PHYSICO-CHEMICAL ANALYSIS, ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM DRINKING WATER IN GUMMA PANCHAYATH, VISAKHAPATNAM, ANDHRA PRADESH.

Laxmi Sowmya, K., *Sandhya Deepika, D. and N.S.M Jyothi

Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh.

Article Received on 08/12/2014               Article Revised on 29/12/2014               Article Accepted on 20/01/2015

ABSTRACT
The present study was undertaken to evaluate the water quality of the Gumma panchayat of Ananthagiri mandal in Visakhapatnam district with affable means. The physicochemical and the microbial studies are most important regions by which we are able to test the portability of water. The isolation and characterization of the pathogenic microorganism from the water sample collected were the main emphasized area of the study. In this study drinking water samples were collected from a hand bore and a stream for a period of one year i.e., from April 2011 to March 2012. The various constituents monitored include the physicochemical characters, the bacterial parameters like Total plate count (TPC), Most probable number (MPN) and isolation and identification of pathogenic bacteria. The physicochemical characters of all the two drinking water samples were with in the recommended permissible level of WHO. The total plate count was above the WHO guidelines values (<10CFU’s/ml) in the Two water samples studied and the highest count was during August and September. The bacteria isolated were E. coli, Salmonella, Shigella, Staphylococcus, Group D Streptococcus, Vibrio cholera and V. parahaemolyticus and Klebsiella pneumoniae. The samples were inoculated and were incubated at 37°C for 24 hrs or 48hrs.for appropriate bacterial growths. Thus we can use this study for the assessment of the water and to resolve the hygienic problems of the water.

KEYWORDS: Drinking water, Quality assessment, pathogenic bacteria, Gumma panchayat.
INTRODUCTION

Water is a precious gift of nature to mankind and million of other species living on the earth. It is fast becoming a scare commodity in most part of the world. India is the second most populated country in the world with over 1.2 billion people (Census of India, 2011). More than 3.4 million people die each year from water, sanitation and hygiene related causes. Nearly all deaths 99% occur in the developing countries. Water in India is intricately intertwined with the cultural fabric of the country, and has both economic and social connotations. Clean and plentiful water provides the foundation for prosperous communities. We rely on clean water to survive, yet right now we are heading towards a water crisis. Changing climate patterns are threatening lakes and rivers, and key sources that we tap for drinking water are being overdrawn or tainted with pollution. Dirty water is the world's biggest health risk, and continues to threaten both quality of life and public health. Diarrhoea is more prevalent in the developing world due, in large part, to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. According to the latest available figures, an estimated 2.5 billion people lack improved sanitation facilities, and nearly one billion people do not have access to safe drinking water. These unsanitary environments allow diarrhoea-causing pathogens to spread more easily. 88% of cases of diarrhoea worldwide are attributable to unsafe water, inadequate sanitation or insufficient hygiene. These cases result in 1.5 million deaths each year, most being the deaths of children. That means water crisis claims more lives through disease than any war claims through guns. Childhood underweight causes about 35% of all deaths of children under the age of five years worldwide. An estimated 50% of this underweight or malnutrition is associated with repeated diarrhoea or intestinal nematode infections as a result of unsafe water, in adequate sanitation or insufficient hygiene. Such underweight in children is directly responsible for some 70,000 deaths per year. Underweight children are also more vulnerable to almost all infectious diseases and have a lower prognosis for full recovery. When water from rain and melting snow runs off roofs and roads into our rivers, it picks up toxic chemicals, dirt, trash and disease-carrying organisms along the way. Many of our water resources also lack basic protections, making them vulnerable to pollution from factory farms, industrial plants, and activities like fracking. This can lead to drinking water contamination, habitat degradation and beach closures. This paper basically focused on microbial analysis of various portable water sources at Gumma panchayath in Ananthagiri mandal, Visakhapatnam district, Andhra Pradesh.
MATERIAL AND METHODS

Study Area

Ananthagiri (18°17′14″N, 83°6′43″E) is about 60km away from Visakhapatnam and lies on the top of the Eastern Ghats. The area of the Ananthagiri mandal is roughly 50sq km and the entire area is inhabited by aboriginal tribes.

Of the 25 panchayats in Anantagiri mandal, Gumma panchayat with 20sq.km area was selected for the present study. The total population present in this panchayat is around 4,026 and includes 2,415 literates. The different tribal types present in this panchayat are “Konda Dora, Parena Karja, Petege, Bagatha, Valmiki and Gadaba” and most of them depend on agriculture. The mean temperature is 36°C and receives 1171.0mm normal annual rainfall. Based on their economic status they live in different types of houses such as sheet houses, tiled houses and slab houses. Drinking water sources include 03 hand bores and a small stream running from hills. The stream is the main source of drinking water.

In the present study, Two water samples were collected from Two sources i.e., a hand pump and stream once in a month for a period of 12 month from April 2011 to March 2012, in white plastic bottles, which were previously rinsed with distilled water and sterilized with 70% alcohol. At the collection point, the containers were rinsed thrice with the sample water before being used to collect the samples. The collected samples were placed in a thermocol box. The temperature in the box was maintained at 4°C by using ice packs.

Plating for microbial isolation

The collected samples were serially diluted tenfold in order to reduce the number of microbes in the water samples. The bacteria were isolated by pour plate and spread plate methods using $10^{-3}$ and $10^{-4}$ dilutions.

In pour plate method 1ml of the sample was taken from both $10^{-3}$ and $10^{-4}$ dilutions separately and transferred into two petri dishes. The nutrient agar was autoclaved and then poured in the petri dish. The agar was allowed to solidify and incubated at 37°C for 24-48 hrs. In spread plate method sterile petri dishes were taken and sterilized nutrient agar was poured into them. On the solidified agar surface, 0.1ml of the sample (diluted sample i.e., $10^{-3}$ and $10^{-4}$ dilutions) were poured and spread evenly using a L- shaped bent glass rod (spreader). The plates were incubated at 37°C for 24-48 hrs.
**Microbial analysis and identification of bacteria**

Total plate count was determined by pour plate method. After 48 hrs of incubation colonies were counted by using colony counter and results were expressed as CFU/ml. Coliforms in the water samples were determined by Most probable number (MPN) method (FAO 1997). Water analysis was carried out by multiple tube method. In this method double strength and single strength Mac conkey broth was prepared. Measured volumes of water to be tested were added to tubes containing medium and incubated. Most probable number (MPN) coliforms per 100ml of water sample were calculated from the relevant MPN table.

For identification of bacteria staining, colony characteristics, cultural characteristics, biochemical tests and characteristics of bacteria were used. In staining of bacteria Gram staining, Endospore staining, Capsule staining and Motility test were done. In order to study the morphology of bacteria, cells were heat killed and fixed on the slide. The fixed bacteria were stained and observed for size, shape, arrangement, spore formation and capsulation etc. Hanging drop method was performed to study motility of bacteria. The colony characteristics such as size, shape, margin and elevation were observed on nutrient agar medium. Haemolytic behavior was observed on blood agar. The cultural characteristics of isolates were observed on selective media. The media used were Eosin Methylene Blue (EMB), Salmonella – Shigella agar (SSA), Mac- conkey agar, Manitol salt agar, TCBS agar and Bile esculin agar. Biochemical behavior of bacteria for utilization of specific substrate and enzymatic activity were studied by carbohydrate fermentation, catalase test, gelatin hydrolysis, IMViC test and urease test.

**Analysis of water for physicochemical characters**

The pH of the water samples was measured by using the electrometric methods and other physicochemical parameters such as Total dissolved solids and Fluoride content were analysed by standard methods given in APHA(1989).

**RESULTS**

Water samples collected from Gumma panchayat for a period of one year i.e., during April 2011 to March 2012 were analyzed for physical, chemical and bacteriological characteristics. The physical characteristic measured is pH. Among the chemical characteristics Total dissolved solids (TDS) and fluoride contents were measured. For total number of viable bacteria total plate count (CFU/ml), for faecal and total coliforms most probable number
(MPN/100ml) and for isolation and identification of bacteria staining, biochemical and
growth on selective media were performed.

In steam water it was in the range of 6.89-7.2 and with the mean $pH$ value 7.045. In bore
water it was in the range of 6.7-7.2 with the mean $pH$ value 6.95. The $pH$ value in the Two
water samples is in the safe limit as recommended by WHO. According to Medera et al.,
(1982), the $pH$ of most natural water ranges from 6.5-8.5 while derivation from the neutral 7.0
is as a result of the carbon dioxide/ bicarbonate/ carbonate equilibrium.

The amount of total dissolved solids of the stream water was on the average 129.84mg/l and
Fluoride content on the average was 0.135mg/l. The amount of total dissolved solids of the
bore water on the average was 273.25mg/l and Fluoride content on the average was
0.184mg/l. Both the values in the Two water samples were in the permissible limits as
recommended by WHO.

The total plate counts of bacteria in the two water samples are given in figure1. In stream
water the total plate count fell in the range of 35-64 CFU’s/ml. The water sample showed the
maximum number of CFU’s (64CFU’s/ml) in August and minimum number was noted in
March (35 CFU’s/ml). In bore water the total plate count fell in the range of 58-139
CFU’s/ml. The water sample showed the maximum number of CFU’s (139CFU’s/ml) in
August and minimum number was noted in March (58 CFU’s/ml). Total plate count for
bacteria performed for all water samples showed that the bacteria in all the samples were
above the WHO guideline values(<10CFU’s/ml). The total plate count in all the Two water
samples was highest during the rainy season i.e., August and was due to the contribution of
all the pathogenic bacteria. However the water samples of tap showed relatively higher plate
count throughout the year. This may be due to the presence of sewage surrounding the well
which continuously seeps into the well water. This study is in conformation with the result of
Zaky et al., (2006) who reported increased bacterial content in the water of Manzala Lake,
Egypt which is polluted by drainage and sewage.

The MPN values for Coliforms present in all the water samples are presented in Figure 2. In
stream water the MPN index ranged from 3-15/100ml. The maximum MPN index was
recorded in (15/100ml) August and October. The minimum MPN index was recorded in
(3/100ml) April and May. In bore water the MPN index ranged from 9-28/100ml. The
maximum MPN index was recorded in (28/100ml) August. The minimum MPN index was recorded in (9/100ml) January and March.

During the study period all the two water samples (i.e. stream and bore) showed the presence of the eight pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Group D Streptococcus*, *Vibrio cholerae* and *V. parahaemolytics*. (Table 1)

**Escherichia coli (E.coli)**
It is a gram negative rod. It forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar. *E.coli* is the causal agent of gastroenteritis, urinary tract infections, and neonatal meningitis.

**Staphylococcus aureus (S.aureus)**
It is a gram positive coccus, non spore forming and non- motile bacteria. It forms circular, low convex with entire margin, smooth, medium opaque colony on nutrient agar. It forms yellow coloured colonies on mannitol salt agar. *S.aureus* incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It causes a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.

**Group D Streptococcus**
It is a gram positive coccus. It forms thin, even growth on nutrient agar. Black (or) Brown coloured colonies were observed on Bile esculin agar. *Group D Streptococcus* causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.

**Vibrio cholerae: (V.cholerae)**
It is a gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar. *Vibrio cholerae* is responsible for the occurrence of cholera.
**Vibrio parahaemolytics: (V.parahaemolyticus)**

It is a gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar. *V. parahaemolytics* is responsible for gastrointestinal illness in humans.

**Klebsiella pneumoniae: (K.pneumoniae)**

It is a gram negative rod. It forms slimy, white somewhat translucent, raised growth on nutrient agar and dark pink coloured colonies on mac - conkey agar. *Klebsiella pneumonia* is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrohe, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia.

**Salmonella typhi: (S.typhi)**

It is a gram negative rod. It forms thin even greyish growth on nutrient agar and dark green colonies on SS agar. *Salmonella typhi* causes typhoid.

**Shigella dysenteriae: (S.dysenteriae)**

It is a gram negative rod. It forms greyish growth on nutrient agar and colourless colonies on SS agar. *Shigella dysenteriae* is the bacillary dysentery causing bacterium.

**Table 1: Biochemical Characteristics of isolates**

<table>
<thead>
<tr>
<th>Test</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Methyl-red</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vogel-Proskauer</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate Utilization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogen sulphide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate Utilization</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose fermentation</td>
<td>-</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Glucose fermentation | A | A | AG | AG | AG | A
Sucrose fermentation | A | A | A(+) | AG | AG | A+/-
Organism | Staphylococcus | Streptococcus | E. coil | Vibrio | Salmonella | Shigella

A = Acid production only
AG = Acid and gas production
+/− = Variable reaction
+ = Positive
− = Negative
(+) = Late Positive

Figure 1: Total Plate Count (CFU/ml) of Bacteria in two water samples

Figure 2: Most Probable Number (per 100ml) of Coliforms in two water samples
DISCUSSION

Water is a colorless, transparent, odorless, tasteless liquid that forms the seas, lakes, rivers, and rain fall as well as the basis of the fluids to living organisms (Michael, 2000). Water is a combination of hydrogen and oxygen atoms, with a chemical formula H₂O and known to be the most abundant compound (70%) on earth's surface (Osei, 2005). However, for water to be potable it must be microbiologically safe and in order to achieve this, an approach that will eliminate pathogenic organisms from the source of water supply must be ensured. Retra (2002), described water in its pure form as a substance that has a pH value of 7.0, freezing point of 0°C and boiling point of 100°C at 760mmHg. Water is capable of dissolving other substances more than any other known solvent and therefore, it is called a universal solvent. Since the beginning or recorded history, water has been recognized as a potential carrier of germs and diseases Retra (2002). Ground water sources, wells, boreholes and springs; that are properly located produce water of a very good quality. The majority of the infections that is associated with the lack of accessibility to Potable water supply and poor environmental sanitation especially in developing countries. The following are micro-organisms associated with water; *Pseudomonas aeruginosa, Salmonella, Mycobacteria, Escherichia coli, Proteus, Shigellasonnei, Klebsiella, Cyanobacteria* (Chris, 2004). Water borne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated water is consumed. Cholera is a good example of water borne disease and it is endemic in some parts of India. In 1991, more than 16,000 people died worldwide from half a million case of cholera. Improved treatment has reduced the death rate dramatically, but it is still a serious disease (UNPE, 1997).

The ensuring of good quality drinking water is a basic factor in guaranteeing public health, the protection of the environment and sustainable development (Ranjini et al., 2010). Water of good drinking quality is of basic importance to human physiology and man’s continued existence depends very much on its availability (Lemikanra, 1999; FAO, 1997). The provision of Potable water to rural and urban population is necessary to prevent health hazards associated with poor drinking water (Nikoladze and Akastal 1989; Lemo, 2002). A significant proportion of the world’s population use potable water for drinking, cooking, personal and home hygiene (WHO, 2004). Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is potable and safe for drinking (Tebutt, 1983). Potable water is defined
as water that is free from disease producing microorganisms and chemical substances deleterious to health (Ihekoronye and Ngoddy, 1985).

Total Dissolved Solid (TDS) is a measurement of inorganic salts, organic matter and other dissolved materials in water. The experimental values for TDS of water samples were found to be 129.8 mg/litre (stream) and 273.25 mg/litre (bore) which was lesser than FAO standard (1200 mg/litre).

pH is a term used universally to express the intensity of the acid or alkaline condition of a solution. pH of water samples were 7.045 (stream) and 6.95 (bore) which lies in the range of FAO standard (6.5 to 8.4).

Fluoride of water samples was 0.135mg/l (stream) and 0.184mg/l (bore) which lies in the range of FAO standard (0.5mg/l). Fluoride testing in water quality analysis should be given importance because fluoride is know to cause a variety of health problems viz dental fluorosis and non skeletal manifestations when the level beyond the limit. Fluoride has come to stay as number one parameter in causing toxicological and geo-environmental problems in various countries. The fluoride content of all the two water samples was within the permissible limit.

The results of microbial analysis of the water were presented in figure 1 and 2. The presence of pathogenic bacterial such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Group D Streptococcus*, *Vibrio cholerae* and *V. parahaemolytics* indicated that the water is not potable (Shittu et al.,2008). Although variations existed in Total Plate Count and Most Probable Number, the values were high exceeding the acceptable limits for water. The present results obtained for Total Plate Count and Most Probable Number were similar to the results obtained by Okonko et al., (2008) and Oluyege Jacob Ololuo et al., (2010).

This finding agrees with similar studies by other workers who reported that the sources of heterotrophic bacteria in water are human and animal wastes, runoffs, pasture, natural soil or plant bacteria, sewage, and other unsanitary practices (Edema et al., 2001; Ibe and Okplenye, 2005; Kiman-Murage and Ngindu, 2007). Runoffs, sewage, agricultural waste are usually high in organic matter and nutrients and could cause increase in the microbial flora of the water bodies thereby resulting in high heterotrophic Bacterial counts (Obire and Aguda, 2004). The higher number of bacterial count recorded in stream water samples could
probably be as a result of the increased surface area of the stream which exposes the water to contaminants as well as human activities like swimming, washing, dipping of dirty legs or hands and cans inside the stream while fetching water (Welch et al., 2000; Shittu et al., 2008; Manjula et al., 2011). None of the water samples complied with the standards (WHO, 2004) for coliforms in drinking water. The total coliform for all the samples were higher than the WHO standards of zero MPN per 100ml. However, according to WHO (2004), drinking water can be graded into four categories depending on their MPN value. Water with MPN of zero is excellent, MPN of 1 – 3 is satisfactory, MPN of 4 – 10 is suspicious and MPN above 10 is unsatisfactory. Water with MPN greater than 3 is not suitable for drinking (WHO, 2004). The high coliforms obtained may be an indication that the water samples were faecally contaminated (Ajayi and Akonai, 2005). The presence of \textit{E. coli}, \textit{Vibrio}, \textit{Klebsiella}, \textit{Enterobacter} species and other bacteria not only make the water unsuitable for human consumption, but also poses serious health concerns (WHO, 2011). Similar studies reported the presence of these bacteria in drinking water sources (Okonko et al., 2008; Adejuwon and Adelakun, 2012) and attributed it to indiscriminate human and animal defeacation and general poor sanitation.

This study reveals that the increased in the microbial loads at the consumer points (i.e. stream and bore) was due to the observed activities. At some points, the direct washing of human clothing and washing of other household utensils around the sampling point. The presence of animals and the intense agricultural related activities going on around the consumer point could lead to contamination. The direct washing of legs, hands, clothes and utensils in the stream could also lead to contamination (Banwo, 2006).

Thus, potable and domestic water should be harmless for the health of man and should have organoleptic properties and should be suitable for domestic use. Water quality should be controlled in order to minimize acute health problems of water related disease in humans.

The following three points approach is suggested for improving the quality of water supplied to the tribal communities of Gumma panchayath studied.

- Investigate the source of contamination of pipe borne water supplies to delineate the roles of the water delivery system and of household water storage system.
- Institute a system to monitor the quality of untreated water sources so that water collection can be restricted to uncontaminated sources and or water treatment advisories can be issued appropriately.
Educate the public on appropriate water handling storage and treatment methods. It is evident that until these recommendations are implemented water supplied to the tribal communities in Gumma panchayath of ananthagiri mandal, Visakhapatnam district will continue to pose a health hazard to the population.

REFERENCES


