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IDENTIFICATION OF MICROORGANISMS IN STAGNANT WATER – A NOTE

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ABSTRACT

One of the greatest concerns for the water consumers with respect to the quality of drinking water is contamination with pathogenic microorganisms. Certain microorganisms, including various bacteria, viruses, and parasites, are well-known water contaminants, of which several may lead to waterborne disease and epidemics. The aim of the present study is to identify the bacterial contaminant. The collected sample of stagnant water were processed for bacterial isolation using the nutrient broth and EMB agar. The conventional methods of swabbing and streaking were used. Obtain an EMB agar plate and streak it with the appropriate bacterial culture using the quadrant streak plate method. This will result in the isolation of individual colonies. Nutrient broth inoculated with Escherichia coli (a gram-negative bacterium) demonstrating growth with whitish colonies. Gram's staining method type of bacteria either gram positive or gram negative was conformed.

INTRODUCTION

Water a vital nutrient in chemical metabolism which plays an important role in the digestion, absorption of food, transportation of nutrients in the body and elimination of waste products via urine. Physical, chemical and microbiological qualities water has a fundamental importance in chemical industry. The role of water in spreading communicable diseases is much evident due to combined source of water Contaminated water with fecal coliform severely affects the performance of humans. Salmonella, Camphylobacter spp, Staphylococcus aureus, Pseudomonas eruginosa, Clostridium botulinum, *Vibrio cholerae* and Escherichia coli are the main human pathogens responsible for water contamination. Water quality used for chemical industry and health is one of the most significant segments in health management. Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogens from Pseudomonas Sps, aeromonas, staphylococcus and other microorganisms groups, being able to generate infections by contact with skin, mucous membrane, nosopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Pyogenic infection of injuries, meningitis, urinary system, respiratory system, inflammation of the middle ear and eyes are typical diseases caused by contaminated water. Wound infections, peritonitis, meningitis, endocarditis, septicemia, corneal ulcers, nosocomial infections, urinary tract infections, gastroenteritis of people who bathe or use water in other ways are caused *by Aeromona shydrophila*. Infections of skin, nosopharyngeal cavity, eyes, outer ear among bathing people could be caused by recreational waters polluted by S.aureus. All the above mentioned species of bacteria survive in water longer than classical indicators of sanitary state and they are not connected with faecal contamination present in water.

MATERIALS AND METHODS

Materials: Nutrient Broth-4gm/500ml, Distilled Water, Incubator

Composition of EMB Agar:10 g of peptone, 5 g of lactose, 5 g of sucrose, 2 g of dipotassium phosphate, 13.5 g of Bacto agar, 0.4 g of Bacto eosin Y. Distilled water to bring final volume to 1 liter. EMB agar is commercially available in premixed form from biological supply companies.

Gram staining: Christal violet, Grams iodine, Decolouriser, Saffrine

Other necessities: Petri plates, Test tubes, Cotton, Glass rod, Conical Flask, Filter Paper, Mortar and Pestle, Pipettes Surgical Spirit (for Cleaning)

METHODS

Sterilization: Sterilization (or sterilization) is a term referring to any process that eliminates (removes) or kills all forms of life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure, and filtration or combinations thereof. A widely used method for heat sterilization is the autoclave, sometimes called a converter. Autoclaves commonly use steam heated to 121–134 °C (250–273 °F). To achieve sterility, a holding time of at least 15 minutes at 121 °C (250 °F) is required. Additional sterilizing time is usually required for liquids and instruments packed in layers of cloth, as they may take longer to reach the required temperature (unnecessary in machines that grind the contents prior to sterilization). Following sterilization, liquids in a pressurized autoclave must be cooled slowly to avoid boiling over when the pressure is released. Modern converters operate around this problem by gradually depressurizing the sterilization chamber and allowing liquids to evaporate under a negative pressure, while cooling the contents. Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant.

EMB agar medium preparation: Add required amount of EMB agar to 100 ml of water. Adjust pH to 7.2. Boil to completely dissolve agar. Sterilize in an autoclave for 15 minutes at 15 psi (121oC). Cool to 60oC. If any precipitate is apparent in the medium, disperse by gently swirling before pouring into sterile Petri dishes.

Nutrient broth preparation: Add required amount of EMB agar to 100 ml of water. Adjust pH to 7.2. Boil to completely dissolve agar. Plug non-absorbent cotton to avoid solidification and contamination. Sterilize in an autoclave for 15 minutes.

Gram staining: Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure. The addition of iodide, which binds to crystal violet and traps it in the cell, Rapid decolorization with ethanol or acetone, and *Counterstaining* with safranin. Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.

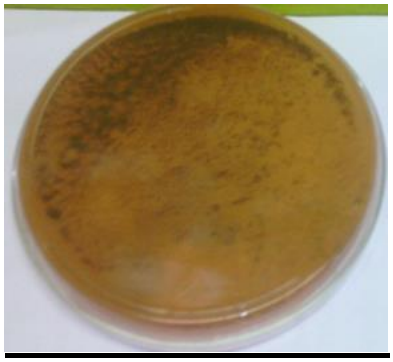
PROCEDURE

Culturing of micro-organism from the sample: Obtain an EMB agar plate and streak it with the appropriate bacterial culture using the quadrant streak plate method. This will result in the isolation of individual colonies. Eosin-methylene blue agar is selective for gram-negative bacteria against gram-positive bacteria. In addition, EMB agar is useful in isolation and differentiation of the various gram-negative bacilli and enteric bacilli, generally known coliforms and fecal coliforms respectively. Colonies of micro-organism will be formed after incubation of 24 hours. The colonies will appear as metallic green or blue.

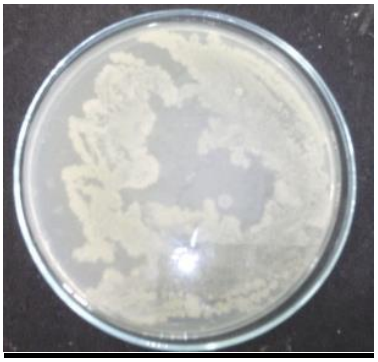
Subculture preparation: From the prepared mother culture desired colonies of microorganism are selected. These colonies are inoculated in the prepared nutrient broth using streak plating in the petridish. After 24 hours of incubation new colonies of microorganism will be formed. Subculture is therefore used to produce a new culture with a lower density of cells than the original culture.

Gram staining: Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure. The addition of iodide, which binds to crystal violet and traps it in the cell, Rapid decolorization with ethanol or acetone, and *Counterstaining* with safranin. Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.

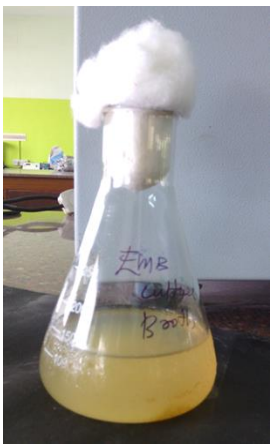
RESULTS AND DISCUSSION



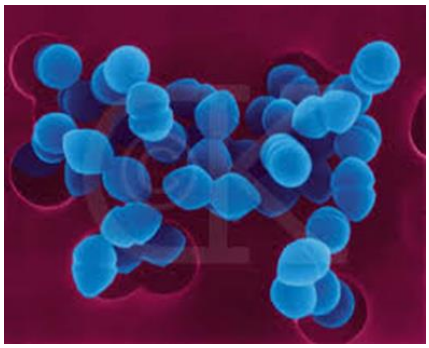
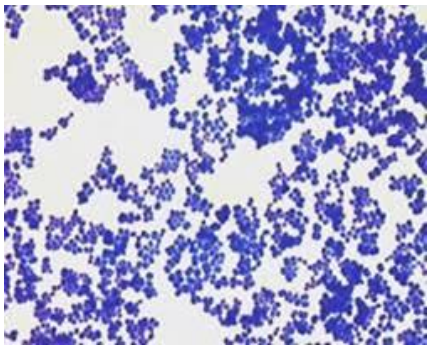
EMB agar plate



Subculture



EMB culture broth

Microscopic view of *E.Coli* – cocci shaped clusters

DISCUSSION

This study has clearly indicated that the water sample is highly contaminated by bacteria. The bacteria isolated in this study are known to present in all sorts of environment of human involvement, majority of them are human as well as animal pathogen. The water analyzed in this study has clearly shown that they are loaded with indicator organisms which are the indication of faecal pollution and thus the human interference. Basically the bacteria are responsible for the degradation of organic and inorganic compounds. They derive their nutritional requirement from the compounds presented to them in the waste water. They are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the food, together with other elements. The energy from oxidizing either organic compounds (chemoorganotrophic metabolism), or organic compounds (chemolithotrophic metabolism), such as reduced sulfur or nitrogen compounds. They use the energy for their bodily functions, reproduction and growth. Many researchers found that gram-negative bacteria constituted the most genera bacteria isolated from stagnant water. The results of this study also indicated that gram-negative *E.coli* bacteria constituted the majority of bacteria in the lake water.

CONCLUSION

Thus the blue coloured cocci shaped clusters of bacteria were obtained. And it was identified as *Escherichia coli.*

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