Microbiological analysis of drinking water supply in wards/ ICU of LN Hospital

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INTRODUCTION

Water is the major requirement for every individual on this earth. Contaminated water if consumed by people, can lead to an outbreak of water borne diseases. Water from a protected source such as properly constructed well or a source which has been subjected to some kind of treatment i.e. chlorination, UV rays etc is only considered safe for use. Water bacteriology aims at testing the drinking water for the presence of coliforms and other pathogen, indicating a recent contamination of the water source by fecal matter.

CDC (Center for Disease Control and Prevention) defines a disease as water borne when >2 persons experience similar symptoms after exposure to water encountered in drinking. Approximately 4 billion cases of diarrhea occur worldwide every year. In the year 2002, 1.8 million people were killed due to diarrheal diseases.

Significant pathogens causing water borne diseases include Escherichia coli, Clostridium spp., Vibrio cholerae, Salmonella spp., Shigella spp., Brucella spp., Listeria monocytogens etc.

Provision of clean drinking water has now been included as a Millennium Developmental Goal by APMCHUD.

Bacterial indicators of water pollution.(1):

The bacteria present in the water act as indicators of fecal pollution. If pathogens are present then it is a clear indicator that it has been derived from the human colon but the normal commensals present in the drinking water or the water sample is more reliable of it being polluted by human feces because they are far more in number than the pathogens. The presence of any spores of the bacteria indicates that the water may have been polluted in the past times rather than its recent pollution. Main indicator bacteria are:

Coliforms: These are bacteria which occur in large number in feces and sewage but are also found in the environment in the absence of fecal contamination. Thus their presence in water does not necessarily signify fecal contamination. These are lactose fermenting Gram negative bacilli and include typical or fecal (Escherichia coli) and atypical (Klebsiella aerogenes). The typical coliforms are

exclusively derived from the human intestine and are commensals in normal human beings and they die in a few days to weeks after leaving the human intestine, there by indicating recent pollution, whereas that of atypical ones is not necessarily so as they can be can be derived from the normal vegetation or environment.

Fecal streptococci: They are also the normal commensals of the intestine and include Gram positive catalase negative bacteria i.e. Streptococcus faecalis, Streptococcus bovis etc, members of family Enterococci. Their presence in the water is a strong indicator of the pollution of water by feces but their absence does not render the water pure.

Sulphite reducing Clostridium species: These are the members of genus Clostridium and reduce sulphite to sulphide. Although it is less numerous but its spores can survive for a long time thereby indicating remote contamination. Main organisms of this genus are

Cl.perfringens, Cl.difficile.

Nosocomial infections by bacteria present in water ie, Clostridium difficile (Cl. difficile): People are most often nosocomially infected in hospitals, nursing homes, or institutions, although C. difficile infection is increasing in the community and outpatient setting. (2)C. difficile-associated diarrhea (aka CDAD) is most strongly associated with the use of fluoroquinolones.

Pseudomonas aeruginosa: It can multiply in aquatic environment but absent in feces. It is not a good indicator of water pollution but it is an opportunistic pathogen.

AIMS AND OBJECTIVES OF THE PROJECT

1. To test the samples of water taken from different wards and ICUs of Lok Nayak hospital for the presence of coliforms & other pathogenic bacteria

2. To determine the Presumptive coliform count in each water sample and comment on the safety of its use.

3. To perform the antibiotic susceptibility testing for all the isolated pathogenic bacteria

METHODOLOGY.(3)

Collection of the sample: About 100 ml of each sample to be tested will be collected in a sterile bottle. When collecting sample from a running tap, the water will be allowed to run waste for 2-3 min before collection. The bottle will be stoppered and labeled with full details of the water source, time and date of collection of the sample.

Bacteriological count in water:

The routine tests generally used in bacteriological examination of water are:

1) Presumptive coliform count by multiple tube method: It is the quantitative test for all the coliform bacilli. The estimation of the coliform count is generally made by adding varying quahtities of water (from 0.1ml to 50ml) to bile salt lactose peptone water (with indicator of acidity) contained in bottles with Durham's tubes to show the formation of gas; acid and gas formation indicates the growth of coliform bacteria.

Multiple tube method: Liquid culture medium ie, MacConkey's broth is used at 50 and 10 ml volumes of double strength concentration and 5ml volumes of single strength in suitable test tubes having an inverted Durham's tube to detect the production of gas.

Water samples to be tested will be aseptically added with sterile graduated pipettes to MacConkey's broth medium in the following amounts:

- one 50 ml quantity to 50 ml double strength medium
- five 10 ml quantities each to 10 ml double strength medium
- five 1 ml quantities each to 5 ml medium at single strength
- five 0.1 ml quantities each to 5 ml medium at single strength

The inoculated medium will be incubated at 370:C for 24 hrs and the results will be noted by comparative analysis with the standard MPN (Most probable number) charts. The identification of the micro-organisms will be confirmed by sub-culturing on to MacConkey's and blood agar media and following the standard identification protocols

2) Differential coliform count: To ascertain whether the coliforms detected in the presumptive tests are E.coli ,the Eijkman test will be employed. This depends on the ability of E.coli to produce gas when growing in bile salt lactose peptone water at 44.0C and inability of atypical to do so. After the presumptive test the subcultures are made from the bottles showing acid and gas production into fresh tubes of single strength Mac Conkey's broth. They are incubated at 440C and examined after 24 hrs.

Gas production in Mac Conkey's broth at 440C

Indole production at 440C

+

+

Typical coliform bacilli

+

-

Irregular forms of coliform organisms

-

+/-

Other coliform organisms

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Antibiotic susceptibility testing: The susceptibility of all the isolated pathogen in the water samples will be tested against antibiotics using disc diffusion (Kirby Bauer's) method.