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Full Length Research Paper

Enteric pathogens and diarrhea disease potentials of water sources in Ahiazu Mbaise, Eastern Nigeria

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This research was targeted at determining the load of enteric pathogens and possible diarrheal disease potentials of the water sources to prevent possible disease outbreak through improved portable water supply for the inhabitants. Water samples were collected from boreholes, underground tanks, and streams and subjected to standard microbiological analysis. The result of total heterotrophic bacterial count (THBC) and total coliform bacterial count (TCBC) (cfu/ml) ranged between 2.0×10^5 to 1.2×10^2 and 4.8×10^3 to 2.0×10^1 cfu/ml respectively. The isolates occurred thus: *Escherichia coli* (50.0%), *Salmonella* spp. (100%), *Shigella* spp. (100%), *Streptococcus* spp. (50.0%), *Vibro* spp. (20.0%), *Proteus* spp. (30.0%), *Klebsiella* spp. (80.0%), and *Enterobacter* spp. (50.0%). This showed that *Salmonella* and *Shigella* spp. occurred highest (100%) in water samples followed by *Klebsiella* (80.0%); while the lowest occurrence was recorded by *Vibro* spp. (20.0%). The occurrence of total isolates in water samples showed thus: samples A (50.0%), B (37.5%), C (37.5), D (50.0%), E (25.0), F (75.0%), G (50.0%), H (87.5%), I (100%), and J (87.5%). These results show that stream water sources had more enteric pathogens followed by underground tank sources and borehole water sources being the least contaminated. Water sources in Ahiazu Mbaise are not free from enteric pathogens and might expose users to diarrhea.

Key words: Enteric pathogens, water sources, Ahiazu Mbaise, diarrhea, morbidity, mortality.

INTRODUCTION

A study in 1990 estimated that more than one billion people in developing countries lacked access to safe drinking water (WHO, 2000). A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common. The lack of water supply and sanitation, that is, water that is easily accessible, adequate in quantity, free from contamination, safe and readily available throughout the year, is the primary reason why diarrheal diseases are so common in developing countries (Aderigbe et al., 2008; Park, 2002). Two and a half billion people have no access to improved sanitation, and more than 1.5 million children die each year from diarrhea diseases (Fenwick, 2006). Diarrhea accounts for 10% disease burden in developing countries (Park, 2002). According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more than 50% are microbial intestinal infections (Cabral, 2010). Coliform bacteria are used as microbiologic indicators for water quality. Freedom from contamination with fecal matter is the most important parameter of water quality because human fecal matter is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens that are agents of diarrhea (Scott et al., 2008). Ahiazu Mbaise, Imo State, Nigeria, is made up of about 500,000 people and the major sources of water supply include underground water tanks, streams, and boreholes, which, if contaminated, might serve as a major source of enteric pathogens responsible for diarrhea disease. Therefore, this research was targeted at determining the load of enteric pathogens and possible diarrheal disease potentials of the water sources to prevent possible disease outbreak through improved portable water supply

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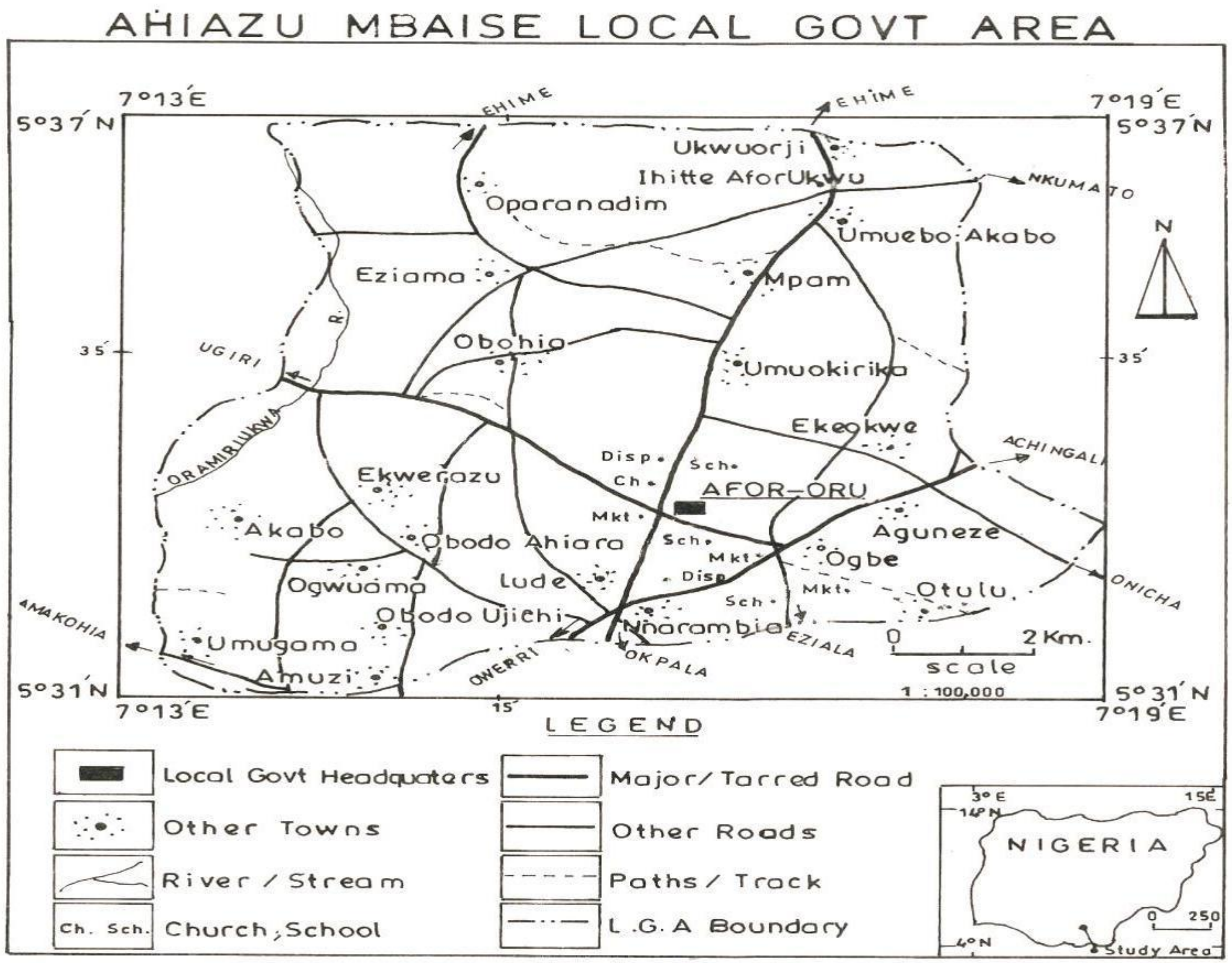

Figure 1. Map of the study area.

for the inhabitants.

MATERIALS AND METHODS

Study area

The study area is as shown in Figure 1. Ahiazu Mbaise is a local Government in Imo State of Nigeria and is made up of sixteen (16) communities which include Amuzi, Eziama, Isiala Oparanadim, Mpam, Nnarambia, Obodo Ahiara Ihenweorie, Obohia/Ekwereazu, Ogbe, Ogbennishi, Ogbor, Okirika Nweke I, Okirika Nweke II, Okirika Nwenkwo, Oru/Lude, Otulu/Aguneze, and Umuno/Umuchieze. The population is estimated to be made up of about five hundred thousand people. Sources of water in Ahiazu Mbaise include rain water capture (underground tanks, containers), boreholes, and pipe borne water from reservoirs, rivers, and surface ponds. These rivers are Iyi Obohia, Iyi Ekwerazu, and Iyi Okponkume. These rivers are seen around its boundaries with Mbaitoli/Ikeduru, Etiti and Mbano Local Government areas, but they also serve as source of water to neighboring communities in Ahiazu.

Sample collection

Water samples for bacteriological examination were collected with clean sterile water sampling bottles and analyzed within 3 h of collection. The borehole water samples were collected by first scrubbing the tap nozzle with 10% ethanol and flaming with flamer gun to burn off the ethanol. This was to sterilize the nozzle from external contamination. Thereafter, the borehole tap was opened to run for about 5 min before samples were aseptically collected. River water samples were collected from the surface by grab sampling method, while underground tank water samples were collected with clean sterile bottles.

Microbiological analysis

Sterilization of media was carried out by moist heat sterilization method using autoclave at 121°C, 15 psi for 15 min. Heat stable materials were sterilized using hot air oven at 160°C for 1 h as described by Cruickshank et al. (1982). Heat labile materials were aseptically rinsed with alcohol and distilled water. The water samples were aseptically subjected to 10 fold serial dilutions to

Table 1. Bacterial counts (cfu/ml) of water samples.

Sample	THBC	TCBC
Borehole water		
A	1.2×10^2	3.0×10^1
B	2.0×10^2	5.0×10^1
C	3.4×10^2	2.0×10^1
D	1.5×10^2	4.0×10^1
E	3.0×10^2	2.0×10^1
Underground water		
F	3.4×10^3	2.2×10^2
G	4.7×10^3	2.6×10^2
H	2.4×10^4	2.9×10^2
River		
I	1.8×10^4	4.8×10^3
J	2.0×10^5	1.9×10^4

THBC- total heterotrophic bacterial count; TCBC- total coliform bacterial count.

dilute the population of microorganism sufficiently in sterile blanks of 9 ml peptone water and then plated to produce discrete colonies for easy enumeration. The media used include nutrient agar, MacConkey agar, Eosin Methylene Blue agar, TCBS, and *Salmonella* - *Shigella* agar. All media were prepared as directed by the manufacturer. The method was adopted for the inoculation of media. Spread plates of appropriately diluted samples were incubated at 37°C for 24 h for heterotrophic bacterial count (THBC) while total coliform bacterial count (TCBC) were determined after incubation at 45°C for 24 h in MacConkey agar. Identification of isolates was based on the scheme described by Cheesbrough (1984) while serological test confirmed the pathogenicity of isolates based on the methods described by Vandepitte et al. (2003).

RESULTS

The result of total heterotrophic bacterial count (THBC) and total coliform bacterial count (TCBC) (cfu/ml) is as shown in Table 1. Bore hole water examples includes samples A, B, C, D and E, and the THBC counts ranged between 3.4×10^2 and 1.2×10^2 while the TCBC count ranged between 5.0×10^1 and 2.0×10^1 cfu/ml. Underground tank water samples includes samples F, G, H and the THBC count ranged between 2.4×10^4 and 3.4×10^3 cfu/ml while the TCBC count ranged between 2.9×10^2 and 2.2×10^2 cfu/ml. River water samples include samples I and J, and the THBC count ranged between 2.0×10^5 and 1.8×10^4 cfu/ml while the TCBC count ranged between 1.9×10^4 and 4.8×10^3 cfu/ml.

Table 2 shows the occurrence of bacterial isolates in water samples in percentage (%). The isolates occurred thus: *Escherichia coli* (50.0%), *Salmonella* spp. (100%), *Shigella* spp. 100% *Streptococcus* spp. (50.0%), *Vibro* spp. (20.0%), *Proteus* spp. (30.0%), *Klebsiella* spp. (80.0%), and *Enterobacter* spp. (50.0%). This showed that *Salmonella* and *Shigella* spp. occurred highest (100%) in water samples followed by *Klebsiella* (80.0%), while the lowest occurrence was recorded by *Vibro* spp. (20.0%).

The occurrence of total isolates in samples showed thus: sample A (50.0%), B (37.5%), C (37.5), D (50.0%), E (25.0), F (75.0%), G (50.0%), H (87.5%), I (100%), and J (87.5). Result showed that the sample with the highest population of isolates was samples I (100%) followed by samples H and J (87.5) which were river underground water samples.

DISCUSSION

The total heterotrophic bacteria count (THBC) test also called “total count” “plate count” is assured to provide an estimate of the total number of bacteria in a water sample that will develop into colonies during a period of incubation in a nutrient. This test detects a wide array of bacteria including pathogens, and opportunistic pathogens, but could not report all the bacteria in the works of Ihejirika et al. (2011). High THBC may be an indicator of poor general biological quality of drinking water (USEPA, 2003). A recommended maximum contaminant level (MCL) for human drinking water has not yet been proposed. The upper limit for portable water is usually 500 cfu/ml (Ihejirika et al., 2011). According to Dezuane (1990), water with counts under 100 cfu/ml should be considered “portable” and values 100 to 500 cfu/ml “questionable”. Therefore, all the water sources from Ahiazu Mbaise have questionable water quality.

The coliform test is a liable indicator of the possible presence of fecal contamination and is consequently

Table 2. Occurrence of bacterial isolates in water samples.

Water samples	*Escherichia coli*	*Salmonella*	*Shigella*	*Streptooccus*	*Vibro*	*Proteus*	*Klebsiella*	*Enterobacter*	Occurrence (%)
Borehole									
A	-	+	+	+	-	-	+	-	50.0
B	-	+	+	-	-	-	+	-	37.5
C	-	+	+	-	-	-	-	+	37.5
D	-	+	+	-	-	-	+	+	50.0
E	-	+	+	-	-	-	-	-	25.0
Underground tank									
F	+	+	+	+	-	-	+	+	75.0
G	+	+	+	-	-	-	+	-	50.0
H	+	+	+	+	+	+	+	-	87.5
Stream									
I	+	+	+	+	+	+	+	+	100
J	-	+	+	+	-	+	+	+	87.5
% Occurrence[b]	50.0	100.0	100.0	50.0	20.0	30.0	80.0	50.0	

correlated with pathogens. The USEPA MCL is less than one coliform per 100 ml. Many human facilities periodically test drinking water for coliform and use this limit (Dreeszen, 1996). The result of coliform test from all the water sources in Ahiazu Mbaise is above the USEPA MCL of less than one coliform per 100 ml. Borehole water samples were between 2.0×10^2 and 5.0×10^2 coliform per 100 ml, and underground tank water samples between 2.2×10^3 and 2.9×10^4 coliform per 100 ml, while stream water samples were between 4.8×10^5 and 1.9×10^6 coliform per 100 ml. This result shows that stream water sources had more enteric pathogens followed by undergrounded tank sources and borehole water sources being the least contaminated.

All the isolates from the different water sources are members of the family *Enterobacteriaceae* excepting *Streptococcus* spp. and *Vibro* spp. (Prescott et al., 2005). As *E. coli* (50%) was isolated from underground tank water and stream water samples only, it indicated recent fecal contamination of the different sources. This result is supported by the works of Health Canada (2006), Cabral (2010) and Ihejirika et al. (2011). While most strains of *E. coli* are non-pathogenic, some can cause serious diarrheal infections in human (Health Canada, 2006).

Salmonella spp. (100%) was isolated from all the water sources samples. This might be due to contamination from domestic sewage, agricultural pollution and storm water runoffs. This argument is supported by the reports of WHO (2008) and Arvanitidon et al. (2005). *Salmonella* spp. are responsible for two types of salmonellosis: 1) typhoid and paratyphoid fever; and 2) gastroenteritis (Le Minor, 2003). This implies that controlled sewage water systems and personal hygiene will reduce the incidence of gastroenteritis and typhoid fever (Popoff et al., 2005) that might result from the use of all the water sources in Ahiazu Mbaise.

The presence of *Shigella* spp. (100%) in all the samples might be due to unsanitary condition of the environment and secondary fecal contamination from an intermediary sources (Ihejirika et al., 2011). The implication of this is the risk of possible outbreak of shigellosis. This was in agreement with the report of Emch et al. (2008).

Streptococcus spp. (50%) isolated in some of the water sources might be due to fecal contamination of the water sources. This was supported by the report of Pruss (1998). Fecal *Streptococcus* spp. is responsible for gastrointestinal illness among humans (Donovan et al., 2008).

Vibro spp. (20.0%) was isolated from one underground tank water and one river water sample. Its presence in these samples might be due to contamination from birds, frogs, toads, and fishes precent in aquatic environments. This argument is supported by the report of Ali et al. (2001). *Vibro* spp. especially *Vibro cholera* is responsible for the disease cholera in humans (Cabral, 2010).

Proteus spp. (30.0%) is an enteric pathogen associated with the feces of animals including humans (Ihejirika et al., 2011). Its low percent occurrence might be due to the fact that it exists in minority of contaminating human feces. This is supported by the report of Wilson (2005).

Klebsiella spp. (80.0%) are ubiquitous in the environment (Cabral, 2010). They have been associated with contaminants like wastewaters, plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugarcane waste and living trees. *Klebsiella* spp. can cause human disease, ranging from asymptomatic colonization of the intestinal, urinary, or respiratory tract to fatal septicemia (Grimont et al., 2003).

Enterobacter spp. (50.0%) might be an implication of fecal contamination of the water sources. This was supported by the works of Grimont and Grimont (2005). Apart from fecal contamination, *Enterobacter* spp. might have been introduced from other sources like soil, polluted water, and plants (Ihejirika et al., 2011). The presence of *Enterobacter* spp. in 8 out of 10 water sources implied possible risk of nosocomial and health care-associated infection. This argument is supported by the reports of Hirdon et al. (2008) and Ihejirika et al. (2011).

Conclusion

This research work has confirmed that water sources in Ahiazu Mbaise is not free from enteric pathogens and might expose users to enteric diseases like diarrhea. Borehole waters are possibly not treated before usage while underground tanks might serve as source of water, but care should be taken as it is also a reservoir of water-borne pathogenic organisms. That streams are contaminated is not news, but provision of adequate portable water for the teaming population of the communities should be of utmost priority to prevent the morbidity and mortality caused by diarrhea.

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