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Bacteriological and Physical Analysis of Dug Wells in South Darfur (Tolus town) for Indication of Pollution by *Enterococus E. faecalis* and Coliform

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Abstract: ***The provision of safe and adequate water supply for the population has far reaching effects on health, productively and quality of life, as well as socio-economic development of the nation. The present study was undertaken to determine bacteriological quality of water sources in Tolus town, South Darfur state west Sudan. Water samples from fifteen randomly selected, unprotected dug wells were collected during rainy season (2001) and transferred and analyzed in Nyala veterinar research laboratory (N.V.R.L).Presumptive coliform test for determination of most probable number (MPN), followed by a physical confirmatory tests. Where, the investigation revealed that the number of bacteria is inversely proportional to Reynolds's number that is obeyed the laminar flow, which confirm the biological results. The investigation revealed that all the studied water sources were contaminated with faecal bacteria and have presumptive bacteria count above the permissible limits for sanitary water , recommended by (WHO, 2008).***

Keyword: dug-wells, most probable number, faecal coliform Rynold's number

1. Introduction

Water is one of the most important elements for all forms of life. It is indispensible in the maintenance of life on the earth. Despite of this, human beings are continuing to pollute water sources resulting in provoking water related illnesses (WHO, 2008). According to world health organization (WHO) report in 2006 approximately three out of five persons in developing countries do not have access to safe drinking water, and only about one in four has any kind of sanitary facilities.

The quality of water and treatment of waterborne diseases are critical public health issues. Bacterial contamination of drinking water sources is the most common health risk (WHO 2008; Abera *et.al* 2011).

Total and faecal coliform have been used extensively for many years as an indicator of faecal contamination , to express microbiological quality of water as well as a Para-mater to estimate diseases risk and the hygienic condition of water (Redy, 2009) .

In South Darfur state over 70% of communicable diseases are due to poor environmental health condition arising from unsafe and inadequate water-supply (Nada, 2003).

The provision of safe and adequate water supply for the population has far reaching effects on health, productivity and quality of life, as well as on secio-economic development of nations. Therefore the present study was designed to detect the coliform, *Enterococcus faecalis* to assess the quality of water sources and the extent of contamination of water in the studied area with intention to help the concerned bodies with the necessary information to take action, and to provide basic information for further study. The rotation of helicoidal flagella allows the motion of the *E.coli* at Reynolds number (Re) and its caused by the drag anisotropy, (Lauga and Powers,2009).

$$\int \frac{dN}{N} = \int rdt \ : \ N(t)\,|_{t=0} = lnN_0$$

2. Material and Methods

Collection of samples:
Fifteen dug wells distributed along the valley (Wadi Bulbul) were randomly selected to study the possibility of their contamination with *Enterococcus faecalis* and coliform. 100ml of water samples from every well were collected, following WHO methods (1988). The water samples collected using sterile container tied by clean string, rubbers stoppers and transferred to Nyala veterinary research laboratory (NVRL) in ice boxes to be analyzed using two methods.

5 ml of pure water added gradually, to different volume of water contaminated with bacteria. Then, based on the principle of the mechanical fluid, the relationship between MPN and Reynold's number (Re) is found.

Total coliform Bacteria Presumptive test :
The most probable number (MPN) of total coliforms bacteria were determined by multiple tube fermentation technique (APHA, 2001) . A serial dilution from each water sample (10^{-1} – 10^{-5})were inoculated in tubes containing double strength of 10 ml Macconkey broth medium with inverted Durham tubes . All the cultures were incubated aerobically at 37 - 48 C° and examined for gas production.

Confirmatory Tests
Positive tubes for presumptive test were sub-cultured into Brilliant green bile (BGB) broth

and incubated at 37 C° for 24 – 48 hours . Total coliform were calculated from (MPN) table as per 100 ml of sample (APHA,2001).and (Deman,1983).

Examination of Enterococci

A glucose azide broth medium was used for the identification for Enterococci bacteria. All samples positive in the presumptive test, were sub-cultured and incubated at 44C° for 24 – 48 hours. A confirmatory test was carried out on bile asculin azide agar medium incubated at 44 C° – 45 C° for few hours .

Physical Analysis

We encountered some interesting dimensionless groups when performing dimensional analysis of fluid mechanical problems. One problem Reynolds investigated experimentally is the transition of flow from the orderly kind that call the " laminar flow " to the more chaotic type of flow termed " turbulent flow " (O.Reynolds,1883).

We know that, bacteria growth rate is increased with proportion to its initial total number N_0 at time $t = 0$, therefore, let N(t) represents the number of bacteria at time t . So, the increasing of growth rate of bacteria $\frac{dN}{dt}$ can be expressed as follow

$$\frac{dN}{dt} = r\,N$$

Where r is the proportional constant .
Therefore,

$$N(t) = N_0 e^{rt} \text{ ----------------------- } 1$$

According to Fig (2) and Fig(3), we notice that,

$$N(t) = \frac{1}{R_e} = \frac{\eta t}{\rho R L} \text{ --------------------- } 2$$

Where L is length of motion path of bacteria within time t
By differentiating the two sides of eq(2) with respect to time t, we obtain

$$\frac{dN(t)}{dt} = \frac{\eta}{\rho R} \text{ ------------------- } 3$$

From eq(1), we obtain

$$\frac{dN(t)}{dt} = N_0 r e^{rt} \text{ ----------------- } 4$$

By using eq(3) and eq(4), we obtain

$$N(t) = N_0 e^{ln\frac{\eta}{\rho R N(t)} t} \text{ ------------ } 5$$

By using logarithm to the base 10, we can obtain

$$r = \frac{log_{10}x_2 - log_{10}x_1}{t_2 - t_1}$$

Then we can obtain

$$r = \frac{10^{n_2} - 10^{n_1}}{t_2 - t_1}$$

Finally, we obtain

$$N(t) = N_0 e^{ln\frac{10^{\Delta n}}{\Delta t}} \text{ --------- } 6$$

Where x_1 is the number of cells at time t_1 , x_2 is the number of cells at time t_2 , N_0 is the number of bacteria per mL (cell concentration), N(t) is the bacteria concentration at time t, r is the instantaneous growth rate (balance between cell division and cell death, per time unit .Such that η is the viscosity of fluid, , Re is Reynold's number, R is the size of bacteria and ρ is the density of fluid, and Δn is the variance of cells Bacteria motility in water: $v_s \approx 30 \times 10^{-6}\ m/s$, $R \approx 1 \times 10^{-6} m,$ $R_e \approx 1 \times 10^{-5}$, $\eta_{water} \approx 0.001\ Pa.s$, $\rho_{water} = \frac{1000 kg}{m^3}$

3. Results and Discussion

Fifteen water samples were collected from dug-wells cited a long the bank of a seasonal valley (Wadi Bulbul) which represents the main water source of the town . The result of bacterial presumptive test revealed that all the studied water samples have bacterial count MPN above the permissible limits for drinking water. The MPN count of the samples range 17 – $3.5x10^6$/100ml of water as shown in (Table 1)

Sample number 9 has the less MPN count for coliform bacterial test (17/100ml) whereas sample No, 5 has the highest MPN count ($3.5x10^6$/100ml of water).
The presence of *E.faecalis* was confirmed almost in thirteen (86.7%) studied wells. two wells (13.3%) showed negative result for the presence of *E.faecalis* . (Table 2). Sample two showed the less MPN count for *E. faecalis* ($3.9X10^5$) and sample six show the highest MPN count ($160X10^5$). The result displayed in table 1 and 2 revealed that approximately all the studied water sources were contaminated with faecal coliform, which most likely was *Ent feacalis* .

The statistical test (T.test) for the most probable number count of coliform and *E.faecalis* and the comparison between them showed that there were significant differences within ($P \geq 0.05$) as illustrated by Fig (1).

Also, the results in Fig(2), and Fig(3) showed that, the total MPN/ml of the bacteria is inverse to Reynolds's Number (Re), this means high contamination of bacteria is occurred in water for range of Re (0.9×10^{-3} - 0.03×10^{-6}),and for coliform counts. And for ***E. faecalis*** counts, the range of Re is (0.2×10^{-6} – 0.07×10^{-9}).

The contamination of water sources obtained in this research could be attributed to the following facts, the main water sources in the studied area, are surface ground water found in the Wadi aquifer which make it very vulnerable to pollution. Dahiya and Kar,(1999), and Battu *etal*.,(2009), reported that surface water source in general , are not acceptable for drinking purpose as they are often loaded by various organic, inorganic and biological constituents.

The existence of the market place near the water sources, within a distance of less than 30m, which is below WHO recommendation for minimum distance that should exist between human activities and water sources, together with the extensive livestock turnover being held for most of the time in the town and pass the water sources in their daily journey, for the sought of pasture gives a continuous health hazard for the quality of water in the studied area , On top of the above mentioned reasons , the valley which represents the main water source, dries during winter and summer , and its beds become casual latrines both for the residents and the market visitors .

The findings of this research is in agreement with two studies (Nada, 2003) and (Amira, 2011) conducted in Nyala town South Darfur, Sudan for the investigation of drinking water quality. However , to our knowledge the information obtained in this study about faecal contamination of water sources in Tolus town is the first of its kind , and it revealed the hygienic conditions of water sources which consumed by the community. The Beer-Lambert law, as applied spectrophotmetric turbidity studies, correlates the concentration of the organismal growth in a solution that absorb the visible light (Sarah.Spence, 2011)

Table 1: Results of total presumptive Coliform counts in water from dug wells in rainy season.

A	B	C	F	E	G	
I		L	M	P	N	H
R	1	5 5	5 5	3 5	1 5	110,0000
//	2	5 5	2 5	0 5	0 5	49
//	3	5 5	4 5	2 5	0 5	220
//	4	5 5	5 5	3 5	1 5	110,0000
//	5	5 5	5 5	4 5	4 5	350,0000
//	6	4 5	4 5	3 5	1 5	33,00000
//	7	5 5	2 5	2 5	1 5	12,0000
//	8	5 5	5 5	2 5	1 5	70,0000
//	9	5 5	3 5	3 5	0 5	17
//	10	5 5	5 5	4 5	2 5	220,0000
//	11	4 5	3 5	3 5	2 5	20,0000
//	12	5 5	4 5	3 5	3 5	45,0000
//	13	0	0	0	0	0
//	14	5 5	2 5	3 5	0 5	120
//	15	0	0	0	0	0

Where: A: volume of water added to medium., B: serial No., C: 10mLx5x5mL., F: 1mLx5x5mL., E:0.1mLx5x5mL., G: 0.00001mLx5., H: MPN/100mL., I: source of water ezamined., L: No. of coliforms+ve in 10mL double strength medium., M: No. of coliforms+ve in 5mL single strength medium. P: No. of coliforms +ve in 5mL single strength medium. No. of coliforms + ve in 5mL strength medium. R: dug well (open).
MPN: most probable number.

Table 2: Results of total presumptive *E. faecalis* counts in water from dug wells in rainy season.

A	B	C	F	E	G	
I		L	M	P	N	H
R	1	5 5	5 5	4 5	4 5	350,0000
//	2	5 5	4 5	3 5	2 5	39,0000
//	3	5 5	5 5	2 5	3 5	120,0000
//	4	0	0	0	0	0
//	5	5 5	5 5	5 5	3 5	920,0000
//	6	5 5	5 5	5 5	4 5	160,00000
//	7	5 5	5 5	4 5	2 5	220,0000
//	8	5 5	5 5	4 5	3 5	280,0000
//	9	5 5	5 5	5 5	4 5	1600,0000
//	10	5 5	5 5	4 5	4 5	350,0000
//	11	5 5	5 5	3 5	2 5	140,0000
//	12	5 5	5 5	4 5	4 5	540,0000
//	13	5 5	5 5	4 5	4 5	350,0000
//	14	5 5	5 5	5 5	2 5	5400000
//	15	0	0	0	0	0

Where: A: volume of water added to medium., B: serial No., C: 10mLx5x5mL., F: 1mLx5x5mL., E: 0.1mLx5x5mL., G: 0.00001mLx5., H: MPN/100mL., I: source of water examined., L: No. of E.faecalis+ve in 10mL double strength medium., M: No. of E.faecalis +ve in 5mL single strength medium. P: No. of E.faecalis +ve in 5mL single strength medium. No. of E.faecalis + ve in 5mL strength medium. R: dug wells (open)
MPN: most probable number.

Table: 3-a , shows the Independent sample T- test for MPN of Coliform and *E. faecalis* in dug wells.

	A				
	B	C	D	K	J
Q	W	15	8.4E6	1.2E6	3.12E5
	U	15	4.7E6	5.2E6	1.3E6

Key: A: group statistics., B: group., C: sample size., D: mean., K: standard deviation., J: standard error mean., E: power of ten., Q: MPN.

Table: 3b, shows the Independent sample T- test for MPN of Coliform and *E. faecalis* in dug wells.

	A				
	B	C	D	K	J
Q	W	15	8.4E5	1.2E6	3.12E5
	U	15	4.7E6	5.2E6	1.3E6

Key: A: group statistics., B: group., C: sample size., D: mean., K: standard deviation., J: standard error mean., E: power of ten., Q: MPN.

Table: 3c, shows the Independent sample T- test for MPN of Coliform and *E. faecalis* in dug wells.

Independent samples test								
			J		Y			
		F	S	T	L	U	O	
							H	I
Q	W	10.329	0.003	-2.803	28	0.009	-3.8E6	1. 4E6
	Z			-2.803	15.5	0.013	-3.8E6	1.40E6

Key: Y: T- test for equality of mean., J:Levene's test for equality of variances., F: frequency., S: significant., T:T-test., L: degree of freedom., U: sig.2-tailed., O: 95% confidence interval of the difference., H: lower., I: upper., Q: MPN., Z: equal variances not assumed., W: equal variances assumed.

*Standard error difference for W, equal $1.4x10^6$, and standard error difference for Z, equal $1.4x10^6$.

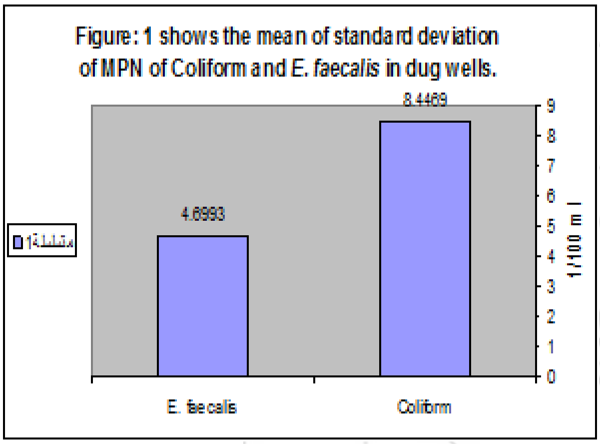

Figure 1: shows the mean of standard deviation of MPN of coliform and *E.faeclis* in dug wells

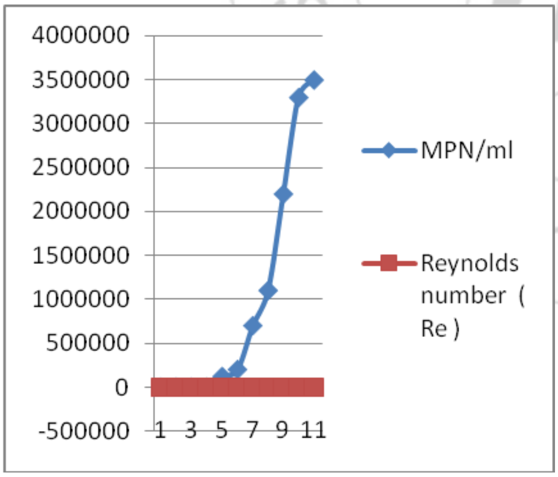

Figure 2: Reynold's number (Re)indicates the E.coliform colonies (MPN/ml) in dug wells in raining seasons.

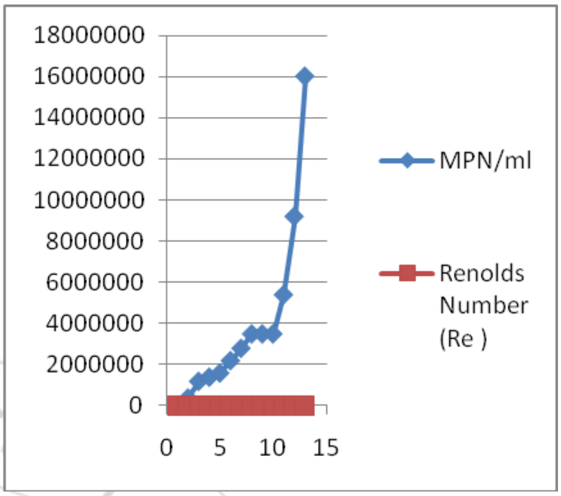

Figure 3: The Reynolds's number (Re) indicates *E. faecalis* (MPN/ml) & in dug wells in raining season.

4. Conclusion

It could be concluded that all the dug wells in Tolus town were contaminated with coliform and *E.faecalis*, and hence with dangerous pathogens. All water samples had total coliform count above the limit of WHO standard for drinking water. As reported by Davies-Colley *et.al.* (2001) that the consumption of drinking water contaminated with pathogenic microbes of faecal origin is a significant risk to human health in the developing world, especially in rural areas . We would like to recommend that a systematic water quality monitoring should be developed in the area to keep the water quality within WHO guidelines. Hands pumps should be installed on open wells to reduce external contamination and to enhance water quality in the study area . Also could be concluded that the nature of slow motion (much less velocity) of the bacteria allow it to accumulate in small area according to the Reynolds's number (Re) which show the type of water flow, so, this indicator for contamination in dugwells of the study area. According to our knowledge its the first time that " Reynolds's number " is used for counting bacterial colonies.

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