RESEARCH ARTICLE

MICROBIOLOGICAL ANALYSIS OF QUALITY OF DRINKING WATER IN THE CYCLONE AFFECTED TWIN DISTRICTS IN TAMIL NADU: A PILOT STUDY.

Illavanji Rajagopal MD[1], Uma Mageswari MD[2], Kalyani MD[3] and Archana Nagarajan MD[4].
Department of Microbiology, SaveethaMedical College, Thandalam, Chennai-105.

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Manuscript Info	Abstract
	Introduction: During cyclone, Human excreta adds to the water bodies causing acute diarrhoeal disease and other waterborne diseases. To prevent Water borne infections, drinking water status should be assessed. Thermo tolerant coliform count is the indicator for drinking water status. The present study was to measure the Thermo tolerant coliform count (TTC) in Vardah cyclone affected areas at Thiruvallur and Kanchipuram districts. **Materials and Methods:** Water collected from 15 drinking water sources from "Vardah" cyclone affected areas during one month (from December 2016–January 2017) at Pazhavaerkadu, Budhur from Thiruvallur district and Kannakinagar from Kanchipuram district. TTC was performed by Most Probable Number (MPN) technique. **Result:** This study results showed the presence of Thermo Tolerant Coliforms in 8 drinking water sources–53.3%. **Conclusion:** Health awareness should be provided to the rural population. Low cost, rapid analysing tool is helpful to detect *E. coli* in drinking water. This would be important to consider in waterborne infection prevention programme.

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Introduction:-
Last two decades Chennai and coastal regions of Tamil Nadu has experienced many cyclones. Tropical cyclones trigger waterborne and mosquito borne infections.

During cyclones, human and animal excreta add to the water bodies causing acute diarrhoeal outbreak (Shah *et al.*, 2016). Post disaster acute diarrhoeal outbreaks are common in developing countries like India.

Open air defecation (OAD) provokes an important risk factor. Globally 12% of population practice open air defecation due to lack of sanitation infrastructure (WHO and UNICEF, 2017). In India 330 million (WHO and UNICEF, 2017) rural population and 44% (Shoba *et al.*, 2013) urban slum population do practice open air defecation. Practising open air defecation not only leads to gastrointestinal infections, it also causes stunting of growth (Spears *et al.*, 2013). Apart from acute diarrhoeal disease (ADD) other water borne infections are also common during cyclones.

Populations at risk to get Water borne infections (WBI) are infants, children under five years, people with lack of sanitation infrastructure, elderly and the sick. There were 10.7 million cases of acute diarrhoeal disease in under five children and 1535 deaths were reported in India during the year 2013 (Govt of India, 2014).

Corresponding Author:- Illavanji Rajagopal.
Address:- Department of Microbiology, SaveethaMedical College, Thandalam, Chennai-105.

Contamination in drinking water sources is detected by indicator organisms. Those organisms inhabit the gut in large numbers and are excreted in human faeces. The presence of indicator organisms in drinking water source is evidence of faecal contamination.Presence of faecal contamination in drinking water sources is by Thermo tolerant coliforms and faecal *streptococci*. But faecal *streptococci* tend to persist longer in the environment thanthermo tolerant coliforms and difficult to detect the recent contamination (Hachich.*et al.,* 2012).Presence of recent faecal contamination in drinking water sources is detected by Thermo tolerant coliform count (TTC count).

This present study was to measure the Thermo tolerant coliform counts in drinking water sources at Vardah cyclone affected twin districts of Thiruvallur and Kanchipuram of Tamil Nadu. Thermo tolerant coliform count is the indicator for quality of drinking water status (Hodge *et al.*, 2016; Water Sampling and Analysing, 1997).

Materials and Methods:-
Place of study: Kannakinagar of Kanchipuram district, Pazhavaerkadu, Budhur of Thiruvallur district were selected as our study area since those places were severely affected by Vardah cyclone.

Study duration: One month (December 2016 – January 2017)

Design of the study: A pilot study.

Objectives:-
1. To assess the microbiological analysis of drinking water at cyclone affected twin districts.
2. To measure the Thermo tolerant coliform count.
3. To isolate and enumerate the Thermo tolerant *E. coli.*
4. To emphasize the treatment plan to overcome the Water Borne Infections.

Methodology:-
In sterilised bottles, water collected from different drinking water sources at Vardah cyclone affected areas of Pazhavaerkadu, Budhur from Thiruvallur district and Kannakinagar from Kanchipuram district. In each area, water samples were collected from five different drinking water sources like bore well, hand pump, uncovered well, corporation water tanks, tap water and private water supply. Collected water samples were transported to the microbiological laboratory and processed for the measurement of Thermo tolerant coliform count by Most Probable Number (MPN) technique (Ananathanarayan&Paniker, 2013; MultipleTube Method for Thermo Tolerant).

Presumptive Test:
This test was considered as presumptive as the reaction could be due to *Escherichia coli* and other coliforms. Hence the presumptive reaction (Acid and gas formation) due to coliform organisms has to be confirmed.

Double strength and single strength Macconkey broth (Himedia Private Ltd) containing phenol red indicator in bottles and tubes with inverted Durham tubes for indication of gas production were used. The procedure was done as follows.

1 sterile graduated bottle with 50ml of double strength Macconkey broth was inoculated with 50ml of water sample. 6 tubes of 10ml double strength tubes were inoculated with 10ml of water sample each and 6 tubes of 5ml single strength tubes were inoculated with 1ml of water sample each.

After inoculation of water samples, tubes and bottles were incubated at 44℃ and examined after 24 and 48 hours for lactose fermentation with acid and gas production. Initially the presumptive positives were read at 24 hours and the remaining negative tubes and bottles were reincubated for another 24 hours. Further positives were added to the previous results. Finally MPN of TTC count was estimated by referring to Mccrady probability table (Multiple Tube Method for Thermo Tolerant).

Confirmatory Test:
Positive growth were streaked in plates of Eosin Methylene Blue Agar (HimediaPrivate Ltd) and incubated at 37℃ for 24 hrs. EMB agar is the selective and differential medium for coliforms and observed for green metallic sheen colonies of *Escherichia coli*(Eosin MethyleneBlue Agar Plates Protocol, 2007).

Fig 1:-Green metallic sheen appearance of *Escherichia coli*

Completed Test:
Completed test was performed by using the organisms which grew on the confirmed test media. They were further subjected to Eijkman's test and biochemical investigations (Koneman'scolor atlas and text book of diagnostic microbiology 2016): Indole, Triple Sugar Iron test, urea hydrolysing test, citrate utilization test, Mannitol Motility Medium, oxidase test, catalase test was carried out.

Result:-

Fig 2:- Most Probable Number (MPN) technique

Presumptive Test:
In this study TTC was estimated to be very high
> 180 MPN/100ml in Kannakinagar – borewell, uncovered well, private water supply, Pazhavaerkadu –uncovered well, private water supply,Budhur– bore well, uncovered well, private water supply, panchayat water tank (Table 1).

> 161 MPN/100ml in Pazhavaerkadu – bore well and Budhur hand pump, low TTC in Pazhavaerkadu handpump and corporationwater supply.

No coliform count was detected in Kannakinagar hand pump, corporation water tanks

Table 1:-

Water sources	Place	Thermo tolerant coliform count/100ml	Organisms
Borewell	Kannakinagar	> 180/100ml	*E. coli&klebsiella*
Uncovered well	Kannakinagar	> 180/100ml	*E. coli&klebsiella*
Private water supply	Kannakinagar	> 180/100ml	*klebsiella*
Hand pump	Kannakinagar	0/100ml	–
Corporation water tank	Kannakinagar	0/100ml	–
Borewell	Pazhavaerkadu	> 161/100ml	*E.coli*
Uncovered well	Pazhavaerkadu	> 180/100ml	*klebsiella*
Private water supply	Pazhavaerkadu	> 180/100ml	*Pseudomonas&klebsiella*
Hand pump	Pazhavaerkadu	35/100 ml	*klebsiella*
Corporation water supply	Pazhavaerkadu	27/100ml	*pseudomonas*
Borewell	Budhur	> 180/100ml	*E. coli&klebsiella*
Uncovered well	Budhur	> 180/100ml	*E. coli&klebsiella*
Tap water	Budhur	> 180/100ml	*E. coli&klebsiella*
Hand pump	Budhur	> 161/100ml	*E. coli&klebsiella*
Panchayat water supply	Budhur	> 180/100ml	*E. coli&klebsiella*

Confirmatory Test:
Table 2:-This study results showed the presence of Thermo Tolerant Coliforms in 8 drinking water sources 53.3%.

Type of Water Sources	Total Number Tested	Positive for Thermo tolerant*E. coli*	Percentage
Borewell	3	3	100%
Uncovered well	3	2	66%
Private water supply	2	0	0%
Corporation/Panchayat water	3	1	33%
Tap water	1	1	100%
Hand pump	3	1	33%
Total	15	8	53.3%

Thermo tolerant *E.Coli* positive water samples
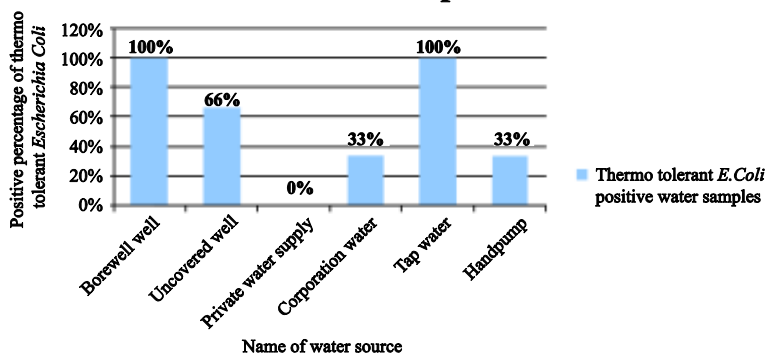
The microbiological analysis of drinking water sources revealed that 3 out of 3 borewell, 1 out of 3 hand pumps, 2 out of 3 uncovered well, 1 out of 3 panchayat water supply, 1 tap water were positive for Thermo Tolerant Escherichia coli.

Pseudomonas aeruginosa species were isolated from 2 samples (private water supply and corporation water at Pazhavaerkadu).

Klebsiella species were isolated from 11 water samples.

(Budhur–Panchayat, tap water, borewell, uncovered well and handpump, Kannakinagar–private water supply, borewell, uncovered well, Pazhavaerkadu–uncovered well, Private water supply and hand pump).

Table 3:-Water samples are classified based on presumptive count

Class	Grading	Coliform count/100 ml
1	Excellent	ni1
2	Satisfactory	1–3
3	Suspicious	4–10
4	Unsatisfactory	> 10

Out of 15 drinking water samples 8 were unsatisfactory and 5 were satisfactory 2 samples were excellent (Table 3)**.**

Table 4:-Completed Test: Biochemical investigations

Name of The Test	*E.coli*	Klebsiella	Pseudomonas
Indole test	+	−	−
Triple sugar test	A/A With +/- gas	A/A With gas	K/K
Urease test	−	+	−
Mannitol motility medium	F/M	F/NM	NF/M
Citrate test	−	+	−
Oxidase test	−	−	+
Catalase test	+	+	+

Discussion:-

Vardah cyclone that struck the Andaman, Nicobar Islands and south India on December 9th 2016 was a natural disaster. In Tamil Nadu, Kanchipuram and Thiruvallur districts were affected by severe cyclonic storm. It is an acute health emergency to protect the population from Water borne infections. Providing clean and safe water during cyclones is the major challenge for the government.

During cyclone, human and animal excreta add to the water bodies causing WBI. Faecal contamination of water is detected by Thermo tolerant Coliform Count. Thermo tolerant Coliforms grow at 44°C, ferment lactose to produce acid and gas. *Enterobacter, Citrobacter, Hafnia, Klebsiella, E. coli* are included in Thermo tolerant Coliforms. Among the total coliforms *E. coli* is the definite indicator of faecal contamination.

Quality of drinking water analysis was done in Vardah cyclone affected twin districts of Thiruvallur and Kanchipuram. Water collected from 15 drinking water sources like bore well, uncovered well, hand pump, corporation water/Panchayat, tap water and private water supply. Thermo tolerant coliform count was estimated by MPN technique.

In this study TTC was estimated to be very high
> 180 MPN/100ml, > 161 MPN/100ml due to water contamination and inappropriate disinfective procedures (Table 1).

No coliform count was detected in Kannaki Nagar hand pump, corporation water tanks due to adequate chlorination.

Klebsiella species were isolated from 11 samples due to decaying of plants during post cyclone period also by surface and soil contaminants.

Pseudomonas aureginosa were isolated from 2 samples which is a microbe of natural water sources. Both are not the indicator organisms. According to BIS desirable limit of coliforms in drinking water 10 MPN/100ml, Escherichia coli 0/100 ml (BUREAU OF INDIAN STANDARDS, 2003-2005).

Consumption of drinking water contaminated with Thermo tolerant *E.coli* is a significant risk factor. Thermotolerant *E. coli* was isolated from eight drinking water sources 53.3% (Table 2).

In the cyclone affected twin districts of Thiruvallur and Kanchipuram few percentages of people only used to buy mineral water for drinking purposes. Rest of the people are using water from the available water sources. During cyclone uprooted trees, lack of transport facilities and extensive damage to the powerlines, made people in Kanchipuram and Thiruvallur districts utilize the water from common uncovered well for drinking purpose and for household works. It might be a cause of diarrhogenic out break at Thiruvallur district during postcyclone period, but not in our study area because of safe drinking water practices.

Thermotolerant *E. coli* was isolated from eight drinking water sources 53.3%. Our study observed relatively high value of *E. coli* in drinking water sources in postcyclone period compared to Rajendran P, *et al.* (*E. coli* – nil)(Rajendran *et al.*, 2006)and Ms Shah, *et al.* (*E. coli* –33%) study (Shah *et al.*, 2016).

Suggestive measures:-
Water should be disinfected from domestic level to community level at desired standards. Domestic level disinfection by boiling drinking water practices which could reduce 86.2% (Rosa *et al.*, 2010) geometric mean of thermo tolerant coliforms. Chlorine tablets, bleaching powder sachets should be provided to the cyclone affected areas to disinfect the private wells at home, water tanks should be cleaned with bleaching powder and to be chlorinated. Incommon uncovered wells it is mandatory to have user friendly movable coverings/lids to prevent direct water contamination also for regular and emergency water usage. Potable water purification machines should be supplied to the cyclone affected areas. Solar water purification system is useful in clean water system. In cyclone affected areas pond and lake water bodies are turbid and sandy, that could be treated by constructing sand filters. Slow sand filters reduce the bacterial counts and *Escherichia coli* by 99.9 to 99.99%(Park 2017). Highly skilled personnel are needed to operate the rapid sand filters which reduce 96 to 99%(Park 2017) bacterial count.

Conclusion:-
In this study traditional MPN technique was carried out, which is a sensitive and useful technique for highly contaminated water.

Health awareness should be provided to the people living in rural areas about open air defection, need of toilet facilities, boiling drinking water practices and hand hygiene.

Low cost, rapid analysing tool is helpful to detect *Escherichia coli* in potable water sample, during cyclone, this would be important to consider for Water Borne Infection prevention programme.

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