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ISOLATION OF WATER FLORA FROM VARIOUS SOURCES

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Abstract

The aim of this study was to isolate and identify environmental bacteria from various raw water sources as well as the drinking water distributions system in Mafikeng, South Africa, and to determine their antibiotic resistance profiles. Water samples from five different sites (raw and drinking water) were analysed for the presence of faecal indicator bacteria as well as *Aeromonas* and *Pseudomonas* species. Faecal and total coliforms were detected in summer in the treated water samples from the Modimola dam and in the mixed water samples, with *Pseudomonas* spp. being the most prevalent organism. The most prevalent multiple antibiotic resistance phenotype observed was KF-AP-C-E-OT-K-TM-A. All organisms tested were resistant to erythromycin, trimethoprim, and amoxicillin. All isolates were susceptible to ciprofloxacin and faecal coliforms and *Pseudomonas* spp. to neomycin and streptomycin. Cluster analysis based on inhibition zone diameter data suggests that the isolates had similar chemical exposure histories. Isolates were identified using *gyrB*, *toxA*, *ecfX*, *aerA*, and *hylH* gene fragments and *gyrB*, *ecfX*, and *hylH* fragments were amplified. These results demonstrate that (i) the drinking water from Mafikeng contains various bacterial species and at times faecal and total coliforms. (ii) The various bacteria are resistant to various classes of antibiotics.

Key words: Environmental, Bacteria, faecal

I. Introduction

Water plays a key role in supporting all forms of life on earth. Water acts as a solvent to dissolve the solutes of human body and also acts a medium for undergoing many metabolic processes. Therefore, it is vital for all known forms of life. Nevertheless, if it contaminated with a variety of contaminants as in sewage water or waste water, it may become the place for the growth of different types of microorganisms which may have a potential for spreading a variety of diseases. Sewage water is nothing but the unprocessed water collected from different sources such as domestic sources, hospitals and industries. Depending on the location, sewage water may contain an array of substances either in solution form or in solid. The different types of waste materials in sewage water include both organic and inorganic wastes, nutrients, toxic chemicals, oils and many more components. Even though sewage contains a lot of wastes, the microorganisms will grow by utilizing the organic and inorganic wastes. These types of microorganisms will have a specific type of characteristics compared to bacteria growing in other environments. [7]. On the other hand, municipal sewage water is a location where different types of substrates from different sources were merged and hence it may act as a best medium for the proliferation of numerous microorganisms. These microorganisms which expose to a variety of substrates will develop novel mechanisms for utilizing them and hence can become a better source for studying their novel mechanisms, novel enzymes and novel bioactive compounds. Therefore, it is important to know which types of bacterial genus are present in the sewage water and there after it is possible to carry out specific studies such as isolation and characterization of specific enzymes or novel bioactive molecules produced by the isolated bacteria [16]. Inadequate use ofpharmaceutically important molecules such as antibiotics may lead to the development of antibiotic resistance in microorganisms.

II. Material & Methodology

2.1 Material

(1) Water Sample
(2) Sterile method nutrient agar tubes
(3) Sterile distilled water tubes
(4) Glass wares, (plates, Sterile 1 ml pipettes)

2.2 Sample collection

Water from Bopal Lake, Ahmedabad, Tap water, Municipal sewage was included in the present study for the assessment of their water quality parameters. Samples were collected from winter season in a sterilized wide mouth bottles of at least 200 ml holding capacity. The bottles were opened and immersed at a depth of 30 cm with its mouth facing the current and the samples were brought to the Microbiology department Parul institute of applied science, Ahmedabad for analysis. The water quality assessment included physical parameters like temperature and biological parameters like MPN of coliforms like bacteria anf fungi.

2.3 Procedure

With sterile pipettes weight 1 ml of water sample on distilled water tubes Transfer the water sample to each 9 ml distilled blank (this gives the 10^{-1} dilution of the water sample) .Mix Contents of the tube vigorously and allow soil particles to settle Prepare 10^{-2},10^{-3},10^{-4}…. Dilution of water sample From each dilution transfer a fixed amount in to sterile melted nutrient agar tube mix well and pour in sterile petri dished. Label plates, clearly indicating the dilution and volume plated respectively After the solidifying media spread 0.1 ml water sample in the plates Incubate all plates at 37·C for overnight .Count the total number of colony develops on the each plates.

Quality of water	**Coliform count/100 ml**	**Thermotolerant E. coli count/100 ml**
Excellent	0	0
Satisfactory	1-3	0
Intermediate	4-9	0
Unsatisfactory	>=10	>=1

Table 1. Quality of Water

2.4 Yeasts

Streaking techniques commonly used for bacterial purification are equally suitable for the isolation of yeasts. A method widely used by yeast specialists is to disperse a portion of a colony in 2 to 3 ml of sterile water, then streak a single loop of this suspension over the whole surface of a plate, moving the loop slowly down from top to bottom while simultaneously moving it rapidly across the plate from side to side. Mter suitable incubation, well separated single colonies should appear in the lower half of the plate. If all of these single colonies appear to be of similar size and appearance (taking into account the effect of crowding), the culture may be judged to be pure. Microscopic checks of some single colonies are also desirable. Disperse a needle point of cells from a colony in a drop of water, add a cover slip and examine by bright field illumination at about 400x. Cell outlines will be clearly visible. Note that, unlike those of bacteria, yeast cell sizes often vary considerably in a pure preparation. Purity is indicated not so much by uniformity of cell size within a preparation as by similarity in microscopic cell appearance from colony to colony. When a culture is considered to be pure, streak it onto an appropriate slant.

2.5 Moulds

Streaking techniques are ineffective for filamentous fungi and are not recommended at all. Isolation depends on picking a small sample of hyphae or spores - judged to be pure by eye, by hand lens or preferably under the stereo microscope - and placing this sample on a fresh plate as a point inoculum. Purity is subsequently judged by uniformity in appearance of the colony which forms after incubation. The appearance of a mixed culture depends on the growth rates of the fungi present. If rates are diverse, a mixed culture is often indicated by a clump of dense hyphae at the inoculum point, surrounded by looser wefts of spreading hyphae. With fungi of approximately equal growth rates, mixtures are often indicated by colonies with sectoring growth: sectors will show differences in mycelial, spore or reverse colours or in radial growth rates. The simplest starting place for isolating fungi is an enumeration plate with well separated colonies. Use a needle, of platinum or nichrome, preferably cut to a chisel point with a pair of pliers, or a steel sewing needle. Sterilise it by heating, then plunge the tip into cold agar and leave until cool - with nichrome or steel this will require several seconds. With the tip of the cold, wet needle pick off a few spores or a tuft of mycelium - just enough to be visible - and inoculate a single point on a plate or slant. The same procedure can be applied to mixed cultures arising from direct plating or surface sampling techniques. It is advisable to keep notes on the appearance of the colony area sampled, as this will give an indication of whether the culture which grows up is the same as that intended to be isolated. It is generally easy to isolate rapidly growing fungi from those which grow more slowly. The outermost hyphal tips are usually free of contamination. The reverse process is often much more difficult. The isolation of slowly growing fungi in the presence of rapidly growing "weeds" often requires skill, patience and ingenuity. It is desirable to watch the point inocula daily over several days at least, because a particular stage in the life cycle may give some advantage. The slow colony may germinate more rapidly, or have a sector accessible to a needle, or spore more freely. The use of higher or lower incubation temperatures, or media of low aw or low nutrient status, or the addition of dichloran (see below) may all be of value in this process. Freeing fungi from bacteria, long considered to be a very difficult procedure, has been greatly simplified in recent years with the advent of media containing antibiotics. With use of the media recommended in the next section, bacterial contamination of cultures should be a rare event. When a pure colony is obtained, it should be inoculated onto a slant of an appropriate medium and incubated until ready for identification. Again, moulds should always be inoculated at a single point, preferably near the centre of the slant. This permits the best colony development and sporulation in most fungi.

III Result & Discussion

Colony Characteristics

	Size	Shape	Surface	Consistency	Margin	Elevation
Isolate:1	Medium	Circular	Smooth	Moist	Entire	Flat
Isolate:2	Medium	Amoeboid	Smooth	Moist	Wavy	Umbonate
Isolate:3	Medium	Round	Smooth	Moist	Entire	Convex
Isolate:4	Medium	Round	Smooth	Moist	Entire	Flat

Bacterial identification

Water samples of 150 ml each were collected from Bopal Lake, Tap water and Municipal sewage water. It was observed that different bacterial colonies were isolated from the water of these three sources. Colonies varied from circuar, irregular margins as well as rhizoidal and filamentous in shape. Different colonies obtained were subjected to gram staining and thus cocci, bacilli and coccobacilli forms were identified. It was found that maximum strains when subjected to microscopic examination revealed that gram negative bacilli were predominant followed by gram positive cocci and gram positive bacilli. The results are tabulated in Table 2.

Morphological and cultural characteristics of the organisms isolated from Bopal Lake water samples			
Samples	**Gram staining**	**Motility**	**Colonies on Nutrient Agar**
Bopal lake	Gram positive rod	Non motile	White rhizoidal
	Gram positive cocci	Non motile	Golden yellow

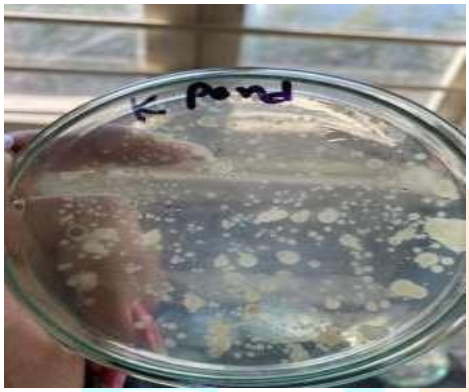

Figure 1: Different colonies obtain from lake water

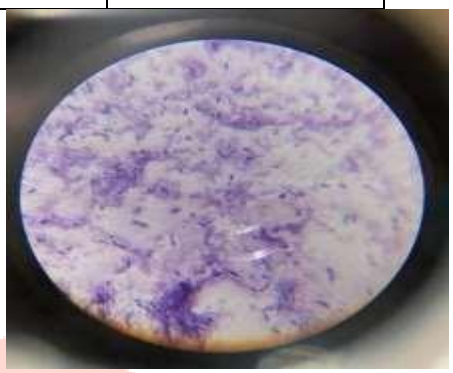

Figure 2: Field observation of microbes from lake water

Classification of the quality of water was based on certain bacteriological tests amongst which MPN count is major. In this study Most probable number (MPN) of water sample collected from all the water bodies was estimated to be very high as much as 810 to > 1600. Quality of water for drinking was UNSATISFACTORY with the coliform count elevated grossly.

Morphological and cultural characteristics of the organisms isolated from Tap water samples			
Samples	**Gram staining**	**Motility**	**Colonies on Nutrient Agar**
Tap water	Gram positive rod	Non motile	White rhizoidal
	Gram negative rod	Motile	Mucoid

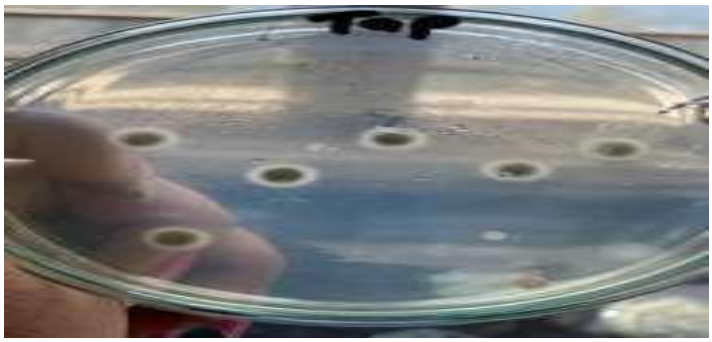

Figure 3: Different colonies obtain from tap water

Classification of the quality of water was based on certain bacteriological tests amongst which MPN count is major. In this study most probable number (MPN) of water sample collected from all the water bodies was estimated to be very low as much as 10 to > 1600. Quality of water for drinking was SATISFACTORY with the coliform count elevated grossly. Bacteria per 100 ml of water sample was also more than 1, which further added to correspond the contamination level in these water bodies.

Morphological and cultural characteristics of the organisms isolated from Sewage water samples			
Samples	**Gram staining**	**Motility**	**Colonies on Nutrient Agar**
Municipal Sewage	Gram positive rod	Non motile	White rhizoidal
	Gram positive cocci	Non motile	Golden yellow
	Gram negative cocci	Motile	Mucoid

Classification of the quality of water was based on certain bacteriological tests amongst which MPN count is major. In this study Most probable number (MPN) of water sample collected from all the water bodies was estimated to be very high as much as 1000 to > 1600. Quality of water for drinking was UNSATISFACTORY with the coliform count elevated grossly. Bacteria per 100 ml of water sample was also more than 1, which further added to correspond the contamination level in these water bodies.

Biochemical Character

Biochemical Test	Bacterial strain
Indole Production	Positive
Methyl red	Positive
Urea Hydrolysis	Negative
Starch Hydrolysis	Positive
Vogas Proskaver	Positive

3.2 Fungi Identification

Water samples of 150 ml each were collected from Bopal Lake, Tap water and Municipal sewage water. It was observed that different fungus were isolated from the water of these three sources.

- Aspergillus

Size	Shape	Margin	Color	Texture
7-12 mm	Circular	Flat	Olive Green	Dusty

- Morphological :- Thick walled ad have “I” shaped unbranched long and swells at the tip pictures resembles like a sunflower under microscope
- Penicillium

Size	Shape	Margin	Color	Texture
5-7 mm	Circular	Flat	Dark Green	Smooth

- Morphology:- Conidiophores are short and branch at the tip in finger like clusters of sterigmata panicillium are usually green or blue color.

3.3 Isolation from Tap water samples

Figure 5 Obtain fungi from tap water

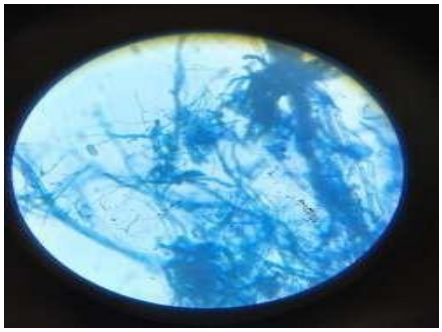

Figure 6 Field observation of microbes from tap water

IV Conclusion

Environmental laws have become stringent, discharge of the effluent within the permissible limit is mandatory in the developed and developing countries. The analysis might be practice at large scale as well as at a small scale level all over the world. The methods practiced by large-scale holders comprise physicochemical methods requiring a large surface area for the set up of effluent treatment plant and technically trained personnel with efficient management skills. It adds to the cost of the treatment process, making it cost intensive and cannot be employed in small scale industries. Therefore, biological treatment methods are considered to be ideal and economical. Wastewater is an enriched media for the microbial growth. It is important to know which types of bacterial genus are present in the sewage water and there after it is possible to carryout specific studies such as isolation and characterization of specific characteristic or novel bioactive molecules properties produced by the isolated bacteria and fungi.

V Reference

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