

A comparative study of microbial analysis of drinking water in Kothuru and Kondiba Panchayats in Ananthagiri Mandal, Visakhapatnam

D. SANDHYA DEEPIKA, K. LAXMI SOWMYA AND P.K. RATNA KUMAR

SUMMARY

A comparitive study was conducted to find out the bacteriological characteristics of drinking water samples in Kothuru and Kondiba Panchayats 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 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 total plate count was above the WHO guidelines values (<10CFU's/ml). In the water samples of Kothuru Panchayat 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. In the water samples of Kondiba Panchayat studied, the highest count was during August and September. Increased presence of coliforms was noticed bacterial isolates *viz., E.coli, Salmonella, Shigella, Staphylococcus, Klebsiella pneumoniae, 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 in the area.

Key Words : Drinking water, Pathogenic bacteria, Microbial analysis

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Atter 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. Though water is available in the universe in huge quantity only 3% is fresh water. Beautiful water body has been the worst victim of the tremendous anthropogenic pressure. The water we use is taken from lakes and rivers, and from

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underground (ground water) and after we have used it and contaminated it - most of it returns to these locations. Water pollution is the introduction into fresh or ocean water of chemical, physical or biological material that degrades the quality of the water and affects the organisms living in it. Water in nature is seldom totally pure. Increase in human population and urbanization in recent years resulted in gradual deterioration of water quality. The uniqueness of the water body has deteriorated as it is getting enriched with pollutants. It is estimated that each year 10 million people die from drinking contaminated water. According to WHO(2006) organization about 80% of all diseases in human is caused by water. Atlas and Bartha (1993) considered that bacteria play an important role in global ecosystems which are major factors in controlling the quality of water and are fate determinators of pollution released to environment. Clark and Pagel (1977)

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considered bacteria as are reliable indicator of contamination. The usefulness of monitoring the microbial community is due, in part of its ability to respond quickly to environmental conditions and major role it plays in biogeochemical cycling process (Griffiths and Babick, 1983). This paper basically focused on microbial analysis of various portable water sources at Kothuru and Kondiba Panchayats in Ananthagiri mandal, Visakhapatnam district, Andhra Pradesh.

MATERIAL AND METHODS

Study area:

Ananthagiri (18º17'14"N, 83 º6'43"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, Kothuru Panchayat with 15sq.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. 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. Kondiba Panchayat with 20sq.km area was also selected for the present study. The total population present in this Panchayat is around 5,000 and includes 1,000 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. Drinking water sources include 12 hand bores; 7 wells 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 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. 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.*, 1994).

RESULTS AND DISCUSSION

Water samples collected from both Kondiba and Kothuru Panchayats for a period of one year *i.e.*, during April 2011 to March 2012 were analyzed for bacteriological characteristics. For total number of viable bacteria total plate count (CFU/ ml), for faecal and total colliforms most propable number (MPN/100ml) and for isolation and identification of bacteria staining, biochemical and growth on selective media were performed.

The total plate counts of bacteria in the stream water samples are given in Fig. 1. In Kothuru Panchayat the total plate count fell in the range of 36-64 CFU's/ml. The water sample showed the maximum number of CFU's (64CFU's/ ml) in August and minimum number was noted in June (36 CFU's/ml). In Kondiba Panchayat the total plate count fell in the range of 39-65 CFU's/ml. The water sample showed the maximum number of CFU's (65CFU's/ml) in August and minimum number was noted in June (39CFU's/ml). The total plate counts of bacteria in the bore water samples are given in Fig. 3. In Kothuru Panchayat the total plate count fell in the range of 39-76 CFU's/ml. The water sample showed the maximum number of CFU's (76CFU's/ml) in August and minimum number was noted in June (39 CFU's/ml). In Kondiba Panchayat the total plate count fell in the range of 31-63 CFU's/ml. The water sample showed the maximum number of CFU's (63CFU's/ml) in August and minimum number was noted in June (31CFU's/ml). The total plate counts of bacteria in the well water samples are given in Fig. 5. In

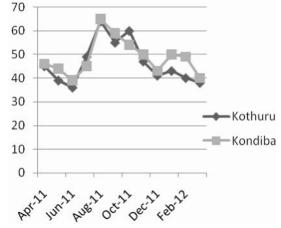


Fig. 1 : Total plate count of stream waters

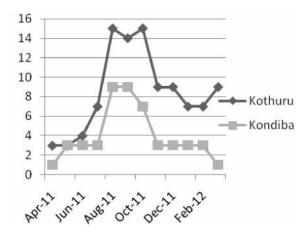


Fig. 2 : Most probable number of stream water

Kothuru Panchayat 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). In Kondiba Panchayat the total plate count fell in the range of 71-52 CFU's/ml. The water sample showed the maximum number of CFU's (71CFU's/ml) in August and minimum number was noted in June (39CFU's/ml). Total plate count for bacteria performed for all water samples showed that the bacteria in all the samples were above the WHO(2006) guideline values(<10CFU's/ml). In both the Panchayts the maximum count was observed during the rainy season *i.e.*, August and was due to the contribution of all the pathogenic bacteria.

The MPN values for coliforms present in stream water samples are presented in Fig. 2. In Kothuru Panchayat 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 Kondiba Panchayat the MPN index ranged from 1-9/ 100ml. The maximum MPN index was recorded in August and September (9/100ml). The minimum MPN index was recorded in April and March (1/100ml). The MPN values for coliforms present in bore water samples are presented in Fig. 4. In Kothuru Panchayat the MPN index ranged from 4-21/ 100ml. The maximum MPN index was recorded in (21/100ml) September. The minimum MPN index was recorded in (4/ 100ml) April and March. In Kondiba Panchayat the MPN index ranged from 1-9/100ml. The maximum MPN index was recorded in August (9/100ml). The minimum MPN index was recorded in January (1/100ml). The MPN values for Coliforms present in well water samples are presented in Fig. 6. In Kothuru Panchayat the MPN index ranged from 28-9/100ml. The maximum MPN index was recorded in (28/100ml) August. The minimum MPN index was recorded in (9/100ml) January and March. In Kondiba Panchayat the MPN index ranged from 3-15/100ml. The maximum MPN index was recorded in June and August (15/100ml). The minimum MPN

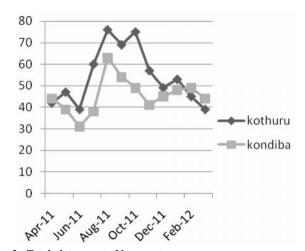


Fig. 3 : Total plate count of bore waters

index was recorded in April, May, January (3/100ml). The coliforms in Kothuru Panchayat showed their increased presence during August – October in stream and hand bore while in well water the increase was noticed during August. The coliforms in Kondiba Panchayat also showed their increased presence during August and September in stream and bore well while in well water the increase was noticed during June, July and August.

During the study period all the water samples from the both Panchayats 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 and 2).

Among the pathogenic bacteria the most dominant species in stream water (Fig. 7) in Kothuru Panchayat the most dominant species of bacterium was *E.coli* (24.26%) followed by *Staphylococcus aureus* with 19.04%, *Group D Streptococcus* with 12.24%, *Vibrio cholerae* and *V. parahaemolytics* with 10.20%, *Shigella dysenteriae* with 9.75%, *Salmonella typhi* with 9.52% and *Klebsiella pneumoniae* with 4.76% contribution. In Kondiba Panchayat was *Escherichia coli* (28.3%) followed by *Staphylococcus aureus* with 15.16%, *Vibrio cholerae* with 12.5%, *Salmonella typhi* with 11.2%, *V. parahaemolytics* with 9.45%, *Shigella dysenteriae* with 8.79%, *Klebsiella pneumoniae* with 7.47% and *Group D Streptococcus* with 7.03%, contribution.

In bore water (Fig. 8) in Kothuru Panchayat the most dominant species of bacterium was *E.coli* (22.25%) followed by *Staphylococcus aureus* with 19.25%, *Group D Streptococcus* with 14.76%, *Salmonella typhi* with 10.28%, *V. parahaemolytics* with 9.71%, *Vibrio cholera* with 9.53%, *Shigella dysenteriae* with 8.59%, and *Klebsiella pneumoniae* with 5.6% contribution. In Kondiba Panchayat the most dominant species of bacterium was *E.coli* (27.9%) followed by *Staphylococcus aureus* with 19.9%, *Salmonella typhi* with 10.6%, *Vibrio cholerae* and *V. parahaemolytics* with 9.84%

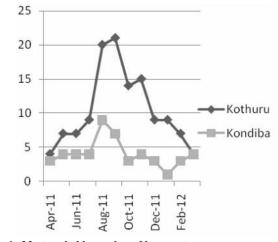


Fig. 4 : Most probable number of bore water

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Table 1 : Morphological and cultural characteristics of organ Morphological and cultural characters	Organism	Disease caused by the organism		
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.	Escherichia coli	Causal agent of gastroenteritis, urinary tract infections, and neonatal meningitis. <i>S.aureus</i> 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.		
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.	Staphylococcus aureus			
Gram positive coccus. It forms thin, even growth on nutrient agar. Black (or) Brown coloured colonies were observed on bile esilin agar.	Group D Streptococcus	<i>Group D Streptococcus</i> causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.		
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar.	Vibrio cholerae	<i>Vibrio cholerae</i> is responsible for the occurrence of cholera.		
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar.	Vibrio parahaemolytics	<i>V. parahaemolytics</i> is responsible for gastrointestinal illness in humans.		
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	<i>Klebsiella pneumonia</i> is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrohea, 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	<i>Shigella dysenteriae</i> is the bacillary dysentery causing bacterium.		

Table 2 : Biochemical characteristics of isolates								
Test								
Catalase	+	-	+	+	+	+		
Oxidase	-	-	-	+	-	-		
Motility	-	-	+	+	-			
Indole	-	-	+	+	-	+		
Methyl-red	-	+	+	-	+	+		
Voge-Proskauer	+	-	-	+	-	-		
Citrate utilization	-	-	-	+	+	-		
Urease	+	-	-	-	-	-		
Hydrogen sulphide	-	-	-		+	-		
Starch hydrolysis	-	-	-	-	-	-		
Nitrate utilization	-	-	+	+	+	+		
Gelatin liquefication	-	-	-	+	-	-		
Lactose fermentation	-	А	AG	AG	-	-		
Glucose fermentation	А	А	AG	AG	AG	А		
Sucrose fermentation	А	А	A(+)	AG	AG	A+/-		
Organism	Staphylococcus	Streptococcus	E. coil	Vibrio	Salmonella	Shigella		
A = Acid production only - = Negative	AG=Acid and gas production (+) = Late positive		+/- = Variable reaction		+ =Positive			

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each, *Shigella dysenteriae* with 8.2%, *Group D Streptococcus* with 8.03% and *Klebsiella pneumoniae* with 5.44%, contribution.

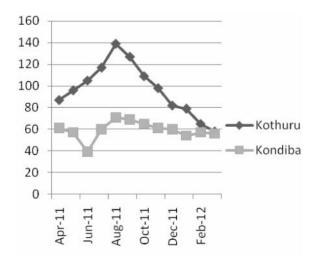


Fig. 5 : Total plate count of well waters

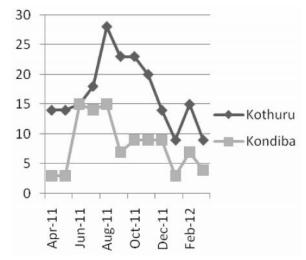


Fig. 6 : Most probable number of well water

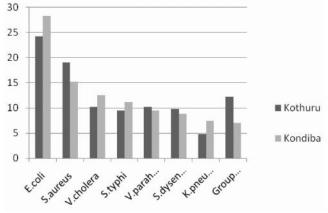


Fig. 7 : % pathogenic Bacteria in stream

Analysis of well water (Fig. 9) in Kothuru Panchayat the most dominant species of bacterium was *E.coli* (24.712%) followed by *Staphylococcus aureus* with 17.69%, *Group D Streptococcus* with 13.82%, *Salmonella typhi* with 10.15%, *Vibrio cholera* with 10.05%, *Shigella dysenteriae* with 9.73%, *V. parahaemolytics* with 8.48% and *Klebsiella pneumoniae* with 5.34% contribution.In Kondiba Panchayat revealed that the predominant bacterium was *E.coli* (26.16%) followed by *Staphylococcus aureus* with 19.57%, *Salmonella typhi* with 12.40%, *Shigella dysenteriae* with 10.65%, *Vibrio cholerae* with 9.30, *V. parahaemolytics* with 8.13%, *Group D Streptococcus* with 7.17% and *Klebsiella pneumoniae* with 6.58%, contribution.

Most common and wide spread health risk associated with drinking water is the bacterial contamination caused either directly or indirectly by human or animal excreta. In the present study the heterotrophic plate count was used to estimate the total amount of bacteria in water which indicates the overall microbial status of water. The factors responsible for high microbial counts in the water samples may be due to technically ill planned sewerage network, damaged sewer lines, rust water pipe lines and poorly maintained disinfection system. Muhammad Anjum Zia *et al.* (2005) found that the

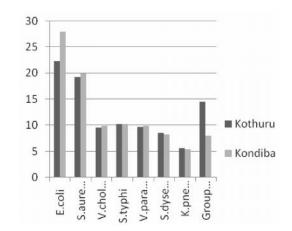
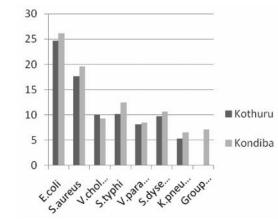


Fig. 8 : % Pathogenic bacteria in bore





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ground water of Faisalabad city showed high bacterial counts. Similarly Prasai *et al.* (2007) revealed that the plate count of water samples of Kathmandu valley crossed the permissible limits, which may be due to failure of the disinfections of raw water at the treatment plant or to the infiltration of contaminated water (sewage) through cross – connection, leakage points and back siphonage.

Obire *et al.* (2009) reported that the drinking water samples of Nigeria showed plate counts above the recommended level, due to faecal pollution of the drinking water. They observed that the sanitary conditions and standard of living of inhabitants in the various locations were improper. They suggested that efficient and proper sanitation check in drinking water supplies has to be executed regularly as well as personal and household hygiene has to be emphasized.

Ashok and Govind (2010) reported that the total plate counts of waters of Tehri dam reservoir were high during rainy season and minimum during winter. He reasoned that the luxurious growth of bacterial population during the summer and monsoon months were due to influx of washed organic matter in the reservoir from the surrounding forest areas. He suggested that the rain brings in particulate matter which serves as sites of adsorption for bacteria, there by increasing the bacterial load.

Out of all the samples well water from the both Panchyats were found to be having high plate count, than the stream and bore samples throughout the year. In Kothuru Panchyats both well and bore water samples showed high microbial counts due to improperly managed water distribution system, insanitary conditions around the sampling points and inadequate disinfection of the water to be delivered. The comparative data of the two Panchayats in all the three season depicted that there was two fold increase in concentration of all indicator organisms during monsoon seasons than in summer and winter seasons. Chitanand *et al.* (2008) in their studies on the suitability of raw ground water for safe drinking purpose and the status of these sites for bacterial contamination throughout year (Ashok and Govind, 2010).

High proportion of indicator organisms was found in all the samples. When these sampling sites were surveyed it was observed that insanitary conditions in the surroundings areas of the collection sites. Omezuruike *et al.* (2008) reported that the water samples collected from Nigeria were found to harbor coliform organism in numbers greater than the required standards for water. Srila *et al.* (2009) reported that the drinking water of Vellore district in Tamilnadu was found to be microbiologically unfit for human consumption.

The pathogenic bacteria thus isolated were mostly belonging to the Enterobacteriaceae. The presence of Enterobacteriaceae members in the tested water samples indicates the faecal pollution. High level of contamination of ground water with faecal coliforms was found in urban areas of Karachi (Zubair and Rippy, 2000). Khan *et al.*, 2000 also found that more than 50% water samples of Peswar, Nowshera and Charsada were polluted with faecal coliforms. These faecal coliforms were also reported from Umian lake water (Rajurkar *et al.*, 2003) and also in different water samples at Sivakasi (Radha Krishnan *et al.*, 2007). Water sources used for drinking or cleaning purpose should not contain any organism of faecal origin (Sabongiri, 1982, Fonseca *et al.*, 2000).

Staphylococcus aureus is washed out from soil and get their entry into the water bodies during heavy rainfall also belong to the allochthonous bacteria (Saleem *et al.*, 2011). The presence of *Staphylococcus aureus* in the water samples more or less similar to the results of Sapkota *et al.*, 2012; Kumar *et al.*, 2012; Oluyege Jacob Olaluwa *et al.*, 2010.

In as much as water is essential for life, it could at the same time prove to be a potential reservoir of microorganisms that could pose significant threat to the consuming citizentry. Thus, it is necessary that all the sources of contamination of water used for drinking and other domestic purposes in rural setting should be subjected to adequate treatment when faecal pollution is suspected. The water sources should be prevented from household activities and other uses. It is necessary to ensure that washing sites of domestic chores are kept at a distance of at least 15m especially in areas where channeled drainages is absent. Animal and humans should also be prevented from entering directly into the dam. Pro-poor informative programs, that focuses on the immediate and longterm effects of indiscriminate waste disposal and contamination. From other anthropogenic activities on the quality of drinking water sources should be embarked upon. Failure of embark on the suggested strategies may lead to increased risk of exposure to water borne pathogens which in turn could portend serious implication on public health outcomes in such settings.

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