ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

ASSESSMENT AND MANAGEMENT OF GROUND WATER-A MICROBIOLOGICAL APPROACH

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ARTICLE INFO

Key words:

Access this article online Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

In water systems with inadequate quality control and sanitation, water could act as a vehicle for pathogenic microorganisms that originate from the faeces of wildlife including birds, livestock and pet animals, as well as humans. In particular, the spread of enteric viruses, e.g. Noroviruses, are repeatedly related to poor water quality Globally, huge efforts are put into improving and monitoring drinking water safety, but still it is estimated that 1.1 billion people have water sources regularly contaminated with faecal microorganisms

INTRODUCTION

Ground water is an essential and vital component of our life support system. The ground water resources are being utilized for drinking, irrigation and industrial purposes. There is growing concern on deterioration of ground water quality due to geogenic and anthropogenic activities. The quality of ground water has undergone a change to an extent that the use of such water could be hazardous. Increase in overall salinity of the ground water and/or presence of high concentrations of fluoride, nitrate, iron, arsenic, total hardness and few toxic metal ions have been noticed in large areas in several states of India. Ground water contains wide varieties of dissolved inorganic chemical constituents in various concentrations as a result of chemical and biochemical interactions between water and the geological materials through which it flows and to a lesser extent because of Contribution from the atmosphere and surface water bodies. Water sources available for drinking and other domestic purposes must possess high degree of purity, free from chemical contamination and micro organisms. But the rapid increase in population and industrialization together with the lack of wisdom to live in harmony with nature had led to the deterioration of good quality of water thus resulting in water pollution. Therefore, pollution of water resources needs a serious and immediate attention through periodical monitoring of water quality. The evaluation of potable water supplies for coliform bacteria is important in determining the sanitary quality of drinking water. High levels of coliform counts indicate a contaminated source, inadequate treatment or post-treatment deficiencies in drinking water. Many developing countries suffer from either chronic shortages of freshwater or the

readily accessible water resources are heavily polluted, Microbiological health risks remain associated with many aspects of water uses, including drinking water in developing countries irrigation reuse of treated wastewater and recreational water contact. It has been reported that drinking water supplies have a long history of association with a wide spectrum of microbial infections. The primary goal of water quality management from a health perspective is to ensure that consumers are not exposed to doses of pathogens that are likely to cause diseases

Bacteriological Analysis: Heterotrophic bacteria and total coliform counts were enumerated using the membrane filtration technique (100 ml of water sample was filtered using 0.45 mm pore size membrane) and the multiple tube fermentation method respectively; The Isolates were further identified using standard biochemical procedures. Isolation of Enteric Pathogens Selenite F broth and Salmonella Shigella Agar are the enrichment broth and selective medium respectively for the enumeration of Salmonella and Shigella. One hundred millilitres of each water sample was introduced into sterile 250ml conical flasks containing an already sterilized (by boiling for 10 minutes) and cooled, 100ml double strength selenite F broth; the broth containing the sample was incubated at room temperature for 12-18 hours. Then, 0.1 ml of the incubated sample was aseptically pipetted on already prepared SSA medium.

The inoculum was spread with a sterile bent glass rod and incubated in an inverted position at 37ºC. After 16-24 hrs, the plates were observed for Salmonella growth. Alkaline peptone water and Thiosulphate Citrate Bile Salt agar are enrichment broth and selective medium, respectively for the enumeration of Vibrio. Ten millilitres of each water sample was pipetted into tubes containing already sterilized and cooled alkaline peptone water; the broth containing the sample was incubated at room temperature for 7-8 hours. Then, 0.1ml of the incubated sample was aseptically pipetted on already prepared TCBS medium. The inoculum was spread with a sterile bent glass rod and incubated in an inverted form at 37ºC. After a 24 hour period, the plates were observed for the formation of visible colonies

Microbial studies are carried out by MPN method Most Probable Number (MPN) technique this method is also called as multiple tube fermentation technique. This technique is used to detect the total coliforms. The test is performed sequentially in three stages namely the presumptive, confirmed, completed tests. Hi-media supplies dehydrated Double & Single strength Mac Conkey liquid media and EMB agar**.**To prepare this media an amount of the dehydrated powder, (as mentioned on the container) is dissolved in 1 liter of distilled water and autoclaved to get the specific medium ready.

Presumptive test: First set of 3 test tubes containing 10.0 ml of double strength MacConkey liquid media and Durham s tube were inoculated aseptically with 10.0 ml of water sample. Similarly 1.0 ml and 0.1 ml of water samples was inoculated aseptically into each of three tubes of 2nd and 3rd sets respectively, each containing 5 ml of single strength Mac Conkey liquid media and **Durham stube:** All tubes were incubated at 37°C for 2days.Tubes was then observed for gas production after 24 and 48 hours. The presence of gas in any tube after 24 hr is a positive presumptive test, the numbers of tubes in each set showing gas production were counted and the most probable count number/100 ml of the water sample was calculated by comparing with McCrady chart, following the standard methods for examination of water given by APHA.

Confirmed test: This test was applied to all samples that givea positive or doubtful presumptive test. Inoculum from the MacConkey liquid media tube showing positive presumptivetest with least quantity of water sample, wastaken and streaked onto a plate of Eosinmethylene blue (EMB) agar and kept forovernight incubation at 37°C. If typical dark colored colonies withgreen metallic sheen developed (mostprobably colonies of E. coli) on the platewithin this period, the confirmed test wasconsidered to be positive.

Completed test: From the EMB-agar plates, a single dark colored colony with metallic sheen (most probably colony of E. coli) was picked upand inoculated into 5ml peptone water and incubated at 37℃. After 4 hrs of incubation of peptone water at 37℃, inoculum from the incubated peptone water was inoculated on to citrate slope. Inoculated citrate media is incubated at37℃, in an incubator. And the previously inoculated peptone water is further incubated at 44℃ in a water bath for overnight incubation. Since, bacteria Escherichia coli (E. coli) and Enterobacteria erogenes (E. aerogenes)bear a close resemblance to each other intheir morphological and cultural characteristics, biochemical tests wereperformed to differentiate between them.

These tests were

Indole test (I): E. coli synthesizes an enzyme, tryptophanase, which forms indole, from tryptophan, i.e. it is positive for indole test, whereas E. aerogenes cannot catabolise tryptophan and is negative for indole test.

Citrate utilization test (C): E. aerogenesis capable of utilizing sodium citrate as its sole source of carbon, i.e. it is positive for citrate test. E. coli does not grow under these circumstances and is citrate negative Categories used for water quality assessment The microbial content is a very important water quality parameter because of its bearing on human health

Phages in water environments: Studies on the incidence of phages in water environments have been reported from most parts of the world for some decades now.Unfortunately the data are not particularly consistent and comparisons are generally not meaningful. One reason for this is that there are many variables that affect the incidence, survival and behaviour of phages in different water environments, including the densities of host bacteria and phages, temperature, pH and so on. Another important reason is the inconsistency in techniques used for the recovery of phages from water environments, and eventual detection and enumeration of the phages. This is not altogether surprising because virology, including phages, is a young and rapidly developing science. Phages can be recovered and detected by many techniques and approaches, and much of this work is still in a research or developmental stage. A major reason for discrepancies in results is the host bacteria used for the detection of various groups of phages. Nonetheless, international collaboration is now leading to meaningful, universally accepted guidelines for the recovery and detection of phages in water environments (such as those produced by the International Organisation for Standardisation)..

Application of monoclonal and polyclonal antibodies

Antibodies (glycoproteins produced by mammals as part of their defence system against foreign matter) possess highly specific binding and recognition domains that can be targeted to specific surface structures of a pathogen (antigen). Immunological methods using antibodies are widely used to detect pathogens in clinical, agricultural and environmental samples. Antisera or polyclonal antibodies, the original source of immune reagents, are obtained from the serum of immunised animals (typically rabbits or sheep). Monoclonal antibodies which are produced in vitro by fusing plasma cells of an immunised animal (usually a mouse or rat) with a cell line that grows continuously in culture (so that the fused cells will grow continuously and secrete only one kind of antibody molecule (Goding 1986)), can be much better standardised (Torrance 1999). Such monoclonal antibodies have been successfully used for the detection of indicator bacteria in water samples (Hübner et al. 1992; Obst et al. 1994). In these studies the water sample was subjected to a precultivation in a selective medium. In this way the complication of detecting dead cells was avoided. Another option for the detection of ‘viable’ indicators is the combination of immunofluorescence with a respiratory activity compound. This approach has been described for the detection of E. coli O157:H7, S. typhimurium and K. pneumoniae in water (Pyle et al. 1995). Detection of Legionella from water samples has also been achieved

with antibodies (Obst et al. 1994; Steinmetz et al. 1992). In general, immunological methods can easily be automated in order to handle high sample numbers. Antibody technology is often used in medicine with enzyme amplification (ELISA – enzyme linked immunosorbent assay), to allow the development of an antigen signal readable by the naked eye. Such an approach is under development for the rapid identification of coliform microcolonies (Sartory and Watkins 1999). As always with immunological techniques, the specificity of the reagents and optimisation of their use is paramount. Although total coliforms are a broad group and likely to be unsuitable immunological targets in environmental waters, E. coli could be identified from other coliforms.

CONCLUSION

Numerous epidemiological studies of waterborne illness in developed countries indicate that the common aetiological agents are more likely to be viruses and parasitic protozoa than bacteria. Given the often lower persistence of vegetative cells of the faecal bacteria compared to the former agents, it is not surprising that poor correlations have been reported between waterborne human viruses or protozoa and thermo tolerant coliforms .

Such a situation is critical to understand, as evident from recent drinking water outbreaks where coliform standards were met . Nonetheless, water regulatory agencies have yet to come to terms with the inherent problems resulting from reliance on faecal indicator bacteria as currently determined.

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