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Bacteriological analysis of tap, processed and filtered water samples

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Abstract
The bacterial and chemical contamination of water is the leading cause of numerous diseases i.e. Cholera, Hepatitis A, Schistosomiasis and many others. Polluted water is dreadfully a big threat to many lives all across the globe. In this present study, a total of 60 water samples i.e., bottled, tap and drinking water samples were collected in sterile containers. By using the techniques of spread plate and filter paper replication methods, colonies were isolated. Further identification was done using microbiological and biochemical tests. The organisms identified were *Escherichia coli*, Pseudomonas spp., *Staphylococus aureus*, Bacillus spp., *Klebsiella pneumonia*, *Proteus vulgaris*, Aeromonas spp. and Micrococcus spp. Their prevalence patterns were also studied accordingly. Attention must be paid for the sustainability of the water and the lives, which are dependent on its consumption. From the microbiological standpoint, a safety UV disinfection step in the water-treatment system is encouraging. To void bacterial re-contamination episodic cleaning and disinfection of the water-treatment and distribution systems should be done.
Keywords: Water quality, pathogenic strains, paper replication method.

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Introduction

Environmental monitoring of micro-organisms is essential for the examination of their activities and for public health security measures. In order to perceive impending sources of pathogens for preventing their invasion in the public health bounds require high degree of sensitivity. Coliform bacteria are usually used as an environmental indicator of potential human faeces contamination and they are commonly used for examining the bacteriologic safety of water supplies. Coliforms even show the presence of enteric pathogens [1].

Apprehensions regarding safe and potable water have increased in the recent years; the reason would be the common contamination of drinking water by Giardia, *Escherichia coli* O157:H7, Cryptosporidium and other pathogens [2]. Non-point source of pollution is intricate to compute and sources are difficult to determine, therefore contrasting groups of interest often identify other sources of pollution for causing the problem without any technical basis. Decision of conflicts the espousal of better management practices and policies require sound scientific data on the bacterial pathogenic strains and their life histories for the implementation of better possible techniques.

Currently, faecal coliforms, *Escherichia coli*, total coliforms and enterococci are the bacterial strains, which are used as indicator in health risk assessments and water quality checking [3]. Each of these bacteria is the normal commensal of the intestines and faeces of the warm-blooded mammals including humans, livestock and wild life. The indicator bacteria are normally not pathogenic, they are used because they are much easier to handle and are economical as well [4]. Faecal bacteria are enumerated either by using multiple tube fermentation technique or by membrane filtration technique [5]. However, the presence of faecal coliform and *Escherichia coli* in the drinking water represents contamination.

An ideal indicator should be non-pathogenic, easily enumerated, rapidly detected and have survival characteristics that are similar to those of pathogens that are of concern. [6].The principle threat to human health is those pathogens, which somehow manage to escape from the water treatment process and therefore, lessen water quality. These pathogens are designated as primary pathogens. Moreover, there is an increased interest in assessing the probability of the normal micro-biota in drinking water supplies. These microbes are however, known as heterotrophic plate count (HPC) bacteria. Epidemiological studies have revealed that the presence of HPC bacteria in drinking water gives mixed results. There is evidence that HPC concentrations show some association with those of enteric bacteria and some of them do not show such results [7].

Several problems are associated with viable culture methods that are used routinely for the observation of bacteriological safety of water supplies. These problems include maintaining the viability of bacteria between the time of enumeration and collection, time required for monitoring,

confirmation of enteric bacteria (days), lack of growth of viable but non-culturable bacteria such as those that are stressed by chemicals in the water and lack of specificity for detection of true faecal coliforms specifically *Escherichia coli* [8,9]. Hence, a study was carried out in order to determine the number and types of bacteria found in three types of drinking water: tap water, bottled water and filtered water.

Materials and methods

Different samples were collected in sterilized containers from three sources i.e., tap, processed (bottled) and filtered water. All the domestic brands were taken for bottled water samples, tap water was collected from different regions of Lahore, Pakistan (vicinity of the University of Veterinary and Animal Sciences) and filtered water was collected from University of Animal and Veterinary Sciences and also from different hostels of Lahore. Ten of each water sample was collected for processing in sterile containers. Isolation of the bacterial strains was carried out on McConkey's and nutrient agar plates. The processing of the water from different sources is given below:

Drinking and bottled water Processing

These samples were processed by filter paper replication method. Measured 100 ml of water and dispensed it slowly over the filter paper set in the conical flask. As the whole water passed through the filter paper, took the filter paper with the help of sterilized forceps and placed over the media plates and waited for about 5 minutes so that the material on the filter paper could stick well to the media plates. Took out the filter paper with the help of forceps and cover the Petri plates. The plates were incubated for about 24 hours at 37°C.

Tap water processing

One ml of water sample was spread over the media plates with the help of spreader and those plates were then incubated for 24 hours at 37°C.
After incubation every colony that appeared to be different morphologically was sub-cultured and streaked on separate plates.

Identification of colonies

For the identification of various bacteria, Gram's staining was performed and the results were noted for each. Once the bacterial groups were generally divided on the basis of Gram's staining then different biochemical tests were performed accordingly. Before biochemical testing, the motility test was performed to check whether the concerned groups are motile or not. Indole test, methyl red, Voges-Proskauer test, manitol fermentation test, catalase test, urease test, oxidase test, coagulase test, glucose fermentation test, Dnase and sugar hydrolysis test were performed to identify the isolated cultures on species level. Each of the pure isolate was bio-chemically characterized and confirmed by using already described standard protocols [10].

Results and discussions

A study was conducted to determine the microbial quality of commonly available water sources. For this purpose, a total of 60 water samples (from 3 different consumable water sources) were checked for the presence of micro-flora by using various identification methods. The data is shown in Table 1.

Table 1: Percentage of bacteria isolated from filtered, tap and bottled water samples.

Species	Filtered water (%)	Tap water (%)	Bottled water (%)
Staphylococcus aurous	50	80	50
Bacillus cereus	40	80	40
Escherichia coli	60	70	60
Klebsiella pneumonia	40	70	30
Pseudomonas aeruginosa	40	70	30
Proteus vulgaris	30	60	20
Aeromonas	30	90	20
Micrococcus varians	30	60	20

Pathogenic bacterial strains like *Escherichia coli*, Pseudomonas spp. and Salmonella spp. were detected in the water samples which have been demonstrated to survive and multiply in bottled water with a potential to cause outbreaks in consumers (11, 12). It was alarming that out of all the 10 selected domestic brands of bottled water, the prevalence of coliform bacteria was estimated to be 75% which suggests that proper legislation and manufacturing protocols should be standardized for bringing potable water to the consumers.

In bottled water, bacteria may be ethnic from the natural source of the water or may be introduced during handling [11, 13]. It has also been documented that a number of these bacteria could multiply during storage to reach infective doses for

consumers [13, 14]. Different opinions have been expressed about the public health significance of *Pseudomonas* and related species in bottled water. They are reported to be resistant to several antimicrobial agents [15]. However, other studies have shown a minimal risk of infection from drinking water from these sources [16].

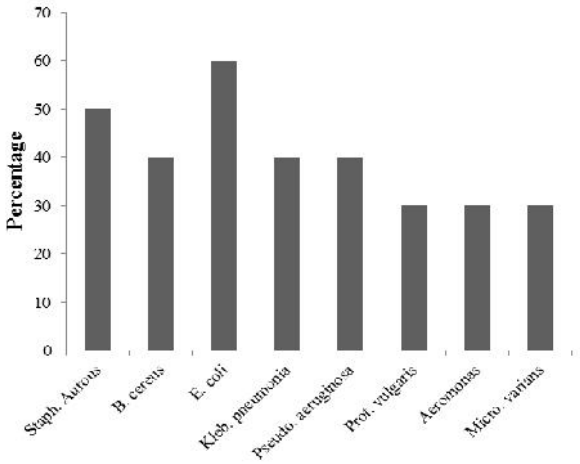

Fig. 1: Percentage of the bacterial strains in filtered water

Of the three sources of water focused in our study, a total of 60 water samples were taken. Out of these, *Staphylococcus aurous* had a prevalence of 50%, 80% and 50% in distilled water, tap water and bottled water, respectively. Studies of Guo-Jane and Shou-Chin (1997) showed that *Staphylococcus aurous* belongs to the contaminating bacteria. Hence the isolation of *Staphylococcus aurous* from bottled water clearly indicates that it was contaminated at the time of bottling [17].

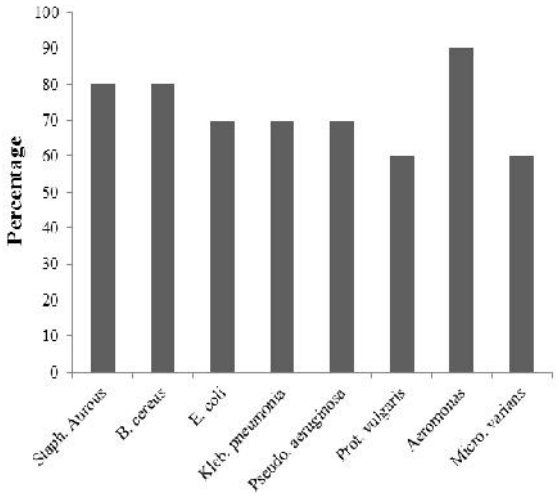

Fig. 2: Percentage of the bacterial strains in tap water

Escherichia coli is primarily associated with human faeces; it is a useful indicator of human faecal contamination of water and the appropriate focus of monitoring for indicators of potential enteric pathogens in environment of potable waters [18]. In our study, *Escherichia coli* showed a prevalence of 60%, 70% and 60% in the samples of distilled water, tap water and bottled water, respectively. It is alarming that based on the prevalence of *Escherichia coli* in tap water; it validated the severe contamination of faecal origin, which renders it unsuitable for human consumption.

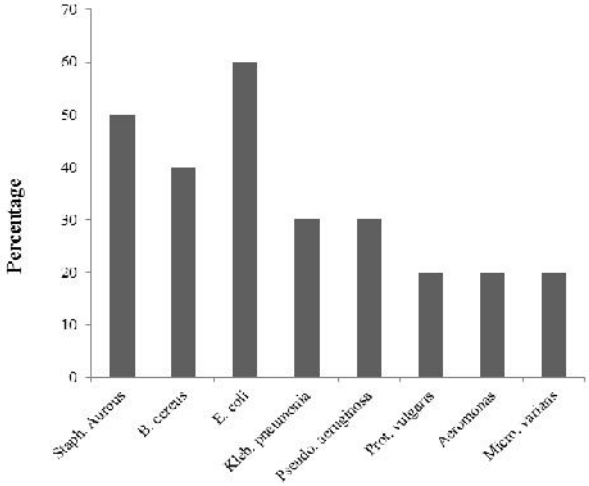

Fig. 3: Percentage of the bacterial strains in bottled water

Pseudomonas aeruginosa showed a prevalence of 40%, 70% and 30% in the distilled water, tap water and bottled water, respectively. It indicated that 46% of the all water samples tested were positive for *Pseudomonas aeruginosa.* Other bacteria isolated from water samples included *Bacillus cereus*, which was recovered from 40%, 80% and 40% of the samples collected from distilled water, tap water and bottled water, respectively. Out of 60 water samples tested, 28 (46%), 22 (36%), 28 (46%) and 22 (36%) were positive for *Klebsiella pneumoniae*, *Proteus vulagarius*, *Aeromonas* and *Micrococcus varians*, respectively. Of the all water samples tested, *Proteus vulgarius* and *Micrococcus varians* showed the lowest prevalence of 36%, while *Escherichia coli* had the highest prevalence of 63%. The occurrence of the bacterial strains isolated from the sources and their frequency is given in the Fig. 1, 2, 3, respectively.

Many of the species of bacteria found in water were not recovered by traditional plating procedures. However, seminal levels of culturable heterotrophic bacteria can offer useful information regarding the microbiological quality during the storage, treatment

and distribution of drinking water. In this study significant relationships were observed among three different sources of water and also the prevalence of bacterial pathogens was also carried out. This type of data may provide useful information for water utilities involved in the operation and maintenance of water distribution systems.

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