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Bacteriological Status of Untreated Drinking Water Sources in Baen Town, Khana Local Government Area, Rivers State

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Abstract: Good quality water is a challenge in most towns and cities in Nigeria and households have for years depended on other sources of water to supplement their activities. The introduction of borehole waters to consumers was to provide safe, hygienic and affordable drinking water to the public. Although this is a valiant idea, current trends seem to suggest that site or location of bore-hole drinking water sources could be a route of transmission of diseases. This current paper investigated the bacteriological status of untreated drinking water sources in Baen town, Khana Local Government Area, Rivers State. Six water samples from the six villages (Wiiga, Gaken, Luuzue, Gui, Gba and Nyorwii) that make up the town were analyzed using Standard microbiological methods. The total viable count ranged from $1.15x10^5$ to $1.9x10^6$cfu/ml while the total coliform count ranged from $1.85x10^4$ to $5.9x10^4$cfu/ml. Salmonella species was detected in three sample sourced from Wiiga, Luuzue and Gba with counts of $3.8x10^2$, $4.8x10^2$ and $3.1x10^2$cfu/ml respectively while staphylococcus count ranged from $2.3x10^2$ to $4.6x10^2$cfu/ml in all samples studied. The most prevalent species isolated include *Micrococcus* species, *Bacillus* and *Staphylococcus* species (22.7%) followed by *Salmonella* sp. (13.63%); *Serratia* and *Enteroccoccus* species (9.09%). Treatment of water before drinking and strict regulations of borehole drillers to ensure that requirements for siting borehole facilities are met before drilling is encouraged as most rural dwellers have inadequate information and are only concerned with getting water for their day to day activities.
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1.0 Introduction

Water is very important for life to be sustained. Accessibility to potable water supply has been a top priority for most developing societies in Sub-Saharan Africa like Nigeria. It must be available to all if a healthy living must be attained in our communities and hence the need for reasonable effort to be made to make drinking water as safe as possible (W.H.O, 2008). Over a billon people globally cannot acsess potatble water which has resulted to more than 1.5 million children dying each year from diarrheal related diseases (Fenwick 2006; João, 2010) as a result of intake of contaminated water. W.H.O recorded that mortality resulting from intake of contaminated water exceeds 5 million people per year of which over 50% results from enteric infections, with cholera majorly implicated (João, 2010). All forms of pathogenic microbes including bacteria, viruses, fungi, protozoans and cyanobacteria are capable of causing deviations from the normal state of human health due to ingestion of contaminated water. While the pathogenic bacteria, viruses and protozoa reproduce within the body and cause disease, some Cyanobacteria and fungi produce poisonous substances (toxins) when they contaminate water sources. When more than tolerable volumes of these secretions are ingested through contaminated water, they become toxic to the body and result in adverse health challenges.

Generally, the greatest microbial risks are related with intake of water that is contaminated with human or animal feces (George *et al.,* 2001). Acute diarrheal diseases resulting from ingesting microbe contaminated water of major public health concern even in developing societies. Most People who report to hospitals for diarrheal diseases are low income earners with reduced hygienic orientations. Children under five, primarily in Asian and African countries, are the most affected by microbial diseases transmitted through water (Seas *et al.,* 2000; João, 2010).

Developed nations are also affected by microbial waterborne diseases. The United states for instance has an estimated 560,000 persons experiencing severe microbe related waterborne diseases, as about 7.1 million suffering from a mild to moderate infections could resulted in estimated 12,000 deaths a year (Medema *et al.,* 2003).

In Baen town, Borehole water has gradually replaced the ancient ways of going to fetch water from the stream for drinking. Only persons in farms go to streams to get water at noon to quench their taste while farming. The dependence on borehole water supply by over 90 percent of households in Baen (Barine and Ogbu, 2019) has has necessitated the need to investigate their microbiological quality and their fitness for consumption. Most of these borehole do not receive any form of treatment before being taken. Also, most borehole water supplies are not dug to standards and indigenes may be drinking underground water that are not fit for human consumption as there is a general belief among indegens that borehole water is the most suitable source of water to dring aside rain water that has been greatly affected by industrial activities.

2.0 Materials and Methods

2.1 Study Area/Sampling sites

Baen is a town in khana Local Government Area of Rivers State. The people of Baen are predominantly farmers and depend on borehole water supplies to meet their daily needs. The town is made up of Six autonomous villages namely Wiiga, Gaken, Luuzue, Gui, Gba and Nyorwii.

2.2 Sample Collection

Six (6) water samples from different borehole supplies sourced fron the different villages were aseptically collected. To obtain the samples, the nozzle of each of the taps were sterilized with cotton wool soaked in ethanol and the taps were left to run for two minutes to avoid water left on the pipe from being used as samples. Samples were collected using sterile containers from each sampling points. The samples were transported to the laboratory for analysis in coolers containing ice cubes.

2.3 Microbiological analysis of Samples

2.3.1 MPN Analysis

MacConkey broth was prepared according to manufacturer's instruction and dispensed into five tubes each containing 10ml,1 ml and 0.1ml. Durham's tubes were inverted onto the medium and tubes were autoclaved. After autoclaving and allowed to cool, 10ml of the sample was inoculated into each of the double strength tubes while 1ml and 0.1ml were inoculated into the single strength tubes. Tubes for total coliform were incubated at 37°C while those for feacal coliform were incubated at 44.5°C. Gas production in some of the tubes was presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample was be estimated by the number of positive tubes. One loop full of broth from tubes positive for presumptive tests were streaked on EMB agar to confirm the presence of fecal contaminants.

2.3.2 Enumeration of Microorganisms

2.3.2.1 Dilution of Samples

Ten-fold serial dilution was performed on the samples. 10ml of the water Sample was suspended in 90ml sterile normal saline (0.85% w/v, Nacl) to make a stock solution. 1ml of the aliquot was pipetted into a test-tube containing 9ml sterile normal saline to make 10^{-2}, 10^{-3},10^{-4},1 and 10^{-5}

2.3.2.2 Determination of Total Viable Count.

0.1ml of each of the dilution were inoculated into nutrient agar plates by the spread plate technique. The inocula was spread evenly using a sterile glass rod. Incubation of the petri dishes was carried out in an inverted position at 37^0C for 24 hours. The number of colonies were enumerated after incubation of the plates that yielded between 30 to 300 colonies. The inoculation was done in duplicates so as to minimize error and the average was taken afterwards.

2.3.2.3 Determination of Total Coliform Count

MacConkey agar was prepared according to manufacturer's instruction. 0.1ml of diluted sample was inoculated onto the solidified agar and spread using a sterilized hockey stick amd incubated inverted for 24hrs at 37oC. Pink colonies were enumerated.

2.3.2.4 Salmonella Count

Salmonella-Shegella agar was prepared according to manufacturer's instruction. 0.1ml of diluted sample was inoculated onto the solidified agar and spread using a sterilized hockey stick and incubated inverted for 24hrs at 37oC. Black colonies were sub-cultured counted.

2.3.2.4 Staphylococcus Count

Mannitol salt agar was used for the isolation of *Staphylococcus* sp. 111 g of the agar was suspended into 1000 ml of distilled water, swirled and boiled by heating to dissolve completely. After which it was sterilized by autoclaved at 121°C for 15 min and at 15 psi. 0.1ml of diluted sample was inoculated onto the solidified agar and spread using a sterilized hockey stick and incubated inverted for

24hrs at 37oC. Yellow colonies characteristic of *Staphylococcus* species were counted.

2.4 Characterization and Identification of Bacterial Isolates

Characterization and identification of bacterial isolates were based on cell morphology on nutrient agar plates and included shape, size, opacity, colour, edge, elevation. Also Gram stain and biochemical tests including catalase, oxidase, methyl red, sugar fermentation were carried out according to standard microbiological methods as described by Vashist *et al.,* (2013).

3.0 RESULT

Table 3.1: Total Coliform Count Using Most Probable Number (MPN) Based On The Presumptive Test Tubes.

Sample/ml	10mls	1.0ml	0.1ml	MPN/100ml
Wiiga	0	0	0	<2
Gaken	2	1	0	9
Luuzue	0	0	0	<2
Gui	0	1	0	2
Gba	0	0	0	<2
Nyowii	2	1	1	9

Table 3.2: Total Viable Bacteria Count of Borehole water within Baen

Sample source	Dilution	Mean Count	Cfu/ml	Log (Cfu/ml)
Wiiga	10^{-3}	43	$4.3x10^{5}$	5.633
Gaken	10^{-4}	109	$1.09x10^{6}$	6.037
Luuzue	10^{-3}	39	$3.9x10^{5}$	5.591
Gui	10^{-2}	73	$7.3x10^{5}$	5.863
Gba	10^{-3}	190	$1.9x10^{6}$	6.278
Nyowii	10^{-2}	115	$1.15x10^{5}$	5.060

Table 3.3: Total Coliform Count obtained from Borehole water within Baen

Sample source	Dilution	Mean Count	Cfu/ml	Log (Cfu/ml)
Wiiga	10^{-2}	35	$3.5x10^{4}$	4.544
Gaken	10^{-3}	59	$5.9x10^{5}$	5.770
Luuzue	10^{-2}	60	$6.0x10^{4}$	4.778
Gui	10^{-1}	185	$1.85x10^{4}$	4.267
Gba	10^{-1}	190	$1.90x10^{4}$	4.278
Nyowii	10^{-2}	81	$8.1x10^{4}$	4.908

Table 3.4: Salmonella Count obtained from Borehole water within Baen

Sample code	Dilution	Mean Count	Cfu/ml	Log (Cfu/m)
Wiiga	10^0	38	$3.8x10^2$	2.579
Gaken	10^0	-	-	-
Luuzue	10^0	48	$4.8x10^2$	2.681
Gui	10^0	-	-	-
Gba	10^0	31	$3.1x10^2$	2.491
Nyowii	10^0	33	-	-

Table 3.5: Staphylococcus Count obtained from Borehole water within Baen

Sample code	Dilution	Mean Count	Cfu/ml	Log (Cfu/m)
Wiiga	10^0	30	$3.0x10^2$	$3.0x10^2$
Gaken	10^0	46	$4.6x10^2$	2.662
Luuzue	10^0	31.5	$3.15x10^2$	2.498
Gui	10^0	23	$2.3x10^2$	2.361
Gba	10^0	27.5	$2.75x10^2$	2.439
Nyowii	10^0	45	$4.5x10^2$	2.653

Table 3.6: Morphological Characteristic of Isolates from Borehole water within Baen

Sample I.D	Colour	Size	Shape	Edge	Elevation	Surface	Texture	Opacity
Wiiga 1	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Wiiga 2	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Wiiga 3	Cream	3mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Wiiga 4	Cream	2mm	Round	Entire	Raides	Dull	Smooth	opaque
Gaken 1	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gaken 2	Cream	3mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gaken 3	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Luuzue 1	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Luuzue 2	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Luuzue 3	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Luuzue 4	Cream	2mm	Round	Entire	Raides	Dull	Smooth	opaque
Luuzue 5	Red	2mm	Irregular	Irregular	convex	Dull	Smooth	opaque
Gui 1	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gui 2	Cream	3mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gui 3	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gui 4	Cream	3mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gba 1	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gba 2	Cream	3mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Gba 3	Red	2mm	Irregular	Irregular	Flat	Dull	Smooth	Transparent
Gba 4	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Nyorwii 1	Cream	2mm	Round	Entire	Raised	Shiny	Smooth	Opaque
Nyowii 2	Cream	4mm	Round	Entire	Raised	Shiny	Smooth	Opaque

Table 3.7: Grams Reaction and biochemical characteristics of bacterial isolates from Borehole water within Baen

Isolate	Grams Reaction	Catalase	Oxidas	Indol	MR	VP	Citrate	Glu	Lac	Suc	Slant	Butt	Gas	H_2S	Motility	Most Probable Organism
Wiiga 1	+ Cocci	+	+	-	-	-	-	+	-	-	B	A	-		+	*Micrococcus* sp.
Wiiga 2	+ Rod	+	+	-	-	+	+	+	-		B	A	-	-	+	*Bacillus* sp.
Wiiga 3	+ Cocci	+	-	-	-	+	+	+	+		A	A	-	-	-	*Staphylococcus* sp.
Wiiga 4	- Rod	+	-	-	+	-	-	+	-	-	A	A	-	+	+	*Salmonella* sp.
Gaken 1	+ Rod	+	+	-	-	-	-	+	-	-	B	A	-	-	+	*Micrococcus* sp.
Gaken 2	+ Rod	+	+	-	-	+	+	+	-		B	A	-	-	+	*Bacillus* sp.
Gaken 3	+ Cocci	+	-	-	-	+	+	+	+		A	A	-	-	-	*Staphylococcus* sp.
Luuzue 1	+ Cocci	+	+	-	-	-	-	+	-	-	B	A	-		+	*Micrococcus* sp.
Luuzue 2	+ Rod	+	+	-	-	-	-	+	-	-	B	A	-	-	+	*Micrococcus* sp.
Luuzue 3	- Rod	+	-	-	+	-	-	+	-	-	A	A	-	+	+	*Salmonella* sp.
Luuzue 4	+ Cocci	+	-	-	-	+	+	+	+		A	A	-	-	-	*Staphylococcus* sp.
Luuzue 5	- Rod	+	-	-	-	+	+	+	-	+	B	A	-	-	+	*Serrattia* sp.
Gui 1	+ Rod	+	+	-	-	+	+	+	-		B	A	-	-	+	*Bacillus* sp.
Gui 2	+ Cocci	+	-	-	-	+	+	+	+		A	A	-	-	-	*Staphycoccus* sp.
Gui 3	+ Rod	+	+	-	-	+	+	+	-		B	A	-	-	+	Bacillus sp.
Gui 4	+ Cocci	-	-	-	-	+	-	A/G	A/G	A/G	A	A	-	-	-	*Enterococus* sp.
Gba 1	+ Cocci	+	-	-	-	+	+	+	+		A	A	-	-	-	*Staphycoccus* sp.
Gba 2	+ Cocci	-	-	-	-	+	-	A/G	A/G	A/G	A	A	-	-	-	*Enterococus* sp.
Gba 3	- Rod	+	-	-	-	+	+	+	-	+	B	A	-	-	+	*Serratia* sp.
Gba 4	- Rod	+	-	-	+	-	-	+	-	-	A	A	-	+	+	*Salmonella* sp.
Nyorwii 1	+ Rod	+	+	-	-	+	+	+	-		B	A	-	-	+	*Bacillus* sp.
Nyowii 2	+ Rod	+	+	-	-	-	-	+	-	-	B	A	-	-	+	*Micrococcus* sp.

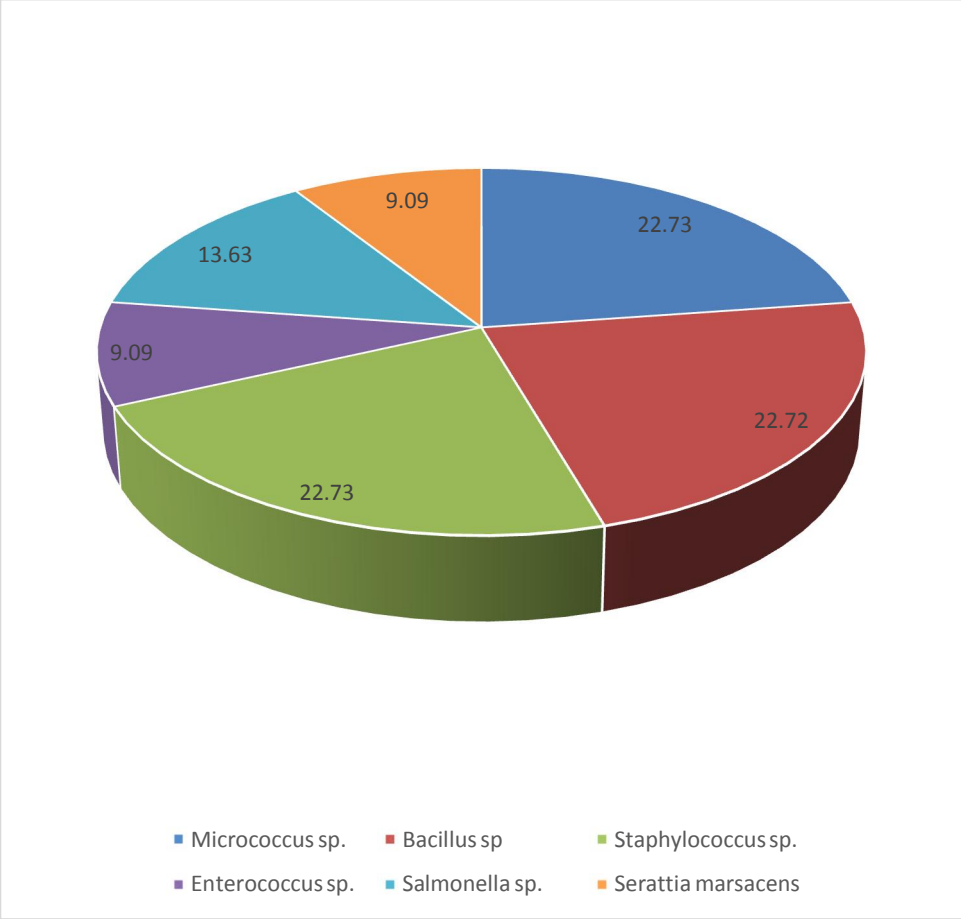

Fig 1: Prevalence of Bacteria Isolated from Borehole water sources in Baen

4.0 Discussions

Good quality water is a challenge in most towns and cities in Nigeria and households have for years depended on other sources of water to supplement their activities. The introduction of borehole waters to consumers was to provide safe, hygienic and affordable drinking water to the public. Although this is a valiant idea, current trends seem to suggest that site or location of bore-hole drinking water sources could be a route of transmission of diseases.

Potable water is meant for human consumption as they are used for drinking and cooking, hence should be free of pathogens and other objectionable materials including colour, odour and taste. The six communities that make up Baen town over the years have depended totally on borehole as source of water supply for drinking and domestic purposes. This study investigated the bacterial quality of the most used of these borehole supplies in the representative communities. Six (6) genera of microorganisms were identified from a total of 6 samples according to Bergey's Manual of determinative bacteriology, (1993). These genera include *Bacillus* spp. 5(22.73%), *Salmonella* spp. 3(13.63%), *Enterococcus* spp. 2(9.09%), *Staphylococcus* spp. 5(22.73%), *Micrococcus* spp. (22.73%) and *Serratia* spp. 2(9.09%) ,respectively. This result indicates that the most prevalent organisms found in Borehole used for both domestic purposes and drinking in Baem town are Bacillus, Micrococcus and Staphylococcus. This is in contrast with the work of Foka *et al.,* (2018) who reported that *Pseudomonas* and *E. coli* were more prevalent in Borehole water sourced from four crowded areas of Benin City. The total coliform analysis usin MPN method as described by Chessbrough (2002) showed that samples from Gaken and Nyorwii had the

highest number of probable coliforms per 100mls of water analyzed (9MPN/100mls, while Luzzue, Wiiga and Gba, all had less than 2MPN/100mls of water studied. However, Gui sample had just pone positive tube indicating a the possibility of 2MPN/100mls total coliform present. These are not in agreement with similar study done by Onuora *et al.,* (2019) who reported 10 colonies/100ml of borehole water sourced from Ogbaru community. The total viable count however, showed that Gaken sample had a

Also, Staphylococcus count in all samples studied ranged from $2.3x10^2$ to $4.6x10^2$ cfu/ml. However, Salmonella was found in only three samples from Wiiga, Luuzue and Gba with counts of $3.8x10^2$, $4.8x10^2$ and $3.1x10^2$ cfu/ml respectively. This may be linked to the fact that these water sources are close to places where open defecation and refuse dumping has been practiced in the past. It could also be attributed to the fact that the borehole may be sited less than 50m downstream of the closest septic reservoir and environmental factors whereby some domestic animals visit the site to drinking water. When drinking, they lick the mouth of the borehole tapss and defecate around the borehole. These activities could enhance bacterial spore to contaminate the water through the opening side of the borehole. The results from these study revealed high bacteria count in water which indicates high coliform count, World Health Organization standard for faecal coliform in drinking water is zero faecal coliform per 100ml (WHO, 2011). Therefore water for human consumption should be of good quality. Most of the samples were contaminated with both non-faecal and faecal coliform bacteria. The samples with low bacterial counts and total coliform counts could be considered to be of better quality for domestic use than the ones with the highest counts of both bacterial counts and total coliform counts. However. No bacteria pathogen should be present in water (Public Health association,2005)

5.0 Conclusion and Recommendations

Portable drinking water for everyone is one of the major challenges faced by developing nations in Africa and Asia. Drinking water sources are most times exposed to feacal contamination and adequate priority should be channeled to curtail the incidence animal and human excretions. Poor treatment of drinking water could lead to water borene dieases and so routine microbiological analysis of drinking water should be carried out by assaying the presence of these pathogens by culture methods. The Government affected nations should devote part of her financial resources to reaserch and implement recommendations emanating from them in other to reduce morbidity and mortality resulting from human

THBC of $5.1x10^5$cfu/ml representing the highest count recorded. Samples from Wiiga, Luuzue, Gab and Nyorwii had viable bacterial counts of $3.5x20^4$ cfu/ml, $6.0x10^4$ cfu/ml, $1.8x10^4$ cfu/ml, $1.9x10^4$ cfu/ml, and $8.1x10^4$cfu/ml respectively.

Coliform counts on MacConkey agar in this study revealed counts ranging from $1.85x10^4$ to $5.9x10^5$cfu/ml (Table 3.3) in all samples studied This is in agreement with Adesiyun *et al*,(1999) who reported similar result. and animal fecal contamination of drinking water sources.

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