Microbial Analysis of the Drinking Water of Manchar Town Water Supply

S. D. SHINDE

Department of Physics Research Center, Mahatma Phule Mahavidyalaya Pimpri Pune-411017 India
Email: sdsa404@gmail.com

Abstract

The microbial analysis of water was carried out using standard methods and the isolated micro-organisms were identified by using standard tests. The series of tests were performed for exact detection or identification of microbial and contaminated water sources. It was observed that drinking water of Manchar town contain Logionella pneumophila, Shigella sonnei, Campylobacter jejuni and Aeromonas hydrophila microorganisms. These isolates were identified and confirmed, based on the selective, differential media and biochemical tests. The presence of the micro and nano-organisms in a drinking water supply was evidence of drinking water contamination.

Keywords: microbial analysis, Manchar town, contamination.

Introduction:

The bacteriological (nano and micro-organisms) examination of water can be performed routinely by water utilities to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery. Since human fecal pathogens vary in kind and in number, it is impossible to test each water sample for each pathogen. Instead, it is easier to test for the presence of non pathogenic intestinal organisms.

In order to determine whether water has been contaminated by fecal material, a series of tests can be used to demonstrate the presence or absence of bacteria's. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed.

Bacteria like *Salmonella Spp., Campylobacter Spp.* and *E. coli* can contaminate the food through contact with sewage and contaminated water [1]. Contamination from raw materials and equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens [2]. In the investigation, the samples showed occurrence of high bacterial load. The presence of these microbes in food can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing and dilution, cross

contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife and trays [3,4]. The reliability of the Kirby-Bauer Disc Diffusion method for detecting Methicillin-resistant strains of *Staphylococus aureus* had been tested and put forth various advantages by Drew and his co-workers [5]. Hence, the investigation of Bauer *et al*. about the antibiotic susceptibility testing is very important and can be studied by standard single disc method [6]. The potential disease causing bacteria are found to be different in different water sources. The contamination may be due to human faces or animal faces or by any means [7]. All these investigations reported that it is essential to check the contamination with potential disease-causing microorganisms and purity of the drinking water. Hence we had taken such investigation.

Materials and Methods

Microbial analysis of water sample

Standard water analysis was carried out in a systematic way as follows.

Isolation of microorganisms from water samples

1) Three samples of water were collected from Manchar, Grampanchayat (town) drinking water supply.
2) These samples were diluted in a ratio from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.
3) After dilution, the samples were spread on Nutrient Agar plates.

The M-H Agar medium was prepared by using standard material and method.

i. Peptone-5 gm/lit.
ii. Nacl-5 gm/lit.
iii. Yeast extract-2 gm/lit.
iv. Beef extract-1gm/lit.
v. Distilled Water-1000 ml.

4) ***Inoculation***

Inoculation was carried out by using spread plate technique. 0.1ml sample was inoculated and spread with the help of sterile glass spreader under aseptic conditions. This procedure was performed in triplicate. Plates were incubated in an inverted position at 37 ^{0}C temperature for 24h. On the next day each bacterial colony was picked up with sterile needle and transferred to separate nutrient agar slant. Thus different colonies were obtained and they were isolated. These slants were designated as pure culture. Pure cultures were stored in refrigerator at 4^{0}C for further studies.

5) Gram staining: Microorganisms were first tested by Gram staining.

Principle: In gram staining, bacterial cells were divided into two major groups as gram positive and gram negative. The gram stain reaction is based on the difference in the chemical composition of bacterial

cell walls. Gram positive cells have a thick peptidoglycan layer, whereas the peptidoglycan layer in gram negative cells is much thinner and surrounded by outer lipid containing layers.

Method:

i) Heat-fixed smear was prepared.
ii) The smear was flooded with crystal violet and allowed to stand for 30 seconds.
iii) The smear was rinsed with water for 5 seconds.
iv) The smear was covered with Grams iodine mordant and allowed to stand for 1 minute.
v) It was rinsed with water for 5 seconds.
vi) It was decolorized with 95% ethanol for 15 to 30 seconds.
vii) Then rinsed with water for 5 seconds.
viii) It was counterstained with safranin for about 60 to 80 seconds.
ix) Then rinsed with water for 5 seconds.
x) Lastly blotted dry with bibulous paper and examined under oil immersion.
xi) Gram staining, differentiating bacterial species into two large groups was performed (gram-positive and gram-negative).
xii) Thus micro-organisms were isolated.

6) Characterization and Identification of microorganisms:

The isolated micro-organisms were identified by using following tests:-

i) Catalase Test
ii) Glucose utilizing Media
iii) Gelatin, Starch, Ceasin-Hydrolysis Test
iv) Oxidase Test
v) IMViC Test

The isolated colonies were pure cultured and gram stained. Identification was carried out according to Bergey's Manual of determinative Bacteriology, 9^{th} edition. Following tests were used for genus identification.

a) Catalase Test

Method:

A capillary tube was dipped into 3% H_2O_2 solution. By touching a colony, the tube was observed for bubble indicating a positive reaction.

Result:

If there are bubbles, then reaction is positive and if there are no bubbles, then reaction is negative. In this way reaction gave information.

b) Glucose utilization from media

Principle:

Glucose is metabolized through different metabolic pathways depending on types of microbial species and aerobic or anaerobic environment. If fermenting bacteria are grown in a liquid culture medium containing carbohydrate, they may produce organic acids as by-product of the fermentation. These acids are released into the medium and so lower pH of the pH indicator such as phenol red is included in the medium, the acid production will change the medium from its original color.

Gases produced during fermentation process can be detected by using a small, inverted tube, called Durham tube, within the liquid medium. If gas is produced, the liquid medium inside the Durham tube will be replaced by the gas in the form of bubble.

Medium used:

The medium used was Glucose fermenting medium. The medium was a nutrient broth to which phenol red was added.

Method:

i) The glucose fermenting broth was prepared.
ii) By using the sterile inoculating loop, inoculums from the culture tube of the unknown bacterium picked up and inoculated in broth.
iii) The inoculated broth was kept into the incubator at 370C.

c) ***Gelatin hydrolysis:***

The purpose was to see if the microbe can use the protein-gelatin as a source of carbon and energy for growth. Use of gelatin was accomplished by the enzyme gelatinase.

Medium used:

The medium used was nutrient gelatin. The medium was a nutrient broth to which 12% gelatin is added, converting it into a semisolid medium.

Method:

i) The gelatin medium plate was prepared.
ii) By using the sterile inoculating loop, the inoculums from the culture tube of the unknown bacterium were picked up.
iii) Immediately the inoculums were spread into the nutrient gelatin medium plate.
iv) The inoculated plate was placed into the incubator at 37 ^{0}C.

Results and Discussion:

The examination of water was intended to identify water sources which were contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly sewage treatment systems.

Results of the microbial analysis of water samples are presented in Table-1, Fig-1 and Fig-2. The photographs of grown strains are showed in Fig-3. A water sample examined in this study showed pH 6.7. It was observed that water sample contained four different bacterial isolates viz. *Logionella pneumophila, Shigella sonnei, Campylobacter jejuni and Aeromonas hydrophila* (nano and micro in size) These isolates were identified and confirmed, based on the selective, differential media and biochemical tests [8-10].

Table-1: Bacteria identified in Manchar town Water supply

Sr. No.	Isolate No.	Bacteria – Species Name
1	Isolate 1	*Legionella pneumophila*
2	Isolate 2	*Shigella sonnei*
3	Isolate 3	*Campylobacter jejuni*
4	Isolate 4	*Aeromonas hydrophila*

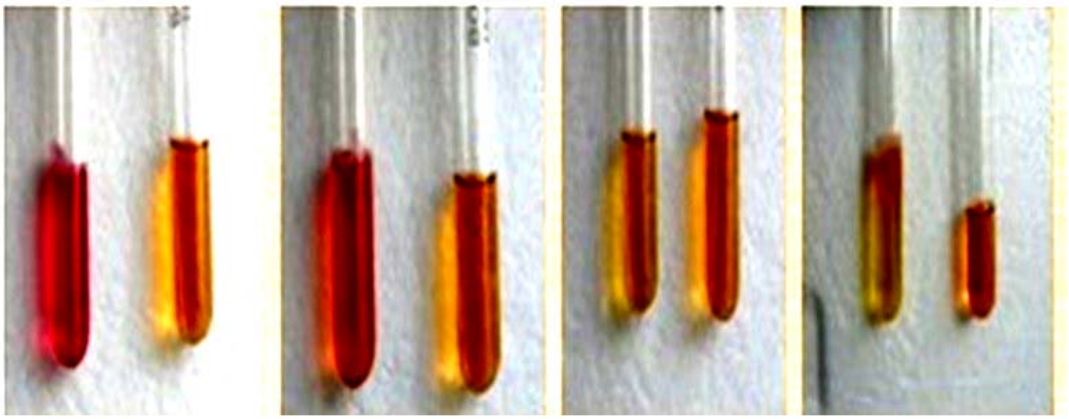


Fig-1: Identification of bacteria

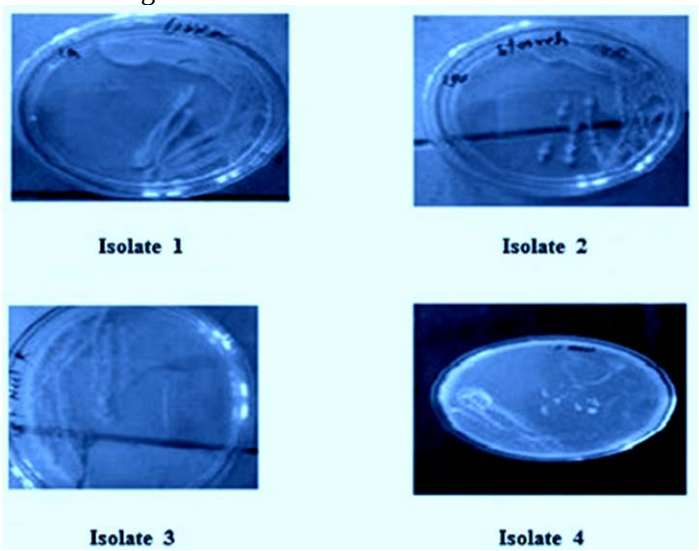
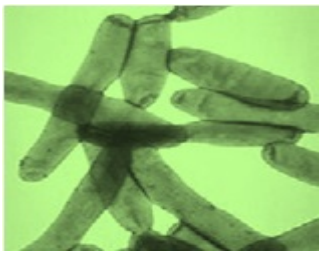
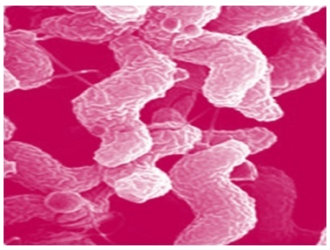


Fig-2: Identified bacteria

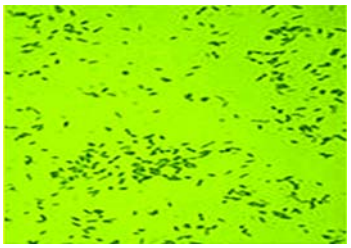


L. pneumophila

S. sonnai



Campilobacter jejuni



A. hydrophila

Fig-3: Growth of identified bacteria

Table-2: Conformation of identified bacteria:

Sr. No.	Characteristics	*Legionella pneumophila*	*Shigella sonnei*	*Campylobacter jejuni*	*Aeromonas hydrophila*
1	Gram reaction	Negative	Negative	Negative	Negative
2	Cell shape	Straight Rod	Rod	Helical	Short rod
3	Motility	+	-	-	+
4	Catalase test	-	+	+	
5	Acid from glucose	-	+		+
6	Indole from tryptophan		-	-	+
9	Hydrolysis of 1) Casein 2) Gelatin 3) Starch 4) Urea		 _ -		 + -
10	Methyl red	-	+		+
11	Voges proskauer		-		+
12	malonate		-		-
13	Oxydase	+	-	+	+

Legionella pneumophila

L. pneumophila is a gram-negative, non-encapsulated, aerobic bacillus with a single, polar flagellum. In nutrient-deficient media, it becomes long and filamentous. The Legionellaceae are intracellular parasites of free-living protozoa, in their natural environment. These organisms inhabit man-made water distribution systems. It is a common cause of community acquired and nosocomial pneumonia in adults. The potable water distribution system was determined to be the culprit behind hospital-acquired Legionnaires disease in one research study [9]. The researchers hypothesized that the natural microbial population and sediment were growth-promoting factors of *L. pneumophila*. *L. pneumophila* is not sensitive to antimicrobials e.g., penicillin, cephalosporin, amino glycosides that are excluded by the plasma membrane. So, it is treated only with antibiotics that can enter the host cells e.g., macrolides, quinolones, tetracycline's

Shigella sonnei

The Shigella sonnei is a non-motile, nonspore-forming, facultative anaerobic Gram-negative bacterium. Its non-motile characteristic showed that species did not have flagella to facilitate its movement like other human enterobacteria. The *Shigella sonnei* is a rod-shaped bacterium. It is lactose-fermenting bacterium causing dysentery. Its optimal environmental temperature is 37 ^{0}C, which is similar to the temperature in the human body. Hence, human's gastrointestinal tract appears to be the only found natural host of *Shigella sonnei*. . This enterobacterium is transmitted by uncooked food or contaminated water. They produce a toxin called as the Shiga toxin. This may bring potential harm to the neurons, cytoplasm of the cells and enteric epithelial cells. *Shigella sonnei* is a rod-shaped and gram-negative bacterium. Its outer membrane is filled with lipopolyscharride. The bacterium is able to survive in soiled linen for up to seven weeks. It can live up to five days and in salt water for 12-30 hours in fresh water environments. The *Shigella sonnei* is spread mostly by means of fecal-oral transmission. Other possible modes of transmission are from ingestion of contaminated food or water and subcutaneous contact with inanimate objects and mostly sexual contact [10].

Campylobacter jejuni

It is curved, helical shape, non spore forming, Gram-negative and microaerophilic [11]. It is a most common cause of human gastroenteritis's. The food poisoning caused by *Campylobacter* species may be debilitating, but is life threatening. The contaminated drinking water and unpasteurized milk provide an efficient means for distribution. The contaminated food is a major source of isolated infections, with incorrectly prepared meat and poultry as the primary source of the bacteria. The infection with *C. jejuni* results in enteritis, which is characterized by abdominal pain, fever, and diarrhea. They only grow in scanty amounts on the plates, since the colonies are oxidase positive. The drug of choice is usually erythromycin.

Aeromonas hydrophila

Aeromonas hydrophila is gram-negative straight rods with rounded ends (bacilli to coccibacilli shape). They are 0.003 to 1 micrometer (3nm to1µm) in width, and one to three micrometers in length. They can grow in temperatures as low as four degrees celsius. These bacteria are motile by a polar flagellum. It is very toxic to many organisms because of its structure. It can produce Arolysin Cytotoxic Enterotoxin a toxin that causes tissue damage. *hydrophila* infections occur often during environmental changes, change in the temperature, in contaminated environments and when an organism is infected with a virus or another bacterium. These organisms live in aquatic environments. It can cause gastroenterrtis disease. This disease can affect anyone, but it occurs in young children and people who have compromised immune systems [12]. Antibiotic agents such as chloramphenicol, florenicol, tetracycline, sulfonamide, tetramycin and nitrofuron derivatives are used to eliminate and control the infection of *Aeromonas hydrophila*.

Conclusions:

The microbial analysis showed that the drinking water of Manchar town consists of different micro and nano-organism which can cause diseases. Hence it is necessary to avoid or remove conditions of contamination of water. The surrounding conditions of water supply system as well as the position of the storage tank should be under the strict observation and check. The drawbacks in the distribution of water system should be removed, particularly leakages at or nearby drainages, bathrooms, toilets, different wastes should be avoided. There should be safety in the supply of water used for drinking, swimming, industrial and domestic purposes.

Acknowledgement:

Our sincere thanks are due to Dr. Arun Andhale Principal, Mahatma Phule College, Pimpri, Pune and Dr. Pandurang Gaikawad Principal, A. A. College, Manchar, Dist. Pune for providing laboratory facilities and encouragement. I am also indebted to Dr. Nalwade A. R, Head, Department of Biotechnology, A. A. College, Manchar Pune for valuable guidance and inspiration.

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