Coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination [7]. *Salmonella* is a genus of rod-shaped (bacillus) gram-negative bacteria of the Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. Strains of *Salmonella* cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning [8].

*Corynebacterium* is a genus of gram-positive, aerobic, rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous. Some are useful in industrial settings such as *C. glutamicum*. Others can cause human disease, including most notably diphtheria, which is caused by *C. diphtheriae* [9]. *Vibrio* is a genus of Gram-negative bacteria, possessing a curved-rod shape (comma shape), several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in salt water, Vibrio species are facultative anaerobes that test positive for oxidase and do not form spores [10].

This study is aimed to find the frequency and identification of different bacterial isolates (total coliforms, and *Pseudomonas spp*) from water samples which are widely used for the evaluation of contaminated water.

**MATERIAL & METHODS**

A descriptive study was conducted at National Institute of Health (NIH) Islamabad, Pakistan over a period of six months i.e. 30th Aug 2015 to 28th Feb 2016. Water samples for this study were gathered in Microbiology Department of NIH. A total number of 521 samples were taken in this study. The entire portable and drinking water samples received from different areas were submitted to microbiology department, NIH. Three types of water samples were included in the study (i.e. samples from 1 = Tap water, 2 = Filtration plants & 3 = Tube well or Bore water). The samples received from study area but not used as drinking water like recreational water, lake water, and river water were not included for the research study purpose. The water samples were collected aseptically and carefully to avoid any kind of cross contamination.
contamination that can produce false positive or false negative results. Strong and thick-walled glass bottles with screw caps of at least 300 ml volume were used for water sample collection. Samples were transported to laboratory within 2-3 hours maintaining the temperature at 4°C or below. The samples were transported in an isolated cold box to the microbiology lab of NIH and processed within 4-6 hours of the collection. All the samples were physically examined for their appearance and concluded either clear or presence of impurity in the form of brown elements. Using pH meter, pH was determined for all the water samples.

All water samples were analyzed through Milli-pore membrane filtration technique (MFT). In this technique, a particular type of filter paper having regularly sized apertures (openings) is used through which bacteria can’t be passed and persist on the surface of the filter paper. Drinking water samples were filtered from the Millipore membrane filters (pore size= 0.45 µm and diameter= 47 mm) with a vacuum speed of 5 to 15 mmHg. Organisms got concentrated on the surface of the membranes. The samples were then inoculated on the MacConkey agar media and incubated at 37 C° for 24 hours. After 24 hours, these plates were examined. Positive cultures were further processed for the identification of these pathogens by Gram’s stain and by performing different types of Biochemical tests [Oxidase Test, Indole test, Triple Sugar Iron (TSI) test]. Frequencies and percentages of data were calculated. All the data was presented in form of graphs& tables and analyzed using SPSS version 17.

RESULTS

Three types of drinking water samples were analyzed (Table 1). Out of total 521 water samples, 353 (67.8%) water samples were found to be unsatisfactory and the other 168 (32.2%) water samples were satisfactory for drinking purpose. In type 1, out of 199 Tap water samples taken at different areas of Rawalpindi, 30 (15.07%) water samples were satisfactory and the other 169 (84.92%) samples were unsatisfactory for drinking purpose. In type 2, out of 202 water samples taken from Filtration plants at different areas of Rawalpindi, 96 (48%) water samples were satisfactory and the other 106 (53%) samples were unsatisfactory for drinking purpose. In type 3, out of 120 water samples taken from Bore/Tube wells at different areas of Rawalpindi, 42 (35%) water samples were found satisfactory and the other 78 (65%) samples were unsatisfactory for drinking purpose (figure-1). All the samples were analyzed for different micro-organisms. In all the samples, Coliform was the most common organisms isolated (figure- 2).

Table-1: Types of Water Samples used for drinking purpose (n=521)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample Type</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tap water</td>
<td>199</td>
</tr>
<tr>
<td>2</td>
<td>Filtration plants water</td>
<td>202</td>
</tr>
<tr>
<td>3</td>
<td>Tube well/ Bore water</td>
<td>120</td>
</tr>
</tbody>
</table>

Figure-1: Frequency of satisfactory and unsatisfactory water samples used for drinking purpose (n=521).

Figure-2: Frequency of Pseudomonas and coliforms found in water samples used for drinking purpose (n=521).
DISCUSSION
Water pollution is affecting the lives of many people throughout the world as organic and inorganic pollution as well as fecal matters load in natural water is being increased. In Pakistan, contaminations of drinking water have been testified to be the leading health problems. In Pakistan, 20 - 40% of the hospitalized patients are suffering from diseases resulting from contamination of drinking water e.g. dysentery, diarrhea, cholera and typhoid [14]. In this study we found that only 29.9% water samples were found to be satisfactory for drinking purposes where as 70.06 % water samples were unsatisfactory for drinking purpose. 90% of the total population suffers from water related infections in Punjab, e.g. dysentery, diarrhea, cholera and typhoid. In this study, we found that in study areas of Rawalpindi and Islamabad, the prevalence of Coliform and Pseudomonas spp was 29.9% and 20% respectively as according to WHO the coliform count should be below 2.2/100ml of water but our study is in accordance with another study done in Lahore in which out of 100 samples, 42% (n = 42) showed growth of E. coli. Another related study conducted in Peshawar shows that 43% of the water samples are polluted with E. coli [15].
We also found that the population of Rawalpindi and Islamabad with poor hygienic status was more prone to have waterborne diseases as here was a strong association between hygienic status and waterborne diseases (P=0.02) and this work is in line with Bangladesh Rehabilitation assistance corporation report. This might exist due to the fact that the health employees are less experienced and indicates that the water and sanitation facilities are below the standards. There are several causes including broken water supplies, leakage in pipe lines, contamination from sewerage pipes and contamination because of human actions. Reduced health promotion and personal hygienic activities are also the leading contributing aspect. Poor health information system causes severe problems with respect to monitoring, arrangement, controlling and management of these situations.

CONCLUSION
This study has shown that there is a high incidence of contamination of water by pathogenic organisms and the data in this study indicates that two (02) main bacteria are responsible for water-borne diseases. These include Pseudomonas and more importantly Total Coliforms because it is a major fecal contamination indicator.

RECOMMENDATION
1. Contamination of drinking water should be stopped by all means and at all levels.
2. Priority should be given to prevent contamination of water sources as first line of defense.
3. All the agencies that supply drinking water should be trained up to the finalized standards.
4. Prevention of prevalence of fecal coliforms in drinking water must be done in the study area.
5. Awareness about water purification mechanisms through cost effective methods e.g. boiling must be advised to public.
6. Personal hygiene like “hand washing” before meal and after using the toilet should be made necessary.
7. Waste products from industries should be disposed by a suitable method.
8. The purity of water lakes/reservoirs should be made compulsory for users.
9. Increase coverage of water supply and water treatment facilities.

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AUTHORS CONTRIBUTION

Muhammad Jaseem Khan: Concept, research design, review.
Muhammad Ferman Ali: Sample collection, analysis
Muhammad Hassan: Statistical analysis
Anwarullah: Manuscript Writing

REFERENCES