Identification and Isolation of Escherichia Coli from Sewage Water Source

(Case Study at Wollega University Main Campus, Nekemte, Ethiopia)

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Abstract: Unsafe water sources, sewage water, was the most contaminated water. Before this study method of E. coli isolation and identification was not documented. Thus study was conducted to identify and document appropriate method for E. coli isolation. Three sites were selected in Wollega university main campus for this study. The representative samples from these sites were collected and analyzed at Wollega university main campus, in biological science laboratory. Indicator bacteria (E. coli) were isolated from all samples. Water analysis documented that all water samples in the study by E. coli. The sample from shower, cafeteria and spring contain 100%, 85.7% and 71.4% E. coli (indicator) colony respectively. The high concentration of bacteria in all samples of this study suggested the preference of pathogenic organisms. The unsafe water source is highly contaminated by pathogenic organisms and so has impact on health of individual. This study was focused on collection of the water sources samples for isolation and identification of E. coli.

Keywords: Escherichia Coli; Pathogenic; Indicator bacteria; Isolation

1. INTRODUCTION

Water is the most abundant and vital substance for life activities in nature. It is the most essential element for life and its nature sources for human use are rivers, underground and lakes water. These sources of water could be contaminated by organic and inorganic chemicals, sewage disposal, running water and living organisms such as protozoans, fungi, viruses and bacteria (Al-Zubeiry, A. H. S. (Taiz University, 2005).

Sewage is contaminated water sources by industrial sewage, drainage, and black water, human wastes such as feces and toilets, showers and from different home activities. It consists of pathogens such as bacteria, parasitic worms, as well as organic and inorganic particles. These contaminants can be water quality indicators, fecal microbes, total coliform, fecal coliform, fecal streptococci and
viruses. Microbiological water contamination is detected by microbial water quality indicator. These bacteria indicate fecal pollution and possible presence and ingestion of the other pathogens with polluted water.

Indicator organisms are selected to demonstrate the presence of human and animal wastes, pathogens and usually originated from warm blooded animals and humans intestine containing pathogens such as salmonella, shigella and E.coli(muyima and Ngcakani,1998; Bitton ,2005). Coliform bacteria are facultative, anaerobic, rod shaped, gram-negative and non-spore forming that ferment lactose at 350c and produce acid and gas with in forty eight hours(48hrs) (“Appendix A : Translation of 1986 Criteria Risk to Equivalent Risk Levels for Use with New Health Data Developed Using Rapid Methods for Measuring Water Quality”, 1986).

Among the coliform bacteria, Escherichia coli is the most abundant and best indicator of water quality and presence of pathogens. It comprises n seven (97%) of fecal coliform bacteria in human faces and available indicator of fecal contamination(Nold, 2005).

Escherichia coli is belong to domain bacteria, phylum proteo bacteria, order Entereo bacteria, class Gamma proteo bacteria, family Entereo bacteraceae, Genus Escherichia and species Escherichia coli (Scheutz & Strockbine, 1996). E.coli is a type of coliform, gram-negative, rod shaped and non-spore forming bacteria which commonly found in warm blooded animals. It is the most widely studied prokaryotic organisms and the most important in the field of microbiology and biotechnology (1990).

E. coli was first discovered by T. Escherichia in 1885 for feacall of health individuals and in 1891, frank land stated it as organisms with sewage characteristics that provide evidence for potentially dangerous pollution and so must be identified (Hutchinson, 1994). Event though, E.coli is indicator of contamination and pathogen presence, some of its strain such as 0157:H7, enter hemorrhagic and entro invasive are pathogenic and causes illness in mammals including humans (Perfomance, Mssc, & Pdf, n.d.).

More than 80% of disease in the world are attributed to unsafe drinking water or in adequate sanitation practices (WHO, 2003). In Ethiopia, 3/4th of the children are affected by unsafe water and sanitation problems. As a result 46% mortality rate in children is due to diarrhea and dehydration (Ethiopia Ministry of Health, 2006). In rural areas and villages of Ethiopia, water for human consumption, drinking, washing (bathing, laundry), for preparation of food etc is obtained from rivers, streams, shallow wells, springs, lakes, ponds and rain fall. These sources are contaminated by animals and human wastes, effluents due to open field detection practices. This contaminated water expose communities to various water born disease (Amenu, Menkir, & Gobena, 2013).

The microbial water quality analysis, by using microbial indicator was done at some Ethiopian universities in different areas. For instance at Addis Ababa university, the assessment of contamination level of water and major sources of contaminants in central highland of Ethiopia(yudo –lege batu) was conducted by Birhanu million July in 2008(Birhanu ,2008). Before the present study, however, microbial water quality analysis, specifically E.coli isolation and identification from sewage water as well as methods of isolation did not conducted and documented in wollega university microbiological laboratory.

Objective of the study;

a. General objective. The present study was carried out in order to identify and isolate E.coli from sewage water sources at wollega university main campus;

b. Specific objectives
   1) To identify E.coli from sewage water sample at present study
   2) To isolate the E.coli from water source samples at present study.
   3) To recommend appropriate method to isolate E.coli from sewage water sample and document.

2. RESEARCH METHODOLOGY

2.1. Materials and Methods

2.1.1. Study Area

The study was conducted at wollega university main campus, Nekemte. Nekemte town is located at distance of 331km to the west of Addis Ababa. The elevation of Nekemte town is range from1350 to 2088m above sea level. The climate of the area is woina dega and the mean annual rain fall is 1400mm. The temperature of the area is range from 160c (lowest) to310c (the highest). Wollega
university main campus is found in Nekemte town. It was established in 2007 by Ethiopian ministry of education on 150 hectares of lands. It is found on latitude of 905'E and longitude 36033'E.

2.1.2. Study Design
A cross sectional study was carried out to isolate and identify E.coli from sewage water sample in the study area. The study was conducted in Wollega University main campus from March to June 2014. The study was conducted by collecting water sample from sewage water sources. Finally the collected water samples were processed to analyze the E.coli isolation and identification.

Water samples were collected from three waste disposal: student’s shower, main cafeteria and unprotected spring found west of natural science faculty. During sampling, the outlets of these water sources were chosen. Selected sites were 100m-120m away from each other. The samples were collected acceptably and kept in ice box during transportation.

Serial dilution, lactose broth, plate count and selective media (EMB) were used to detect and isolate total coliform (E.coli) from the three water samples the following (Water, Association, & Environment, 1999) procedure.

2.1.3. Sample Size and Sampling Point
The water samples were taken from each sites: shower, cafeteria and springs between March to June 2014. The sample were collected following (Water et al., 1999)sampling procedure. In each sampling, two samples (shower and cafeteria) were taken nearby their sources and the other sample was taken 100m far from its sources. The water sample were handled acceptically in sterile glass bottles, labeled and kept in an ice box during transportation to the laboratory biology department of Wollega University. Three of each water samples were taken and analyzed in the laboratory.

Totally three water samples were analyzed after its PH and temperature measured.

2.1.4. Sample Analysis
In the laboratory, the three sample from each site were subjected to serial dilution for analysis of E.coli. after each samples were serially diluted in seven test tubes(dilution 10⁻⁷), one ml of water from each dilution were acceptically transferred to prepared seven lactose broth test tubes for detection of E.coli (coliform) presence.

The prepared tubes were incubated for 48hrs at 450c upon completion of the incubation period, the positive test tubes (tubes with bubble gas formed) counted. From each positive test tubes, the samples were acceptically transferred to selective E.coli media for further culturing and identifying it and incubated for 24hrs at 44.50c, upon completion of incubation period, the colony of each petridish were counted and recorded. Further identification was done by examining the colonies by gram staining technique and observed under compound light microscope (Güldemann, 2000) .

3. RESEARCH RESULT
3.1. Bacteriological Analysis
Bacteriological analysis of samples from the three sites at Wollega university main campus showed that all samples were positive for Escherichia coli. This indicator bacterium was often encountered in all samples from water sources of the study area. Less occurrence of indicator (E.coli) was observed at samples from the spring sites. (Table 1).
Table 1. Bacteriological analysis of water samples from shower, cafeteria and spring in Wollega University main campus between March to June.

<table>
<thead>
<tr>
<th>Sample Water Sites</th>
<th>Shower, n=7</th>
<th>Cafeteria, n=7</th>
<th>Spring, n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of organisms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of +ve sample</td>
<td>No of +ve sample</td>
<td>No of +ve sample</td>
<td>No of +ve sample</td>
</tr>
<tr>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>Total Coliform (E.coli)</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Percent (%)</td>
<td>100</td>
<td>85.7</td>
<td>71.42</td>
</tr>
</tbody>
</table>

n=number of test tubes used.

3.2. Concentration of E.coli in Water Samples

The highest average E.coli colony counts were observed at shower (203.57 cfu/100ml) and the lowest mean counts, 80.7 cfu/100 ml. E.coli colony were found in spring sample. Sample from cafeteria had 138.57 cfu/100ml average colonies (table 2).

Table 2. The average number of E.coli colony counted on each petridish of samples (shower, cafeteria and spring cfu/100ml).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Sample Water Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shower</td>
</tr>
<tr>
<td>No of colony counted, cfu/100 ml</td>
<td>203.57</td>
</tr>
</tbody>
</table>

4. DISCUSSION

The identification and isolation of E.coli from the sewage water source is collected at Wollega University main campus. The selected water sources were collected from near the campus and within the campus, thus water sample were collected from shower, which is located 100m at east of cafeteria and spring 50m west of cafeteria. These water sources were used for different purposes, most of the students used the water sources from shower for cleaning and as well as for bathing, the spring sources used for cleaning of materials, clothes and other activities.

The average indicator bacteria (E.coli) obtained from all sites were different. The average counts of E.coli was detected in shower sample (203.57 cfu/100ml), cafeteria (138.57 cfu/100ml) and the least count were obtained from spring (80.7 cfu/100ml). According to survey of microbiological quality of natural springs conducted in Seoul, South Korea, from all samples collected from untreated wells streams and rivers. The mean density of E.coli varied from 0 cfu/ml to 15 cfu/m (Grisey, Belle, Dat, Mudry, & Aleya, 2010). US, Environmental protection agency office of water found that the mean density of E.coli from waste water effluent was range from 120 cfu/ml to 280 cfu/ml in all samples (Us Epa, 2004). The result of the present study was in the range of the results but it did not found 0 cfu/ml in all samples.
5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on research finding the following conclusion have been drawn.

All water sources were positive for the presence of E.coli.

E.coli colonies were mostly available in water which drawn from shower. Large colony of E.coli was mostly detected in sample from shower because the origin of E.coli is from warm blooded animals and humans (Muyima and Ngcakani, 1998).

The high counts of indicator (E.coli) in water samples of the study area suggested that the presence of pathogenic organisms.

E.coli isolation and identification methodology has been reported in the literature and the isolated and identified E.coli was preserved in biology laboratory. The base line information generated from this study may be attract other researcher for further studies on E.coli.

5.2 Recommendation

Since the indicator (E.coli) counts in all sampled sites were very large, care should be taken and it should be treated before released to surroundings. The spring should be treated or diverted because it was contaminated. Any scientific investigation now can start on E.coli to make the university “center of the scientific study”. Other scientifically and medically important microorganisms should be identified, isolated and preserved in wollega university biological laboratory.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

17. WHO. 2003. Emerging Issues in Water and Infectious Disease