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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM SEWAGE WATERS IN IMPHAL CITY - A COMMUNITY HEALTH ASPECT

***Sanasam Suranjit and Oinam Shashikumar Singh**

Department of Zoology, D. M. College of Science, Imphal Manipur India

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ABSTRACT

The microbiologists separate bacterial mixed populations into individual species for study. The waste water resulting from various human activities (domestic, agricultural and industrial) is technically referred to as sewage. The sewage is mostly composed of organic and inorganic compounds, toxic substances, heavy metals and pathogenic organisms. The bacterial flora was isolated from the serial diluted sewage sample which was collected from the Naga drain. Microscopic examination, physical and morphological characteristics, biochemical tests of the bacterial growth was investigated. During the investigations, the following genera, viz. Enterobacterium sp., Salmonella typhimurium, Pseudomonas sp., Shigella sp. were isolated. The above mentioned genera were concluded to be enteric gram-negative bacteria, which are pathogenic and resistant to multiple drugs.

Key words: Naga drain, Imphal City, Sewage water, Triplate.

INTRODUCTION

Microorganisms are abundant and ubiquitous in our environment. They occur in air, soil, water, food, sewage, decomposing plants and animal tissues. Microbes encountered by microbiologists include all the microscopic forms of life including the viruses. The microbiologists separate these mixed populations into individual species for study. The waste water resulting from various human activities, namely domestic, agricultural and industrial, is technically referred to as sewage. The sewage is mostly composed of organic and inorganic compounds, toxic substances, heavy metals and pathogenic organisms, etc. (Bahig *et al,* 2008 and Wan Ishak *et al*, 2011). Microorganisms, like all other living organisms, require basic nutrients for sustenance of life. The food materials on which microorganisms are grown in the laboratory is known as a culture medium (pl. media) and the grown itself is called a culture. In other words, the nutrient preparation on or in which a culture (i.e., a population of microorganism) is grown A Culture containing a single unadulterated species of cells is called a pure culture (Aneja, 2007). Several different techniques are applied to isolate and study a pure culture. In nature, microbial populations do not segregate themselves by species but exist as a mixture of many different cell types. These populations can be separated into pure cultures.

Corresponding author: Sanasam Suranjit
Department of Zoology, D. M. College of Science, Imphal Manipur India.

These cultures contain only one type of organism and are suitable for the study of their cultural, morphological, and biochemical properties. Techniques commonly used for isolation of discrete colonies initially require reduction of the number of organisms in the inoculums (Wan Ishak *et al*, 2011). The isolates were grouped according to their colony morphology and cell characteristics. The colony were counted and re-isolated in pure culture using the medium on which they had grown (Njoku *et al,* 1990). Enteric bacteria are normal inhabitants of the intestines of human and other animals. These bacteria are often isolated from aquatic ecosystems due to introduction of sewage into the environment. Sewage contains high numbers of pathogenic enteric bacteria known as faecal coliforms. Coliforms are gram negative, facultative anaerobic bacteria (Rene *et al,* 2007). The present work was carried out to gain an insight into the community health scenario and to isolate and identify the pathogenic bacteria present in the sewage water samples collected from the polluted Naga drain, which flows through the Imphal City.

MATERIALS AND METHODS

Preparation of culture media

The preparation of nutrient agar media, mannitol salt agar media (MSA), eosin methylene blue agar media (EMB) and MacConkey agar media were carried out following Aneja (2007).

Collection of sample

This study was conducted during the period of February 2016 to May 2016. Sewage water samples were collected from a highly polluted Naga Nala, a tributary of the Naga drain, flowing along the southern boundary of D. M. College of Science, Imphal. The samples collected were immediately transferred to sterile glass tubes and brought to the DBT-Institutional Level Biotech-hub laboratory for bacteriological analysis.

Isolation of Bacteria

1 ml of sewage sample was subjected through serial dilution to obtain 10^{-6} dilution factor. 1 ml of the resulting suspension from each dilution was aseptically transferred onto the surface of already prepared nutrient agar plates by pour plate technique, and incubated at 37^0C for 18-24 hrs. The colonies were counted as cfu (colony forming unit) observed on the nutrient plates. Pure culture of the single bacteria populations were obtained from the thin individual colonies of the bacteria which varied in shape and colour by streak plate on nutrient plates. The isolates were kept on nutrient agar slant media at 4^0 C and re-culturing every 4 weeks (Wan Ishak *et al*., 2011; Bahig *et al*., 2008). Further investigation like Gram's staining and biochemical tests were carried out. The physical and morphological characteristic such as colours, appearances and shapes of the isolates were recorded in the laboratory.

which varied in shape and colour by streak plate on nutrient plates (Plate 4).

Gram's staining

The bacterial growth were found that appeared purple and coccid in shape were referred to as gram positive; those appeared rod and bacilli in shape and pink in colour were described gram negative.

Physical and Morphology Characteristics of Agar Plate and Slant Cultural

This gram negative rod is a common contaminant of vegetable matter which forms shiny colonies with entire margins, convex elevation; rod forms mucoid colonies with umbonate elevation was observed on the pure culture plates of nutrient agar.

Selective and Differential Media

Mannitol Salt Agar (MSA):

During investigation, a well defined bacterial colony was not observed on this triplate medium due to the inhibition of gram negative bacteria (Plate 5). Mannitol Salt Agar plate was coated being that it selects for gram-positive bacterial growth.

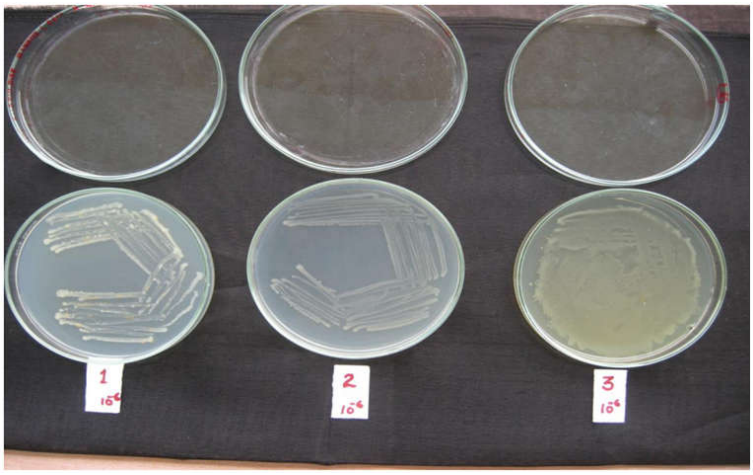


Plate 4. Unknown bacterial pure culture on agar plates

Isolation of Bacteria

After incubation for 24 hrs, the maximum growth of the bacterium culture was found as mixed, non-separated and overlapped growth regarding to the serial dilution factors. The growth of dilutions 10^{-1} to 10^{-6} were enumerated approximately as 300, 210, 145, 80, 35, and 20. The counted colony forming unit (cfu) obtained on nutrient agar plates were 30 x 10^1 CFU/gm, 21 x 10^2 CFU/gm, 14.5 x 10^3 CFU/gm, 8 x 10^4 CFU/gm, 3.5 x 10^5 CFU/gm, and 2 x 10^6 CFU/gm respectively. The result is in agreement with the findings of Bahig *et al* (2008) as the total bacterial count in site (B), where sewage is used directly to irrigate the soil. The bacterial count was ranged from 30 X 10^3 to 45 X 10^4 and from 13 X 10^2 to 30 X 10^3 during hot and cold seasons, respectively. Pure culture of the single bacteria populations were obtained from the thin individual colonies of the bacteria

When checking the results after incubation, there was no growth on this plate, and still no second distinct colony by CPR Louisville (2014).

Eosin Methylene Blue Agar (EMB)

1st tripet – It was observed that a bluish colour that identified as *Salmonella sp.*

2nd triplet – On the same medium, the bacterial species was observed identified as with unbonate elevator.

3rd triplet – This was identified as *Enterobacterium sp.* Gram negative; rod shaped which shiny colonies with entire margin and convex elevator. (Plate 6)

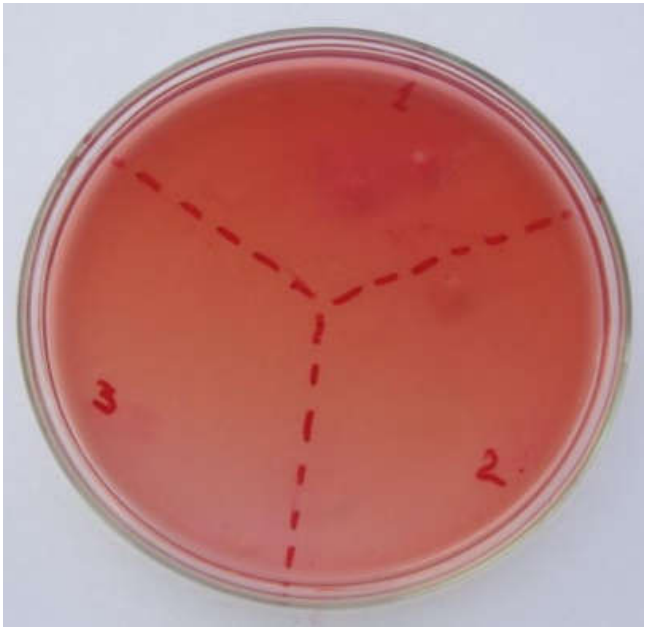
MacConkey Agar

1st tripet – Growth on the plate indicated the organism, *Salmonella typhimurium*, was not inhibit by bile

salts and crystal violet and was a gram-negative bacterium. The absence of colour in the bacterial growth indicated *S. typhimurium* was unable to ferment lactose.

2nd triplet – *Pseudomonas sp.* gram negative, rod forms mucoid colonies would not ferment the lactose and would form off-white colonies.

3rd triplet – *Shigella sp.* Also found as gram negative bacterial colonies would not ferment the lactose and would form off-white colonies (Plate 7).



Plater 5. Triplate of Salt Agar (MSA) plate

Plate 6. Triplate of Eosin Methylene Blue (EMB) Agar plate

DISCUSSION

During the investigation, the finding bacterial genera were characterised as *Enterobacterium sp., Salmonella typhimurium, S. Typhimurium, Pseudomonas sp*., *Shigella sp.*, was pathogenic bacteria which might causes human diseases. Gram-negative bacteria cause infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings.

Plate 7. Triplate of MacConkey Agar Plate

Gram-negative bacteria are resistant to multiple drugs and are increasingly resistant to most available antibiotics. These bacteria have built-in abilities to find new ways to be resistant and can pass along genetic materials that allow other bacteria to become drug-resistant as well (*Centres for Disease Control and Prevention, Atlanta, 2011*). Mannitol Salt Agar (MSA) is used as selective and differential growth medium in microbiology. This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time and contains a high Conc. Of Salt that making it selective for gram positive bacterium *Staphylococci* since the level of NaCl is inhibitory to the most other bacteria (*Laboratory Media and Biochemical Tests*). Eosin Methylene Blue Agar (EMB) is a selective and differential medium. Eosin differentiates between two major coliforms: E. coli (smaller, green-metallic sheen) and *Enterobacter aerogenes* (larger, rose colour). Methylene blue selectively inhibits the growth of Gram+ bacteria. With the media, we can also determine which bacteria are Gram-negative, because only Gram-negative bacteria grow on this special media. The enhanced cell walls of Gram-negative bacteria protect these bacteria from the dye in the EMB plates. The dye is able to enter the cells of Gram positive bacteria and kill them. MacConkey Agar demonstrates the ability of a gram negative bacterium to metabolize Lactose. MacConkey agar is both a selective and differential medium frequently used in culture testing. It contains crystal violet dye and bile salts, both of which inhibit the growth of most gram-positive bacteria. It contains lactose (a sugar) and neutral red indicator (a pH indicator which is yellow in a neutral solution, but turns pink to red in an acidic environment), which allow for differentiation. On MacConkey agar, Escherichia coli and *Enterobacter aerogenes* would ferment the lactose producing acid and would form colonies pink to red in colour. On the same medium, *Salmonella, Shigella, and Pseudomonas* species would not ferment the lactose and would form off-white colonies. The red coloured colonies show that acid was

produced from lactose, meaning the bacteria could utilize lactose as a carbon source. Gram-negative infections include those caused by *Klebsiella*, *Acinetobacter*, *Pseudomonas aeruginosa*, and *E. coli*., as well as many other less common bacteria.

Conclusion

The result revealed that further studies are needed to determine the more accurate in such bacteria medium. However, further work is necessary to undergo the biochemical test such as Amylase production test, Cellulase production test (degradation of cellulose), production of pectolytic enzymes (degradation of pectin), Hydrolysis of gelatine, Casein hydrolysis, Urease test, Hydrogen sulphide production test, IMVic test and catalase test. Hence, it can be concluded that enteric negative bacteria are the pathogens that causing diseases to the human beings.

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REFERENCES

Aneja, K.R. 2007. Experiments in Microbiology, Plant Pathology and Biotechnology. IV Ed. New Age International (P) Limited, Publishers. New Delhi. 145-156.

Bahig, A.E., Aly, E.A., Khaled, A.A and Amel, K.A. 2008. Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt. *Malaysian J. Microbiol.* 4(2): 42- 50.

Bhaswati, G. and Jayati, B. 2014. Identification and Characterization of Extracellular Red Pigment Producing Bacteria Isolated from Soil. Int. J. Curr. Microbiol. App. Sci. 3(9): 169-176.

Louisville, C.P.R. 2014. Enterobacter aerogenes. Retrieved July 1, 2016, from http://acls-bls-louisville.com/563/ enterobacter-aerogenes-how-to-identify-for-micro-unknown-lab-report/

Darjily, D.R., Shanmuga Priya, R., Punam Sen, Muthukrishnan, P. and Saranya, R. 2014. Isolation and Characterization of Red Pigment Producing *Serratia sp.,* from Soil Sample. *J. Sci. Technol.* 2(2): 79-84.

Davis, U.C. 2005. Enteric Bacteria. Retrieved March 28, 2006, from http://ehs.ucdavis.edu/animal/health/enterics.cfm.

Edward Raja, C., Selvam, G.S. and Kiyoshi Omine, 2009. Isolation, identification and characterization of heavy metal resistant bacteria from sewage. Int. Symp. Geadisaster prevention and Geoenviron in Asia. 205-211

George, W.L., Sutter, V.L., Citron, D. and Finegold, S.M. 1979. Selective and differential medium for isolation of *Clostridium difficile.* J. Clin. Microbiol., 9(2): 214–219.

Gram-negative Bacteria Infections in Healthcare Settings. http://www.cdc.gov/hai/organisms/grams-negative-bacteria.html#guideline.

Laboratory Media and Biochemical Tests. Retrieved 30 June, 2016, from http://studylib.net/doc/8267009/bio203-laboratory-media-and-biochemical-tests.

Njoku, H.O., Ogbulie, J.N. and Nnubia, 1990. Microbial Ecology of Traditional Fermentation of African Oil bean seed for Ugba production. J. Food Microbiol. 3:18-28

Rajkumar Nagarajan N and Hema Sudhakar (2012). Isolation of Sulfide Oxidizing Bacteria from Sewage-*Thiobacillus species*. B. Environ., Pharmacol. Life Sci. 1(5): 40 – 42.

Rene Beaudoin N, Danielle DeCesaro R, Debrah Durkee L, and Susan Barbaro E (2007). Isolation of a bacteriophage from sewage sludge and characterization of its bacterial host. Cellrivier Acad. J. 3(1): 1-8.

Teltsch B, Kedmi S, Bonnet L, Borenzstajn-Rotem Y and Katzenelson E (1980). Isolation and identification of pathogenic microorganisms at wastewater-irrigated fields: ratios in air and wastewater. Appl. Environ. Microbiol. 39(6):1183–1190.

Ushijima T, Takahashi M and Ozaki Y (1981). Selective and differential media for isolation and tentative identification of each species of Pityrosporum residing on normal or diseased human skin. Microbiol. Immunol., 25(11):1109-1118.

WMF Wan Ishak S, Jamek NA, Jalanni NF and Mohd Jamaludin (2011). Isolation and Identification of Bacteria from Activated Sludge and Compost for Municipal Solid Waste Treatment System. Proc. Int. Conf. Biol., Environ. Chem. IPCBEE. 24: 450-454.

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