Physico-chemical analysis, isolation and identification of pathogenic bacteria from potable water in Kothuru Panchayath, Visakhapatnam (Andhra Pradesh), India

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Abstract

The study was conducted to find out the physico-chemical and bacteriological characteristics of drinking water samples in Kothuru panchayath of Ananthagiri mandal, Visakhapatnam district, Andhra Pradesh. Water quality is the degree of potability which is determined by the amount and kinds of suspended and dissolved substances in water. The water samples are subjected to physico-chemical analysis by standard methods. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification. The final identification of resulted isolates was done by their biochemical testing mentioned in accordance to the Bergey’s Manual. The physicochemical characters of all the three drinking water samples were within the recommended permissible level of WHO. The total plate count was above the WHO guidelines values (<10CFU’s/ml) in the three water samples studied the highest count was during August. Increased presence of coliforms was noticed during August and October in stream; presence of coliforms was noticed in September in bore well while in well water it was during August. The resulted bacterial isolates viz. E.coli, Salmonella, Shigella, Staphylococcus, Group D Streptococcus, Vibrio cholera and V. parahaemolyticus are highly pathogenic. Poor quality of drinking water was recorded as the major risk factor for the large-scale water borne diseases in the area.

Keywords: drinking water, quality assessment, pathogenic bacteria, Kothuru panchayath

Introduction

No life without water is a common saying, as water is the essential requirement of all life supporting activities. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs and taps (Okonko et al., 2008). In our country, almost 70% of the water has become polluted due to the discharge of domestic sewage and industrial effluents into natural water sources (Sangu and Sharma, 1987). Quality water is vital to the health, social and economic well being of people. Although contaminants in water are divided roughly into physical, chemical and biological, environmental risk assessment today reveals that the exposure to biological contaminants especially water-borne pathogens needs to be given higher priority in treatment and regulatory programs for domestic water supplies. Diseases that spread through the contaminated water principally in areas of poor sanitation are Hepatitis-A, Hepatitis-E, Typhoid fever, diarrhea and dysentery etc. (Light, 2000). Water which looks and tastes good may not necessarily be safe to drink it may be polluted with harmful bacteria, parasites and viruses. These microbes can exist in surface and ground water supplies, and can cause immediate sickness in humans if not properly treated (PFRA, 2003). Quality
standards for treated drinking water vary from place to place. The objective of most treatment schemes is largely to reduce the possibility of the spread of water borne disease to bearest minimum in addition to consideration for its wholesomeness and palatability in all respects (Edema et al., 2001).

Material and Methods

Study area

Ananthagiri (18°17’14"N, 83°6’43”E) is about 60 km away from Visakhapatnam and lies on the top of the Eastern Ghats. The area of the Ananthagiri mandal is roughly 50 sq. km and the entire area is inhabited by aboriginal tribes. Of the 25 panchayats in Anantagiri mandal, Kothuru panchayat with 15 sq. km area was selected for the present study. The total population present in this panchayat is around 4,500 and includes 2,500 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 1171.0 mm 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 4 hand bores; a well and a small stream running from hills. The stream is the main source of drinking water.

In the present study, water samples were collected from three sources i.e. a well, a hand pump and stream once in a month for a period of 12 months 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. 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 analyzed by standard methods given in APHA (1998). The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino, 2009). The final identification of resulted isolates was done by the biochemical tests in accordance to the Bergey’s Manual (Holt et al., 1984).

Results

Water samples collected from Kothuru 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/100 ml) and for isolation and identification of bacteria staining, biochemical and growth on selective media were performed. The mean pH value of stream water was 7. In bore water it was in the range of 7.0-7.2 with the mean pH value 7.06. In well water it was in the range of 6.92-7.1 with mean pH value 7.005. The pH value in the three water samples is in the safe limit as recommended by WHO.

The amount of total dissolved solids of the stream water was on the average 107.84 mg/l and Fluoride content on the average was 0.1 mg/l. The amount of total dissolved solids of the bore water on the average was 273.25 mg/l and Fluoride content on the average was 0.104 mg/l. The amount of total dissolved solids of the well water on the average was 175.08 mg/l and Fluoride content on the
average was 0.109 mg/l. Both the values in the three samples were in the permissible limits as recommended by WHO.

**Fig. 1.** Total Plate Count (CFU/ml) of Bacteria in three water samples

![Graph showing total plate count of bacteria in three water samples.](image)

**Fig. 2.** Most Probable Number (/100 ml) of Coliforms in three water samples

![Graph showing most probable number of coliforms in three water samples.](image)

The total plate counts of bacteria in the three water samples are given in figure 1. In stream water the total plate count fell in the range of 36-64 CFU’s/ml. The water sample showed the maximum number of CFU’s (64 CFU’s/ml) in August and minimum number was noted in June (36 CFU’s/ml). In bore water the total plate count fell in the range of 39-76 CFU’s/ml. The water sample showed the maximum number of CFU’s (76 CFU’s/ml) in August and minimum number was noted in June (39 CFU’s/ml). In well 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 (139 CFU’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 (<10 CFU’s/ml). The total plate count in all the three water samples was highest during the rainy season (i.e.) August 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 (Fig. 2). In stream water the MPN index ranged from 3-15/100 ml. The maximum MPN index was recorded in (15/100 ml) August and October. The minimum MPN index was recorded in (3/100 ml) April and May. In bore water the MPN index ranged from 4-21/100 ml. The maximum MPN index was recorded in (21/100 ml) September. The minimum MPN index was recorded in (4/100 ml) April and March. In well water the MPN index ranged from 28-9/100 ml. The maximum MPN index was recorded in (28/100 ml) August. The minimum MPN index was recorded in (9/100 ml) January and March. The coliforms also showed their increased presence during August-October in stream and hand bore while in well water the increase was noticed during August. During the study period all the three water samples (i.e. stream, bore and well) showed the presence of the eight pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus*
Discussion

Safe drinking water is one of the most important felt needs in public health in developing countries in the 21st century (Sobsey et al., 2003). It is estimated by World Health Organization (WHO) and United Nations International Children’s Emergency Fund (UNICEF) that 1.1 billion people lack access to improved water supplies and 2.6 billion people lack adequate sanitation (Moe et al., 2006). More than half of the world’s population lives in villages in rural areas and most of those without access to safe drinking water supply (Howard et al., 2003; Tambekar et al., 2007). It has been estimated by WHO that diarrheal morbidity that can be reduced by an average of 6-20% with improvements in water supply and by 32% with improvements in sanitation. In India, approximately 72.7% of the rural population does not use any method of water disinfection and 74% have no sanitary toilets (IIPS and NFHS-3, 2005-2006). Provision of safe drinking water has been of primary concern in rural India (Bilas et al., 1981; Kang et al., 2001). The key to providing microbiologically safe drinking water lies in understanding the various mechanisms by which water gets contaminated and formulating interventions at critical points to decrease and prevent contamination of drinking water (Trevett et al., 2004). Physical parameters such as pH, TDS and fluoride content have a major influence on bacterial population growth. The pH values ranging from 3-10.5 could favour both indicator and pathogenic organism’s growth (Zamxaka et al., 2004). The pH provides the information about the acidity or alkalinity of water (Katyal and Satake, 1990). It also provides a means of clarifying and for collecting other characteristics or behavior such as corrosive activity (Ghandour et al., 1985). Eye irritation and exacerbation of skin disorders have been associated with pH values greater than 11. With respect to the water samples the pH values were in safe limit. High TDS was commonly objectional or offensive to taste. A higher concentration of TDS usually serves no health threat to human until the values exceed 10,000 mg/l (Anonymous, 1997). The TDS values of the all the three water samples were within the permissible limit.

Fluoride testing in water quality analysis should be given importance because fluoride is known to cause a variety of health problems viz. dental fluorosis and non skeletal manifestations when the level beyond 1.5 ppm. 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 three water samples was within the permissible limit. The results of physic-chemical analysis of the present study are in agreement with the study of Germs et al. (2004) who reported that the chemical quality of the Chunies River in South Africa was acceptable for domestic as well as for agriculture. Similary, Nevondo et al. (1999) revealed that the chemical quality of all water samples was acceptable. The chemical analysis of water samples from Egypt carried out by Fadaly et al. (1999) showed that the measured parameters were found within the permissible limits.

The results of microbial analysis of the water were presented in figures 1 and 2. The presence of pathogenic bacteria 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.
<table>
<thead>
<tr>
<th>Morphological and Cultural characters</th>
<th>Organism</th>
<th>Disease caused by the organism</th>
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<tbody>
<tr>
<td>Gram negative rod, forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar.</td>
<td><em>Escherichia coli</em></td>
<td>Causal agent of gastroenteritis, urinary tract infections, and neonatal meningitis.</td>
</tr>
<tr>
<td>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.</td>
<td><em>Staphylococcus aureus</em></td>
<td><em>S. aureus</em> 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.</td>
</tr>
<tr>
<td>Gram positive coccus. It forms thin, even growth on nutrient agar. Black (or) Brown coloured colonies were observed on bile esulin agar.</td>
<td>Group D <em>Streptococcus</em></td>
<td>Group D <em>Streptococcus</em> causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.</td>
</tr>
<tr>
<td>Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar.</td>
<td><em>Vibrio cholerae</em></td>
<td><em>Vibrio cholerae</em> is responsible for the occurrence of cholera.</td>
</tr>
<tr>
<td>Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar.</td>
<td><em>Vibrio parahaemolyticus</em></td>
<td><em>V. parahaemolyticus</em> is responsible for gastrointestinal illness in humans.</td>
</tr>
</tbody>
</table>
Gram negative rod. It forms slimy, white somewhat translucent, raised growth on nutrient agar and dark pink coloured colonies on mac - conkey agar.

*Klebsiella pneumoniae*  
*Klebsiella pneumonia* is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhoea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia.

Gram negative rod. It forms thin even grayish growth on nutrient agar and dark green colonies on SS agar.

*Salmonella typhi*

*Salmonella typhi* causes typhoid.

Gram negative rod. It forms grayish growth on nutrient agar and colourless colonies on SS agar.

*Shigella dysenteriae*

*Shigella dysenteriae* is the bacillary dysentery causing bacterium.

<table>
<thead>
<tr>
<th>Test</th>
<th>Staphylococcus</th>
<th>Streptococcus</th>
<th>E. coil</th>
<th>Vibrio</th>
<th>Salmonella</th>
<th>Shigella</th>
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<tr>
<td>Catalase</td>
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<td>Motility</td>
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<td>Methyl-red</td>
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<td>Vogel-Proskauer</td>
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<td>Citrate Utilization</td>
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<td>Urease</td>
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<td>Gelatin liquefication</td>
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<tr>
<td>Lactose fermentation</td>
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<td>AG</td>
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<td>Glucose fermentation</td>
<td>A</td>
<td>A</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>A</td>
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<tr>
<td>Sucrose fermentation</td>
<td>A</td>
<td>A</td>
<td>A(+)</td>
<td>AG</td>
<td>AG</td>
<td>A+/-</td>
</tr>
</tbody>
</table>

Table 2. Biochemical characteristics of isolates

A=Acid production only; AG = Acid and gas production; +/- = Variable reaction; + = Positive; - = Negative; (+) = Late Positive
(Shittu et al., 2008). Although variations existed in the observed 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 (Okonko et al., 2008; Oluyege Jacob Olaoluwa et al., 2010).

Faecal pollution was confirmed by the presence of coliforms in the water samples during the rainy seasons which may arise from animal dung carried by run-off to the rivers during the rainy season. The principal coliforms are *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* sp and *Citrobacter* sp. *E.coli* is abundant found in the gastro intestinal tracts of humans, birds and animals, but rarely found in water therefore their presence in water can indicate faecal contamination. The classified indicator for water analysis is *E. coli* and its presence suggests enteric pathogens (Nwadiaro, 1982). There is a direct relationship between the numbers of *E. coli* and the extent of faecal pollution. The higher the number, the more polluted the sample is. According to Bonde (1977), bacteriological examination of water is a powerful and foremost tool in order to foreclose the presence of microorganisms that might constitute a health hazard. Microorganisms that are used commonly as indicator of water include coliforms. Naturally, waters contain a large number and variety of microorganisms which does not necessarily makes such water not potable. In fact, the sanitary quality of potable water is determined primarily by the kinds of microorganisms present rather than by the microbial count (Bonde, 1977). Water source used for drinking or cleaning purpose should not contain any organism of faecal origin (Sabongari, 1982; Fonseca et al., 2000). In that line, The World Health Organization (WHO, 2006) suggested that treated water entering the distribution system should contain no coliform organisms, and tap water should contain no coliform in 95% of samples taken in any one year and it should not contain more than 3 coliforms per 100 ml or any *E. coli*.

Chan et al. (2007) isolated pathogenic bacteria such as *E. coli*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* from the water samples. Ajibade et al. (2008) confirmed the presence of the coliforms. The bacteria loads of water samples during the rainy seasons were very high and likewise coliform counts. As a result we strongly advise not to drink water from those sources during the rainy seasons without treating them. This study reveals that the increased in the microbial loads at the consumer points (i.e. bore and well) 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).

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