The occurrence of Enterobacteriaceae in the well water of Nallur Divisional Secretariat area of Jaffna district, Sri Lanka.

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ABSTRACT

The access for safe drinking water has become a challenge to human at present due to contamination with the different sources of pollution such as domestic waste, animal excreta and latrine leakage. Therefore, this study was carried out with the focus of determining the microbial quality of dug well water from a highly populated peri-urban area (Nallur Divisional Secretariat) of the Jaffna district in Sri Lanka. The water samples from 20 dug wells were collected from the study area and the information particularly the distance of well from the toilet septic tank, sanitary conditions of well and the surroundings were gathered. The total bacterial count was determined and different bacterial species were identified on Xylose Lysine Deoxycholate (XLD) agar.The t-test was performed to identify the factor/s that significantly influences the total bacterial count. A correlation analysis was performed between the distance of well from septic tank and bacterial count. Nine different bacterial species, that belong to the family Enterobacteriaceae, were identified; Klebsiella, Enterobacter, Escherichia, Salmonella, Shigella, Citrobacter, Yersinia, Serratia and Acinetobacter. Of the 72 bacterial isolates from 20 wells, 37 belong to the coliform group indicating recent faecal contamination. The total bacterial count has an inverse correlation with the distance of the toilet from the well ($R^2=0.506$). Presence of pathogenic bacteria in the well water in the study is a great threat for human health and therefore, regular measures need to be taken to disinfect the water.

Keywords: *Drinking water, Enterobacteriaceae, Faecal contamination, Jaffna district, Microbial contamination.*

INTRODUCTION

The safe drinking water sources are shrinking globally due to various anthropogenic activities such as contamination of chemicals from industries and agriculture, and disease-causing microorganisms (pathogens) from households and animal husbandry. According to the estimation of the World Health Organization (WHO), about 2.3 billion people globally do not have access to safe drinking water (WHO, 2017). Poor quality water is an important risk factor in the occurrence of waterborne illnesses; diarrhoea, cholera, schistosomiasis and gastrointestinal problems. Estimates in North America reveal about 5 - 30% of gastrointestinal diseases occur through water (Payment *et al*., 1997). Further, a recent study reported that around 63% of the children were infected with childhood diarrhoea in developing countries and approximately aetiological agent for 40% of the cases were unidentified (Ugboko *et al*., 2020). Although the drinking water stands impose the threshold level of physical, chemical and microbial parameters, they are not highly considered in the developing countries as people generally access untreated water from natural sources. In Sri Lanka, majority of the people consume untreated water from the natural sources apart from few urban

areas where treated water is provided by the National Water Supply and Drainage Board.

Jaffna district, which is one of the 25 administrative districts of Sri Lanka, is located in the far north of Sri Lanka in the Northern Province. In this region, water is naturally stored in the isolated caves and caverns of the underground limestone beds. This district highly depends on the groundwater for domestic and agricultural water use and retrieves the water from underground using dug wells. Approximately 80% of this groundwater is being used for agriculture, and 20% for domestic consumption in Jaffna (Panabokke and Perera, 2005). The rapid extension of the agriculture sector and increasing population in this region created a heavy competition for groundwater between major consumers. Alongside this, available domestic lands are fragmented due to the increasing population which results in close proximity houses. This increases the chance for contamination of well water with the faecal materials.

Human faecal material is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens (Scott *et al.*, 2003). Faecal contamination can be assessed by detecting the indicator organisms (faecal coliforms) in water. Presence of such bacteria could be taken as an indication of faecal contamination of the well water and the coliform count could be used to assess the severity of the contamination. The higher the level of indicator bacteria, the higher the degree of faecal contamination and the greater the risk of waterborne diseases (Pipes, 1982). A survey showed that globally 1.8 billion people access water sources which are contaminated with faecal matter (Bain *et al.*, 2014). Major bacteria that involve in the causation of waterborne illnesses are enterotoxigenic *Escherichia coli*, possibly enteropathogenic *Escherichia coli*, *Clostridium difficile*, *Salmonella* spp.,

Shigella spp., *Vibrio* spp., *Aeromonas* spp., *Campylobacter jejuni*, and *Yersinia* spp. Excreta from human, animals and birds that can cause direct contamination of water or the proximity of pit latrines to the wells acts as the sources of contamination. The major concern of the waterborne illnesses is the enhanced risk, seen in a weaker population consisting young, old, pregnant and immunocompromised individuals (Leclerc *et al.*, 2002).

The term Salmonellosis includes typhoid fever, paratyphoid fever and gastroenteritis caused by pathogenic species of *Salmonella*. It is one of the serious illnesses that end up in the death of the victim unless the treatment is initiated. Abdominal discomfort, diarrhoea, vomiting and weakness are some of the disease-related signs. The annual incidence of 17 billion cases was recorded worldwide due to Salmonellosis (Typhoid and Paratyphoid fever) (Kindhauser, 2003). In 2009, an outbreak of typhoid fever was reported in Pakistan, in which the involvement of well water was documented (Farooqui *et al.* 2009). Waterborne typhoid fever outbreaks have devastating public health implications. In Sri Lanka, 298 cases of enteric fever were reported in 2003. Of that, Jaffna district had the highest number of cases (107) (Ministry of Health, 2013). In recent years also, the highest number of cases were recorded in Jaffna district (Ministry of Health, 2017). This shows that the drinking water quality in Jaffna district is still under threat.

The recent knowledge about the microbial loading into the drinking water sources in Jaffna district is very limited. In a study, it was reported that out of 38% of sampling locations which were contaminated with *Salmonella* spp., six are being used for drinking purposes and 80% of the tested locations were beyond the drinking water quality standards (Mahagamage *et al.*, 2019). Another study conducted in Kopay MOH area of Jaffna district stated that the

surrounding environment of the wells was highly conductive for contamination and water in the study area was contaminated with faecal material (Arulnesan *et al*., 2015). However, there are no studies available for Nallur Divisional Secretariat of Jaffna district. Therefore, this study was aimed to determine the occurrence of bacterial contamination in the dug well water and possible factors influencing the microbial count in Nallur Divisional Secretariat of Jaffna district, Sri Lanka.

MATERIALS AND METHODS

Study area and sample collection

Thirunelvely, Kandarmadam, and Kalviyangadu of the Nallur Divisional

Secretariat of Jaffna district were selected as sampling regions (Figure 1). The period of this study was between May and July 2017. A total of 20 water samples were collected from the wells manually using a bucket-rope method in randomly selected houses. The water samples were collected in sterile 250 ml plastic sampling bottles soon after drawing from the wells. Collected samples were transported with ice packs to the laboratory within 48 hours. At the time of sampling, the dimensions of the well (depth, diameter), distance of the well from the toilet, presence of aquatic life and floating materials within the well, surrounding environment of the well, history of waterborne diseases and the demography of the household owner were also determined.
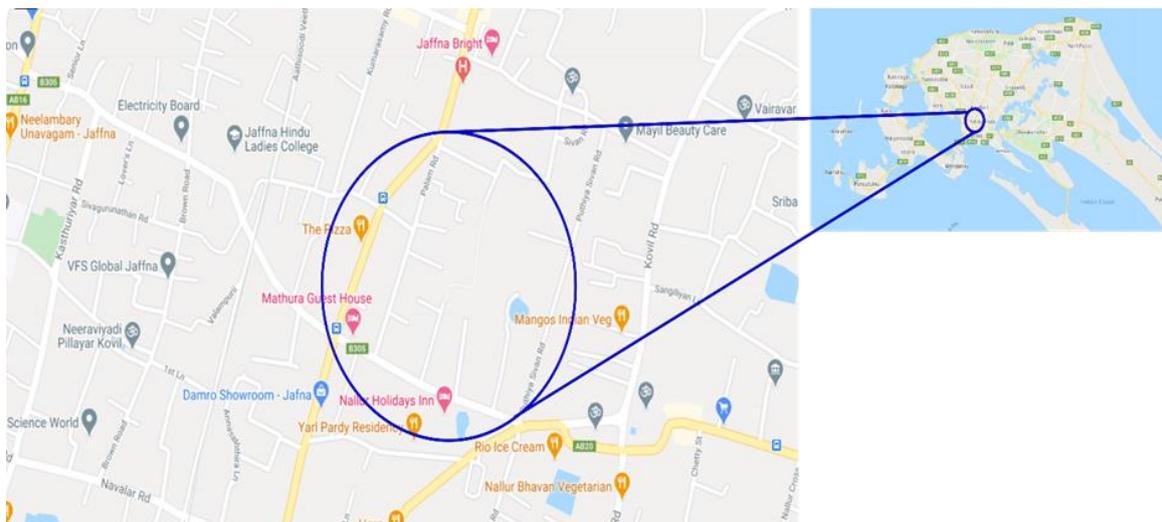

Figure 1. The sampling site of water samples from the wells.

Isolation and identification of bacteria

At the laboratory, 50 ml of the water sample was centrifuged (Sigma 3-18KHS) at 3500 rpm for 10 minutes and the supernatant was discarded until leaving 5 ml of sediment. The sediment was vortexed and 50 µl of it was spread evenly on the XLD agar using a glass spreader. The plates were incubated at 37 ℃ for 24 hours and thereafter the colonies were -

counted using a colony counter. After counting, a total of 72 different types of colonies (1 - 6 colonies per well) were selected and sub-cultured on XLD agar plate. The selected bacterial colonies were identified using phenotypic characters (Gram stain, oxidase, urease, citrate, Triple Sugar Iron test, motility and indole production) (Quinn *et al*., 2002).

Data analysis

The data were analysed using Minitab 16 statistical software (Minitab, 2010). Pearson correlation analysis was performed between the total microbial count and distance of well from the toilet septic tank. The t-test was employed to identify the significance influence ($p < 0.05$) of selected factors on the microbial count.

RESULTS AND DISCUSSION

Species identification and total count of microbial colonies

This study confirms the existence of both coliforms and other pathogenic bacteria in the collected well water samples. Out of the 72 isolates, more than half (56.9%) belonged to Coliform group (*E.coli, Klebsiella, Enterobacter, Citrobacter* and *Serratia*), which indicates the recent faecal contamination (Table 1). Eventhough there were harmless coliforms present in the group, the risk of acquiring waterborne diseases has been always there due to the presence of pathogenic bacteria. Indicator organism of choice for faecal contamination is *E.coli* and the count of this organism should be zero in 100 ml of water (Standridge, 2008). However, 40% of the wells sampled in this study were positive for *E.coli*, which emphasizes the unsuitability of water for human consumption without boiling or other water treatments. In a similar study conducted in Matara district, Sri Lanka, it was reported that 65% of wells were contaminated with faecal matter and it could be the main reason for the reported higher number of faecal-oral diseases in Matara district (Rathnayake and Rathnyake, 2008).

Table 1: Bacterial species identified from the total collected well water samples

Bacterial species	Number of isolates	Occurrence % in total samples
Klebsiella spp	13	65
Escherichia coli	8	40
Salmonella spp.	6	30
Shigella spp.	5	25
Enterobacter spp.	13	65
Citrobacter spp.	4	20
Yersinia spp.	1	5
Serratia spp.	3	15
Acinetobacter spp.	7	35
Unidentified	12	60
Total	72	-

Apart from these, 55.6% of the isolates have the pathogenic potential *(Salmonella, Shigella, Klebsiella, Yersinia, Acinetobacter*, and *E.coli*) (Figure 2). The coliforms including *Klebsiella, Enterobacter* and *E.coli* were the predominant bacteria present in the well water. Further, *Salmonella* and *Shigella* were found in 30% and 25% of the wells, respectively (Table 1). The colony count

ranged between 860-8820 CFU/ml indicating all the selected wells had bacterial contamination.

Salmonella species were positive in the following well numbers; 4, 5, 14, 19 and 20 (Figure 3). Presence of pathogenic bacteria like *Salmonella, Shigella, Klebsiella, E.coli, Yersinia* and *Acinetobacter* is of high public health significance since they are related to the risk of causing diarrhoeal diseases (Ford *et al*., 1999).

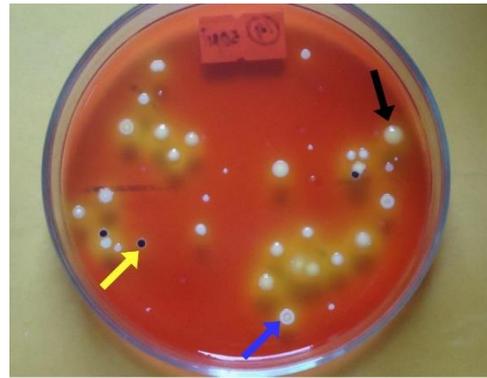In this study, 55.6% of pathogenic bacteria were isolated from the water samples (Table 1).

Figure 2: Different colonies identified on the XLD agar plate (The yellow arrow indicates a Salmonella colony, whereas blue and black arrows indicate *Escherichia coli* and *Klebsiella* colonies, respectively).
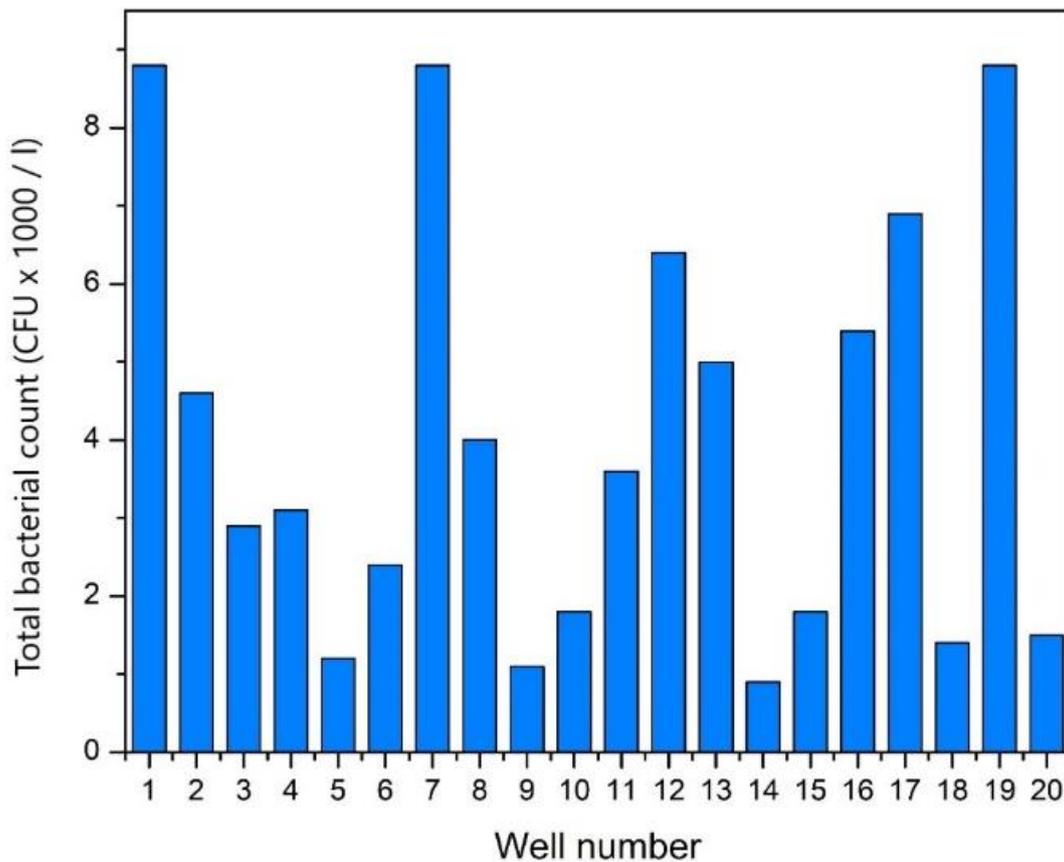

Figure 3:   The total bacterial count in individual well.

The factors possibly involved in the contamination

Table 2: Factors influence the bacterial count in well water

Variable		No of wells	Colony-forming units (CFU/ml)		P-value
			Range	Mean±SE	
Foreign substances on the wall	Present	15	860-8820	4600±690	0.938
	Absent	5	920-3060	3868±1467	
Watering method	Pulley	11	860-8740	4035±822	0.906
	Motor	9	1220-8820	3887±977	
Holes on the wall of the well	Present	4	860-8740	4315±1674	0.822
	Absent	16	920-8820	3881±673	
Birds' faeces near the well	Present	7	860-8740	4917±1103	0.293
	Absent	13	920-8820	3457±726	
Birds on the trees around the well	Present	16	860-8740	4374±709	0.115
	Absent	4	1220-8820	2345±876	
Foreign substances on water	Present	15	1160-8740	4366±671	0.027*
	Absent	5	860-8820	1713±677	
Toilet distance from the well	<10 m	7	1800-8820	6663±963	0.004*
	>10 m	13	860-5020	2517±408	
Occupation of the owner	Gov	9	860-4740	2067±445	0.002*
	NGO	11	1700-8820	5527±796	
Chlorination interval	0-4 weeks	9	860-6480	2722±1822	0.174
	4-8 weeks	3	1220-6880	4440±2910	
	>8 weeks	8	1160-8820	5193±32	

SE = Standard error, Gov = Government organization, NGO = Non-government organization, * Significant difference at 0.05 probability level.

The total bacterial count was significantly influenced by the presence of foreign substances on water (coconuts, coconut leaves, coconut husks, coconut fluorescence, other leaves and shopping bags), toilet distance from the well and occupation of the well owner or tenant (Table 2). The occupation and the educational status of an individual are interconnected. Therefore, the negative correlation for bacterial count with occupation could be ascribed to the awareness on sanitary measures of maintaining clean well water. The total

bacterial count was high when the distance between the toilet pit and the well was less than 10 m (Table 2; Figure 4). Increase in chlorination frequency decreased the bacterial count but this was not significant (Table 2).

The depth of the wells, well diameter, presence of aquatic life, presence of the birds' faecal matter in the surrounding of the well were also analysed to explore the bacterial contamination related to the above factors. However, the results showed no correlation between the bacterial count and the aforementioned factors.

The correlation between total bacterial count and distance between toilet and well

The correlation between total bacterial count and distance from the toilet was moderate ($R^2 = 0.51$) (Figure 4). Similar observation was reported, in which faecal coliform, nitrate and phosphate concentration were negatively correlated to the distance from the toilet (Arnade, 1999).
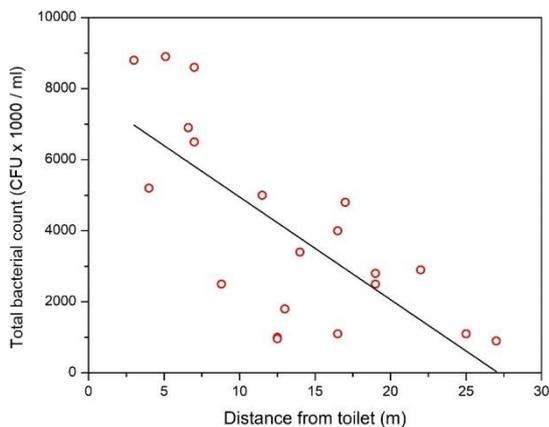

Figure 4: The correlation between the total bacterial count and distance from the toilet pit.

The severity of contamination is seasonal and the possibility is high in the rainy season (Singh *et al.*, 2019) as the well water gets contaminated by the seepage flow of water from the septic tanks (Graham *et al*, 2013). In a flooding condition due to heavy rainfall, both wells and septic tanks can be in direct hydraulic connection if both are located nearby. Therefore, the distance and depth of the well from the septic tank of toilet need to be put up beyond an appropriate distance before planning to build houses. However, increasing population decreases the land size that makes it difficult to maintain the sufficient distance between the septic tank and well.

According to the guidelines of urban development authority of Sri Lanka, the distance between the septic tank and well should not be less than 15 m (Urban development authority, 1986). However, in this study, 60% of the wells studied were not complied with the standards. Similarly the study conducted in Gampaha, Sri Lanka also revealed that over 68% of the toilets were built within 15 m peripheral from the well (Bandara and Najim, 2013). The majority of the people are ignorant of the well water contamination with faecal material and other chemical sources. Also general public have lack of awareness on the transmission and prevention of waterborne diseases (Arulnesan *et al.*, 2015).

In a developing country like Sri Lanka, it is very difficult for the government bodies to monitor the changes of the water in the individual wells in a periodical manner and there have been no support for the regular inspection process. Therefore, it is the responsibility of each well owner to maintain the sanitary condition to safeguard them from waterborne diseases.

Considering the potential risks of contaminated water, remedial measures should be initiated. Chlorination can be used to reduce the disease burden due to contaminated water, since it is one of the simplest, inexpensive, commonly used methods with the lowest operating cost and the longest history for continuous disinfection process (Burch *et al.*, 1998). The success of the water treatment

depends on the amount of chlorine used, the contact time of chlorine and frequency of chlorination. The amount of chlorine to be used should be decided based on the volume of the well. The ideal dosage is 4mg/l or 4 ppm for safe use (EPA, 2013). Boiling the water also a cheap option to kill a significant amount of microbes received from faecal contamination (Clasen *et al*., 2008).

CONCLUSION

This study found that all the dug wells under investigation were contaminated with different bacterial species. The total bacterial count in the water samples ranged from 860 - 8820 CFU/ml indicates a severe degree of contamination. The finding of coliforms strongly suggests the faecal contamination. Further, the detection of pathogenic bacteria (i.e: *Salmonella* and *Shigella*) indicates the risk of water borne infections. The bacterial count showed an inverse correlation with the distance to toilet pit from well and educational status of the owners while a positive correlation was found with the presence of foreign materials. These findings implicit the study area was at high health risk. Therefore, remedial measures are strongly recommended before direct human consumption of water from these sources.

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