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BACTERIAL CONTAMINATES IN DRINKING WATER OF PALAMURU VILLAGES (A.P.)

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ABSTRACT

The quality of drinking water is a potential environmental detriment of health. Assurance of drinking water safety is a foundation for the prevention and control of waterborne diseases. About 10 percent of the rural and urban population does not have access to regular safe drinking water and many more are threatened. Most of them depend on unsafe water sources to meet their daily needs. This study is primarily employed to aim at the analysis of the bacterial contaminants that are prevailing in the drinking water of villages in Palamuru, A.P., India. Drinking water samples were collected from different villages of Palamuru. Bacterial flora was determined by plate culture method followed by 16S rRNA gene sequencing. Drinking water is not expected to be free from microorganisms but the cfu observed in the samples was surprisingly high which indirectly reflects the poor sanitary practices. The concentration of total bacteria isolated from water samples were 217 at maximum and 56 at minimum. The following bacteria were identified: Gram negative bacteria - *Aeromonas hydrophila, E.coli, Citrobacter freundii, Acinetobacter junii, Pseudomonas alcaligens, Delftia lacustris, Pseudomonas aeruginosa* and Gram positive - *Bacillus cereus, Bacillus infantis, Bacillus niabensis, Lysinibacillus fusiformis, Brevibacteria sp., Bacillus sp. and Arthrobacter sp.* The most prevailing bacteria were *Bacillus sp., E.coli* and *Pseudomonas sp.* The highest bacterial contamination (217 cfu/ml) was found in water sample collected from Appanapalle village. The study, on the whole suggests that there is a wide range of bacterial contamination in the rural villages of Palamuru. Continuous

microbiological monitoring of the drinking water, including application of a disinfecting procedure, is necessary.

Keywords: Bacterial contamination, Drinking water, cfu, Villages

INTRODUCTION

Drinking water is defined as having a satisfactory quality in terms of its physical, chemical and bacteriological parameters so that it can be securely used for drinking and cooking. Public health problems such as vector borne and water borne diseases arise due to the percolation of reservoir and other water into the drinking water. Most studies concerning the quality of drinking water deal with the analysis of the Direct Count **[1, 2, 3]** or the determination of the viable counts of different bacteria in the drinking water, mainly focusing on the culturable and pathogenic bacteria. Drinking water is a major source of microbial pathogens in developing regions, although poor sanitation and food sources are integral to enteric pathogen exposure **[4, 5]**. The lack of safe drinking water and adequate sanitation measures lead to a number of diseases such as Cholera, dysentery, Salmonellosis and typhoid and every year millions of lives are claimed in the developing countries **[6]**. Ground water is the main source of drinking water in the villages without any treatment.

The biological contamination in drinking water is a major problem of public health in developing world. WHO estimates that about 1.1 billion people globally drink unsafe water and the vast majority of diarrhea disease in the world (88%) is attributable to unsafe water, sanitation and hygiene **[4]**. It is well known that the quality and safe of the drinking water continues to be an important public health issue **[7, 8]** because its contamination has been frequently described as responsible for the transmission of infectious diseases that have caused serious illness and associated mortality worldwide **[9, 10, 11, 12]**.

MATERIALS AND METHODS

Source of Samples

Seven drinking water samples were collected from different drinking water sources viz., government schools, offices in Palamuru, Andhra Pradesh, India in UV sterilized 1 liter water bottles. The pH of the sample was measured immediately after sampling.

Isolation Method

The samples collected for isolating bacteria should be examined as soon as possible after collection. If samples are to be transported from a long distance, samples must be maintained at 4˚ to 10˚C using cold packs

then transported in three hours & plating should be completed immediately after arrival at the laboratory. For isolation of bacteria, 100ul of the water sample was placed on nutrient agar medium and incubated at 37˚c for 2 days.

41 strains were isolated from these 26 drinking water sources by plating 100 µl of water sample on Nutrient agar medium and incubated at 37˚C. Based on the different colony morphology from each sample, a total of 4 strains were selected and identified in the present study.

Physico-Chemical Tests

These tests are for examination of physical and chemical parameters of water samples. These tests have been done by using multi parameter water testing kit method. It has the following type of tests: pH test, turbidity test, Chloride test, total hardness, Fluoride test, Nitrate test, Iron test, residual (free) Chloride test. Most of these tests are with visual comparison methods.

Identification Methods

For identification of bacteria, a series of biochemical, physiological & serological tests have been performed.

Gram Staining:

The gram staining, probably the most widely used technique in bacteriology is valuable because it enables to differentiate between two bacterial cultures which are morphologically indistinguishable yet of different species. The stain divides bacteria in to two large groups. Those that retain the primary stain crystal violet iodine complex (CVI) throughout the staining procedure, are termed as gram positive & those that loses the CVI complexes upon washing with alcohol but are stained with the color of counter stain (saffranin) are termed gram negative. The staining procedure can be performed on a bacterial smear prepared on glass slide. Smear is gently warmed using a spirit lamp and microscopically observed **[13]**.

Genomic DNA Extraction

Pure bacterial cells are resuspended in 0.8 ml of pre-warmed (60°C) CTAB extraction buffer. After 1 hr add 0.8 ml of chloroform/isoamylalcohol (24:1) solution. Gently mix for 2 min by inverting the tube. Spin for 10 min at maximum speed (14000 x g) at 4°C. Carefully transfer the aqueous phase (above the white interface layer) to a clean micro tube (discard the rest). To do so rapidly, prepare a p1000 with tip on and a p200 with tip on - take the larger volume off with the p1000 and then you can more gently get near the interface with the p200. Add 1 µl RNase (DNase-free) and incubate for 30 min at 37°C. Add 0.6 ml of isopropanol. Gently invert the micro tube to be sure mixing is

complete. Leave to precipitate for 2 hr to overnight at room temperature to allow the formation of the "DNA jellyfish". Spin 15 min at 14000 x g at 4°C to pellet the DNA. Remove the supernatant carefully, then wash the pellet once or twice with 70% cold EtOH, for example: we've done once in with 70% EtOH or 1x in 76% EtOH/10 mM AcNH4; then a second wash 1x70% EtOH Spin 15 min at max speed, 4°C. Remove supernatant and dry the pellet by leaving tube open at RT resuspend pellet in sterile H2O or TE, aliquot & store at -20°C **[14]**.

PCR Analysis

The small subunit rRNA gene of each sample's culture DNA was amplified using 16S rRNA Universal primers. The PCR amplification reaction mixture of 50µl contained 4µl bacterial DNA (nearly 200ng), 1µl Taq-DNA polymerase, 5µl of Taq buffer, 5µl of 2mM dNTP mix, 5 µl of forward primer (10 pM/µl) and 5 µl of reverse primer (10 pM/µl). Amplification was carried out in a Bio-Rad thermo cycler run for 30 cycles. In each cycle denaturation was done for 94ºC for 20s, annealing at 48ºC for 20s and extension were done at 72ºC for 40s and a final extension was carried out for 5min at 72ºC at the end of all 30 cycles. The amplified DNA fragment of approximately 1542 bp was separated on a 1% agarose gel and purified by Quiagen spin columns.

The desired DNA band from the agarose gel was cut weighed and then transferred to a sterile microfuge tube and add QE buffer thrice the volume of weighed excised band. Place it on a thermomixer at 65ºC for 10min and the contents were then transferred to a Quiagen column and spun at 8000 xg for 2min. Then it was washed with 750 µl of PE buffer and eluted with small quantity (30-40µl) of sterile waster. The purified PCR product was then used for sequencing **[14]**.

16S rRNA Gene Sequencing

The purified 1542bp PCR product was sequenced using primers. The resultant almost complete sequence of the 16SrRNA gene sequence of the isolate was subjected to blast sequence similarity search and Ez Taxon to identify the nearest tax a. the entire related 16SrRNA gene Sequence were downloaded from the database (http://www.nbi.nlm.nih-gov) aligned using the clustal – program **[15]**.

RESULTS AND DISCUSSION

Water samples were collected from 7 different villages of Palamuru, A.P. Physical parameters of water samples of the above 7 samples showed varied turbidity, pH, hardness and different Chloride, Fluoride, Nitrate and Iron concentrations. Among these water sample collected from Cristianpalle

showed highest concentrations of Iron and Nitrate and is rich in hardness whereas the least concentrations of Iron and Nitrate were shown by the water sample collected from Appanapalle **(Table 1)**

Different bacterial strains were isolated and identified out of which, a total of 14 selective bacterial strains were recovered from the drinking water samples. The highest bacterial concentration was found in the water sample collected from Appanapalle and least concentration was found in Pothanpally village Microscopic analysis of all the strains isolated from water samples indicated that 7 strains were Gram-positive and 7 strains were gram negative. 16S rRNA gene sequencing indicated that three strains belonged to the genus ***Bacillus (B.cereus, B.niabensis, B.infantis,); Acinetobacter, Brevibacteria, E.coli, Pseudomonas (P.alcaligens, P.aeroginosa), Delftia (D.lacustris), Citrobacter (C.Freundii)*** **and** ***Lysinibacillus (L.fusiformis)*** **[Table 2]**

Bacilli cause an array of infections from ear infections to meningitis and urinary tract infections to septicemia. Mostly they occur as secondary infections in immunodeficient hosts or otherwise compromised hosts. They may exacerbate previous infection by producing tissue-damaging toxins or metabolites that interfere with treatment. Despite the pathogenic capabilities of some bacilli; many other species are used in medical and pharmaceutical processes. These take advantage of the bacteria's ability to synthesize certain proteins and antibiotics. Bacitracin and polymixin, two ingredients in Neosporin, are products of bacilli. Also, innocuous *Bacillus* microbes are useful for studying the virulent bacillus species that are closely related.

Pseudomonas sp. could be considered as an opportunistic pathogen, which can survive on the fish surface or in water or in the gut and may cause disease when unfavorable conditions develop. *P. aeruginosa* is an opportunistic human pathogen, most commonly affecting immuno compromised patients, such as those with cystic fibrosis **[16]** AIDS **[17]** Infection can affect many different parts of the body, but infections typically target the respiratory tract (e.g. patients with CF or those on mechanical ventilation), causing bacterial pneumonia. Treatment of such infections can be difficult due to multiple antibiotic resistances **[18]**.

Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical

infections such as neonatal meningitis and pneumonia. The genus *Escherichia* is named after Theodor Escherich, who isolated the type species of the genus. *Escherichia* organisms are gram-negative bacilli that exist singly or in pairs. *E coli* is facultatively anaerobic with a type of metabolism that is both fermentative and respiratory. They are either nonmotile or motile by peritrichous flagella. *E coli* are a major facultative inhabitant of the large intestine.

Table 1: Multi-Parameter Water Testing

Place of the Sample Collection	pH	Turbi-dity	Chloride test	Total hardnss (ppm)	Fluoride Test (ppm)	Nitrate Test (ppm)	Iron Test (ppm)	Residual free Chloride Test
Allipur	**7.4**	**25 NTU**	**60 ppm/ 10ml**	**225**	**0.5**	**0**	**0.2**	**Absent**
Appanapalle	**7.2**	**5 NTU**	**40 ppm /10ml**	**200**	**0.5**	**0**	**0**	**Absent**
Cristianpalle	**7**	**10 NTU**	**90 ppm /10 ml**	**500**	**0.5**	**10**	**0.4**	**Absent**
Fathepur	**7.6**	**5 NTU**	**30 ppm /10ml**	**375**	**1**	**0**	**0.4**	**Absent**
Palakonda	**7.4**	**10 NTU**	**40ppm /10ml**	**300**	**0**	**0**	**0.3**	**Absent**
Pothanpally	**7.6**	**5 NTU**	**50ppm /10ml**	**250**	**1**	**0**	**0.2**	**Absent**
Yedira	**7.4**	**5 NTU**	**80ppm /10ml**	**350**	**0**	**10**	**0.3**	**Absent**

Table 2: Identification by 16S rRNA Sequencing

Place of the Sample Collection	Type of Water	Names of The Isolates	Cfu/ml
Allipur	**Tap Water**	***Bacillus infantis, Acinetobacter junii, E.coli***	**89**
Appanapalle	**Tap Water**	***Aeromonas hydrophila, Bacillus niabensis***	**217**
Cristianpalle	**Tap Water**	***E.coli, Pseudomonas alcaligenes***	**125**
Fathepur	**Tap Water**	***Brevibacteria sp., Delftia lacustris***	**156**
Palakonda	**Tap Water**	***Pseudomonas aeruginosa, Arthrobacter sp., E.coli***	**89**
Pothanpally	**Tap Water**	***Bacillus sp., Lysinibacillus fusiformis***	**56**
Yedira	**Tap Water**	***Bacillus cereus, Citrobacter freundii***	**169**

CONCLUSION

This is the study, carried out to explore the presence of harmful microbes in the drinking water of villages in Palamuru, A.P. This study further reveals our understanding of the bacterial diversity dynamics and the contamination of the drinking water. The public must also recognize that public drinking water is not frequently monitored by health laboratories for acceptable quality. Accurate identification of bacteria present in treated drinking water is needed in order to better determine the risk of growth of potentially pathogenic organisms within the water distribution systems. The lack of awareness about good sanitation and personal hygienic practices is an important factor for poor drinking water quality in these sources. The surveillance for possible waterborne diseases may be in progress. If a disease outbreak is linked to a water supply, the authorities should have a plan to quickly and effectively contain the illness.

Competing Interests

Author has declared that no competing interests exist.

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