Microbiological Perspective On The Quality And Safety Of Borehole Water In Mubi Metropolis, Nigeria.

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Abstract: The present study was carried out due to increasing concern on the quality and safety of drinking water in Mubi metropolis, Nigeria. The results of the proximity of boreholes to sources of contamination showed that none of the boreholes was correctly cited. None of the boreholes was cited near a pit toilet; however, 31 (77.5%) of the borehole water sampled were cited less than 10m to source of contamination, out of which 21(67.7%) and 10 (32.3%) are cited less than 10m to refuse dump site and septic tank respectively. The result of heterotrophic plate count (HPC) showed that all the boreholes have bacterial count that ranges from $1.1x10^3 – 3.9x10^4$ Cfu/ml, while few other boreholes have too numerous colonies to be counted. Both the total and faecal coliform counts were high and ranges form 09-210 cfu/ml and 0-150 cfu/ml respectively. Based on WHO grouping of faecal coliform bacteria into risk categories, 2 of the borehole water sampled conformed to WHO standard. While 11, 23 and 4 of the borehole water sampled were at low risk, intermediate risk and high risk respectively. The total coliform counts in boreholes from kolere was higher than those of sabonlayi, Nassarawo, Barama, and Lokuwa (p<0.05), but not significantly different from boreholes of other wards (p>0.05). Also, the total coliform count was significantly higher than faecal coliform count (p<0.05). *Escherichia coli* were isolated in fifteen (37.5%) different borehole sampled water from Kolere, Sabonlayi, Barama, Yelwa and Lamurde wards. None was isolated in boreholes from Lokuwa, Nassarawo and Wuropatuji. The results also revealed the presence of *Citrobacter* spp., *Enterobacter aerogenes*, *Salmonella* spp., *Proteus vulgaris* and *Klebsiella pneumoniae*. These organisms are of public health significance.
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Introduction

Water is literally the source of life on earth and it is necessary for all life forms. Because human physiology and man continued existence depends very much on water availability, every effort therefore should be made to achieve a good quality of drinking water (WHO, 2006; FAO, 1997).

Globally, unsafe drinking water coupled with poor sanitation kill at least 1.6 million children under the age of five every year, 84% of them living in rural areas (WHO and UNICEF, 2006). Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation conditions affecting a large part of the world's population (WHO, 2004; Hughes and Koplan, 2005). An estimated 1.1 billion persons (one sixth of the world's population) lack access to clean water and 2.6 billion to adequate sanitation (WHO, 2005; Hughes and Koplan, 2005). Water is an integral part of achieving all of the UN Millennium Development Goals (UNESCO, 2006). The Millennium Development Goals (MDG) target for water is to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation. The WHO (2004) estimates that if

these improvements were to be made in sub-Saharan Africa alone, 434,000 child deaths due to diarrhoea would be averted annually. Safe drinking water is defined by World Health Organization as that water having acceptable quality in terms of physical, chemical and bacteriological parameters. Bacteriological parameters, especially *Escherichia coli* (*E.coli*) and total coliform have been used to determine the general quality of drinking water Worldwide (Ashbolt, 2004; Nevondo and Cloete, 1999; JMP. 2008). The *E. coli* in particular has been found to be the most specific indicator of faecal contamination in drinking-water (Plate *et al*., 2004; JMP. 2008). Its presence indicates contamination of water with faecal waste that may contain other harmful or disease causing organisms, including bacteria, viruses, or parasites (Zvidzai *et al*., 2007). WHO recommends that no faecal coliform be present in 100ml of drinking water. The main origins of pollution of boreholes are industrial, domestic and agricultural and can be accidental or continous. Domestic pollution may involve seepage from broken septic tanks, pit latrines, ceespools and privies. WHO recommends that boreholes should be located at least 30m away from

latrines and 17m from septic tank (Chukwurah, 2001).

For almost two decades, there is either sporadic or no supply of pipe-borne water in Mubi. Hence, people in Mubi largely depend on borehole water for drinking and other domestic purposes. Borehole water in Mubi is mostly vended in 25 litre jericans pushed around by vendors in hand-push truck which may contain 5-10 jericans. These boreholes are designed and located without proper site investigation to determine their proximity to sources of pollution (such as pit toilet, septic tank or soak away, and waste disposal sites). The level of ignorance and illiteracy among the inhabitant contributes to citing of these boreholes and consequently, problems of portable water supply.

MATERIALS AND METHODS
Study Area
Mubi metropolis is vast and large comprising of two Local Government Areas (Mubi north and Mubi south). Mubi is situated in the North-Eastern part of Nigeria, in Adamawa state between latitude $9^o26'$ and $10^o10'$N and between longitude $73^o1'$ and $13^o44'$E. The town is situated near the border between Nigeria and Cameroun Republic and has a land mass of $506.4km^2$ with a population size of 759,045 at a density of 160.5 persons per square kilometre. The area has a tropical wet and dry climate. Dry season last for minimum of five (5) months (November – March), while the wet season spans between April and October. The mean annual rainfall ranges from 700mm to 1050mm. Some of the major ethnic groups in Mubi include Fali, Gude, and Fulani. Other ethnic groups include Kilba, Higgi, Hausa, kanuri, Nzanyi, Mudang and Zilwo (Adebayo, 2004)

Borehole Proximity to Sources of Contamination
The environments where the boreholes are cited were surveyed to examine their sanitary conditions. Factors considered were proximity to pit toilets, proximity to septic tanks, proximity to refuse dump site, proximity to abattoir and industrial discharge/effluent.

Sample Collection
A total of forty (40) boreholes water samples, 5 from each ward in mubi metropolis were collected and analysed. The wards are Lamurde, Sabon Layi, Lokwa, Maiha road, Yelwa, Kolore, Wuropatuji and Nassarawo. Prior to the collection of water samples from the boreholes in these wards, the nozzle of the taps were flamed and sterilized by cleaning with methylated spirit to avoid contamination and the tap was allowed to run for a minute for the initial water to rush out before final collection for analysis. The sample bottles were carefully closed to avoid contamination from fingers. Samples were transported to the laboratory in ice-packed containers and were analysed within 2-3 hours of collection.

BACTERIOLOGICAL QUALITY ASSESSMENT
Heterotrophic Plate Count
Total heterotrophic plate count was obtained using the pour plate method. Dilutions of 10^{-1} to 10^{-6} of the samples were prepared in 0.1% buffered peptone water (oxoid) and 1ml aliquots of each dilution was inoculated into 10ml each of molten Nutrient Agar in universal bottles. These were then thoroughly mixed poured into sterile Petri-dishes and incubated at 37ºC for 24 h. Petri-dishes from dilutions containing between 30 and 300 discrete colonies were counted and the result expressed as the numbers of colony per millilitre (APHA, 2005).

Determination of Total and Faecal Coliform Count
The multiple tube fermentation technique for enumeration of total coliform and *E. coli* as recommended by APHA, (2005) was used to assess the bacteriological quality and safety of borehole water. MacConkey broth was for presumptive test and the tubes incubated at 37^oC and 44.5^oC for 24h for the estimation of total coliforms and faecal coliforms respectively. Eosin methylene blue (EMB) agar was used for the confirmatory test. Organisms with green metallic sheen with dark centres were taken as positive for *E. coli*.

Identification of Bacterial Isolates
Stock cultures of bacterial isolates with different morphological characteristics stored on Nutrient agar slants were identified based on standard method (Washinton *et al*., 2006). The identification of the bacterial isolates were carried out using the following biochemical test, preliminary Gram staining, Motility, use of Kligler iron agar (for sugar fermentation, gas production and hydrogen sulphide production), Voges-proskauer test, Methyl-red test.

Statistical Analysis
Results from the different boreholes were subjected to student T-test to test if there is any significant difference between total and faecal coliform counts. We considered p-value less than 0.05 as statistically significant. All statistical analyses were carried out using SPSS 17.0 window package.

Results
Table 1 shows the proximity of boreholes in all the wards to sources of contamination. The results showed that none of the boreholes was correctly cited. Of the 40 boreholes, 15(37.5%) and 25(62.5%) were cited closely to septic tank and refuse dump site respectively. Although none of the boreholes was cited

near a pit toilet, however, the result showed that 31 (77.5%) of the borehole water sampled were cited less than 10m to source of contamination. Twenty one (67.7%) and 10 (32.3%) of these are cited less than 10m to refuse dump site and septic tank respectively.

The result of heterotrophic plate count (HPC) is shown in Table 2. All the boreholes have bacterial count. The HPC for all the boreholes ranges from $1.1 \times 10^3 – 3.9 \times 10^4$ Cfu/ml. Few other boreholes have too numerous colonies to be counted (TNTC).

Table 3 and 4 showed the results of the total coliform count and faecal coliform count for all the borehole water. The result of the total coliform count ranges from 09-210 CFU/100ml, while that of the faecal coliform count ranges from 0-150 FC/100ml. Based on WHO (1997) grouping of faecal coliform bacteria into risk categories, the results further showed that 2 of the borehole water sampled conformed to WHO standard. While 11, 23 and 4 of the borehole water sample were at low risk, intermediate risk and high risk respectively (Table 5). The total coliform counts in boreholes from kolere was higher than those of sabonlayi, Nassarawo, Barama, and Lokuwa (p<0.05), but not significantly different from boreholes of other wards (p>0.05). Also, the total coliform count was significantly higher than faecal coliform count (p<0.05).

The results of the confirmatory test on EMB agar revealed colonies with green metallic sheen on the borehole water samples from Kolere (3 and 5), Sabonlayi (1-5), Barama (2-5), Lokuwa (2 and 4) and Yelwa (1and 2). The rest of the borehole samples were negative for *E. coli*.

The results of the biochemical test revealed the presence of *Citrobacter* spp., *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella* spp., *Proteus vulgaris* and *Klebsiella pneumoniae*. Their percentage occurrence were as shown in table 6

Discussion

Our study showed that apart from pit toilet and septic tank, refuse heaps and dumps situated near water source could also serve as source of contamination of ground water especially borehole.

The number of heterotrophic plate count bacteria in drinking water varies widely. It depends on the quality of the source water, the types and efficacy of treatment, the type and condition of disinfection residuals, the concentration of the dissolved organics in the treated water, the ambient temperature of the raw and finished water and of course, the HPC method and the temperature of incubation (Allen *et al.*, 2004).

In this study, the heterotrophic plate count bacteria of the borehole samples ranged from 1.1×10^3-3.9×10^4 cfu/ml with none of the borehole samples having count within the limit of 100 cfu/ml

allowed for portable water (NSDWQ, 2007). DeZuane, (1990) suggested that water with counts under 100cfu/ml should be considered "portable" and values 100-500cfu/ml "questionable". Contrary to this however, in the international section of the USP 23, which deals with action guidelines for the microbial control of ingredient water, says "a total microbial (aerobic) count that may be used for source drinking water is 500cfu/ml (USEPA, 1996). Even with this, the heterotrophic plate count bacteria of the borehole water in this study still exceed 500cfu/ml as stipulated by USEPA. USEPA, (2001) revealed that concentration above 500cfu/ml; heterotrophic bacteria can interfere with some total coliform and *E. coli* recovery methods. In accordance with this study, Erah *et al.*, (2002) found unacceptable levels of aerobic bacteria present in borehole water of Teboga district of Benin City. Higher HPC was also reported by Adetunde and Glover, (2010) in their study on Bacteriological quality of borehole water used by students of University for Development Studies, Navrongo Campus in Upper-East Region of Ghana. Contrary to the findings of this study however, Eniola *et al.*, (2007) reported a range of $5.0 \times 10^2 – 7.0 \times 10^2$cfu/ml for stored borehole water samples.

Although there is no epidemiological evidence that higher HPC populations have any public health significance, some have argued that lower HPC bacterial populations in drinking water are more desirable than higher populations.

Almost all the samples from the boreholes were contaminated with both non-faecal and faecal coliform bacteria. The samples with low bacterial count and total coliform counts could be considered to be of better quality for domestic use than those with the highest counts of both bacterial and total coliform. Water samples from borehole Lokuwa2 and Yelwa3 are fit for drinking and other domestic purposes because they had faecal coliform counts of zero MPN/100ml which conformed to the set standard of WHO (1993) which says no water sample should contain faecal coliforms in any 100ml of water sample. Out of the 40 borehole water samples taken, 95% were found to have faecal coliform ranging from 4FC/100ml to 150FC/100ml. Similar findings was reported by Muruka *et al.* (2012) which revealed that 98% of their water samples were found to have faecal coliform ranging from 3FC/100ml to 1100FC/100ml. Similarly, Kimani and Ngindu (2007) revealed in their study that 3% of samples had no faecal contamination where as 97% had. The level of contamination of most of the samples with both non-faecal and faecal bacteria counts may be due to the locations of the boreholes and environmental factors. The presence of coliform in drinking water is a pointer for assuming that a potential health hazard existed because of the possible presence of pathogens.

In our study, *E. coli* was isolated in 35% of

borehole water samples. The untidy nature of our physical environment along with close proximity of some wells/boreholes to toilets, rubbish dump, may be responsible for the presence of somewhat high density of *E. coli* in the drinking water sources. Also, the presence of *E. coli* in water suggests enteric pathogens and faecal pollution (Ivey *et al*., 2006), and has been reported to be the causative agent of diarrhoea, urinary tract infection, haemorrhagic colitis and haemolytic uraemia syndrome (Esrey *et al*., 1985). The findings of this study are closely similar to earlier reports by Okorafor *et al*., (2012) in their studies on physico-chemical and bacteriological characteristics of selected streams and boreholes in Akamkpa and Calabar Municipal, and that of Gwimbi, (2011) in Maseru district Lesotho. The presence of *Citrobacter* spp., *Enterobacter aerogenes*, *Salmonella* spp., *Proteus vulgaris* and *Klebsiella pneumoniae* in varying proportion in some of the borehole water samples are worrisome and unacceptable from the public health point of view. This is because these organisms could be pathogenic and consumption of water containing these organisms could pose a serious public health risk.

Conclusion

The present study was carried out due to increasing concern on the quality and safety of drinking water in Mubi metropolis, Nigeria. The results showed that pipe-borne water supply is beyond reached and alternative sources of drinking water like boreholes are grossly exposed and contaminated with enteric pathogens due to poor town planning, overcrowding, unhygienic environment or poor sanitation. Thus, consuming unsafe drinking water may lead to several water borne diseases and other chronic health related problems. Therefore, provision of safe and quality water to individuals and communities is paramount. On this note, a proper sanitary survey and implementation of water and sanitation projects in this community is highly recommended.

Table 1: **Proximity of Boreholes to Pit toilet, Septic tank and Refuse dump Site**

Distance (M)	No. of wells	Pit toilet	Septic tank	Refuse dump site	% of boreholes
< 10	31	-	10	21	75.5
10-19.9	9	-	5	4	22.5
20-29.9	-	-	-	-	0
≥ 30	-	-	-	-	0
Total	40	-	15	25	100

Table 2: Heterotrophic plate count (HPC) of borehole water

Heterotrophic plate count (cfu/ml)					
Wards	1	2	3	4	5
Kolere	3.0×10^4	TNTC	TNTC	2.5x104	3.0x104
Sabonlayi	3.0x103	2.0x103	1.0x104	1.5x104	4.0x103
Wuropatuji	2.2x104	3.5x103	TNTC	TNTC	TNTC
Nassarawo	TNTC	2.0x104	1.5x104	1.2x104	1.5x104
Barama	2.1x104	3.0x104	2.5x104	3.5x104	3.9x104
Lokuwa	2.3x104	20.x104	1.0x104	6.0x103	1.1x104
Lamurde	TNTC	3.5x103	2.0x103	TNTC	1.5x104
Yelwa	3.0x103	1.1x103	TNTC	1.2x104	3.2x103

KEY: TNTC: Too numerous to be counted

Table 3: **Total coliform count (CFU/100ml)**

LOCATION	BOREHOLE WATER SAMPLES				
	1	2	3	4	5
Kolere	210	210	210	210	210
Sabonlayi	09	20	20	28	150
Wuro patuji	150	15	120	210	210
Nassarawo	210	35	28	35	35
Barama	28	210	27	27	35
Lokuwa	20	28	35	27	20
Lamurde	210	28	210	150	75
Yelwa	210	75	210	210	210

Table 4: **Faecal coliform count (FC/100ml)**

LOCATION	BOREHOLE WATER SAMPLES				
	1	2	3	4	5
Kolere	75	23	75	23	43
Sabonlayi	05	10	10	14	35
Wuro patuji	45	10	75	150	10
Nassarawo	120	11	26	30	30
Barama	15	11	11	07	15
Lokuwa	09	0	04	07	10
Lamurde	27	20	20	75	10
Yelwa	150	35	0	28	150

Table 5: **Risk Category for the presence of faecal coliform**

Categories (cfu/100ml)	No. of Boreholes	Risk category
0	02	Conformity
1-10	11	Low risk
10-100	23	Intermediate risk
100-1000	04	High risk
41000	-	Very high risk
TOTAL	40	

Table 6: **Percentage (%) occurrence of organisms isolated from all the Boreholes**

Organisms	% occurrence
Escherichia coli	37.5
Citrobacter sp.	2.5
Enterobacter aerogenes	12.5
Salmonella sp.	2.5
Proteus vulgaris	27.5
Klebsiella pneumoniae	17.5

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