

## Distribution and Diversity of Bacteria in a Small Tropical Freshwater Body (Aiba Reservoir) in Iwo, Osun State, Nigeria.

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**Abstract:** Environmentalists have become increasingly concerned about the pollution of surface waters. The Aiba reservoir is the second oldest impoundment of Osun river basin and provides potable water to Iwo community. The diversity of bacteria in the Aiba Reservoir was studied by collecting water samples from four locations within the reservoir to reflect human impacts during three different seasons. The most probable number (MPN) of bacteria at the different sampling locations during the sampling seasons, the total non-specific bacteria count, total coliform and *Escherichia coli* count were measured using the tube dilution and pour plate techniques. Significant differences were observed in various parameters of bacterial diversity. Water samples from all sampling points were contaminated with coliforms, the highest being 1100<sup>+</sup> MPN/100ml in location D. the highest number of bacteria was isolated in June at all sampling locations. Seventy-nine bacteria belonging to sixteen genera and twenty-three species were isolated. The genus *Bacillus* was the most diverse with thirty-one isolates and eight species. Other genera isolated were *Aeromonas*, *Citrobacter*, *Listeria*, *Proteus*, *Escherichia*, *Klebsiella*, *Alcaligenes*, *Enterobacter*, *Corynebacterium*, *Lactobacillus*, *Edwardsiella*, *Flavobacterium*, *Staphylococcus*, *Streptococcus* and *Acinetobacter*. Of the 23 genera isolated 10 (43.5%) were Gram negative bacteria, 60% of which are coliform bacteria. Eight (61.5%) of the Gram positive bacteria belonged to the Genus *Bacillus*. The high bacteria count and the type/genera of bacteria isolated are indicative of contamination of the system with soil from agricultural runoff, domestic sewage and fish remains. This on the long run could lead to spread of infective diseases to the populace.

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**Keywords:** Aiba reservoir, anthropogenic influence, bacterial count, coliforms, MPN.

### 1.0 INTRODUCTION

Water is essential to all living organisms including pathogenic microbes. Man, in his efforts to get rid of wastes have introduced into natural water bodies, noxious substances which include organic wastes that promote the growth of pathogenic bacteria, fungi, viruses and protozoans (Adams and Kolo, 2006). High concentrations of bacteria and nitrates discharged into water can occur from animal husbandry operations like grazing and this can result in health hazards to man due to the presence of pathogens (Atiribom et al., 2007). Environmentalists have become increasingly concerned about the pollution of surface waters. The World Health Organization (WHO) estimated that about 80% of ill-health in developing countries especially are water related (Cheesbrough, 2000). Aquaculture has been linked to aquatic disease outbreaks. It leads to greater needs for water treatment chemicals and drugs for disease prophylaxis and treatment which could lead to the development of drug/biocide resistant strains of human pathogens in adjacent waters (Akolisa and Okonji, 2005). Most morbidity and in some cases mortality associated with water-related diseases especially in developing countries is due to infectious agents which can infect man through ingesting pathogenic bacteria, viruses or other parasites in

water polluted by human or animal faeces or/and urine. Diseases in this category include cholera (*Vibrio cholerae*), dysentery (*Shigella* spp), typhoid fever (*Salmonella enteritis* subsp *typhi*) gastroenteritis (other species of *Salmonella*), diarrhea (*Escherichia coli*), hepatitis (Hepatitis A-E viruses). Other diseases caused by infections from water include scabies, skin ulcers, trachoma, paragonimiasis, malaria and schistosomiasis (Obasohan et al., 2010).

Bacteria are important components of the aquatic systems and are exposed to a wide variety of different habitats such as water, leaves and sediments which may affect their success and significance (Leff et al., 1998). Interactions within a stream are confounded by the fact that bacteria in these systems may originate from autochthonous sources such as biofilms, the water column and the benthos or allochthonous sources. Some of these allochthonous cells originate in soils and groundwater while others are introduced by human-mediated processes such as via sewage or industrial effluents (Janakiraman and Leff, 1999).

Aiba Reservoir is the second oldest impoundment of Osun River Basin and was created primarily for the provision of potable water with fisheries development as an ancillary benefit to Iwo

and surrounding communities. The reservoir serves as a daily source of fish protein and livelihood to the Iwo community. There has been a sharp rise in the development of Iwo in recent years as evidenced by the establishment of a private University, proliferation of the banking industry and presence of a television station among others. This has led to an increase in the population of Iwo and a greater dependence on the resources of this meager but renewable aquatic environment. Construction of residential houses is encroaching into the northern part of the reservoir. Other anthropogenic influences in and around the reservoir include intense fishing activity, washing of domestic wares and automobiles, bathing and fetching of water for construction and domestic purposes.

Therefore water and fish contamination with pathogenic resistant bacteria can pose serious health risks to those people within the immediate environment and beyond. This study aims to document for the first time bacteria associated with Aiba Reservoir, and to determine anthropogenic and seasonal influence on the bacterial load of the water body.

## 2.0 MATERIALS AND METHODS

### 2.1. Sampling

Water samples from Aiba Reservoir were collected from four points (A, B, C and D) within the reservoir and three months (October, March and June 2011) representing different seasonal periods. Water samples were collected from each point using a clean freshly purchased 500ml polyethylene bottle previously rinsed with sterile water and then the reservoir water. The water samples were transported to the laboratory within one hour of collection and kept in the refrigerator until ready for analyses.

### 2.2. Bacterial Isolation and Identification

Coliform contamination of water was determined by carrying out the most probable number (MPN) procedure or multiple tube fermentation test. The presumptive, confirmatory and complete tests were carried out as described by Onyuka *et al.*, (2011) and APHA/AWWA/WEF (1998) with slight modifications. MacConkey broth was used instead of phenol red lactose broth and colour change was from purple to a cream or colourless solution. Pure cultures isolated from the tubes were grown on nutrient agar (NA) for Gram staining to be carried out. Cultivation and isolation was carried out using the serial dilution methods as described by Brown (2005). Different dilutions were pour plated on plate count agar for Total Non-specific bacterial count, MacConkey agar or Total Coliform count, Eosin Methylene blue agar for *E. coli* count and these were carried out in

triplicates. Incubation was carried out at appropriate temperatures for the bacteria to be isolated. Numbers of colonies were counted and the means from triplicate experiments were determined and recorded. Distinct colonies were picked and restreaked onto fresh agar plates until pure cultures were gotten. The pure cultures were kept on nutrient agar slants and kept at 4°C.

A total of 79 isolates were characterized. Morphological and biochemical tests including Gram staining, spore staining, colonial morphology, indole, Methyl red, Voges-Proskauer, catalase, citrate, starch and casein hydrolysis were carried out to characterize the isolated bacteria (Pollack *et al.*, 2002). Bergey's manual of systematic bacteriology (Sneath *et al.*, 1986) and the manual of identification of medical bacteria (Barrow and Feltham, 2004) were also used.

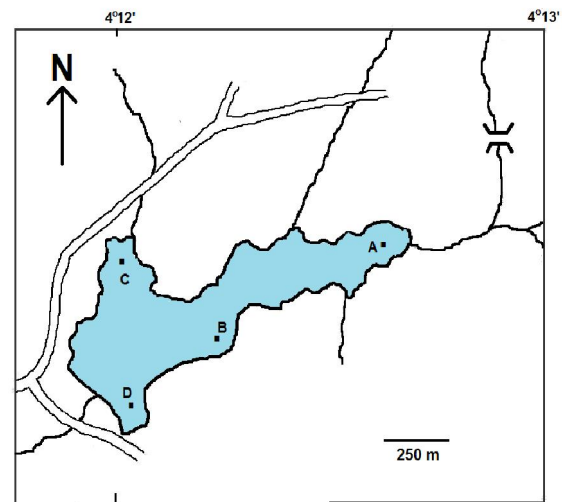


Figure 1: Map of Aiba Reservoir, Iwo, Osun State showing sampling locations.

Key:

- A: North-east, near source of inflow (upstream), little human activity (occasional washing/bathing).
- B: South-east, shares border with land used for agricultural activities, minimal direct human interference
- C: North, rapid residential and occupational encroachment, food-vendor, carpentry, mechanic workshops, washing of vehicles and motorcycles
- D: South, close to outflow (downstream), landing site for fishermen, high human activity-swimming, bathing, domestic washing, and removal of fish intestines.

## 3.0. RESULTS

The most probable number (MPN) of bacteria test was carried out and it was found out that the water samples from all sampling points were contaminated with coliforms (Table 1). In October,

Location B was the most contaminated at 93 MPN100ml<sup>-1</sup>, in March Location A was most contaminated (93 MPN100ml<sup>-1</sup>) while in June Location D was the most contaminated (1100<sup>+</sup> MPN100ml<sup>-1</sup>). Contamination was highest in June over the three sampling months, although there was no significant difference between the sampling months ( $p = 0.199$ ). The highest number of bacteria was isolated in June at all sampling locations followed by October and the least count was in March (Figure 2). The count in June was significantly different when compared to the other two sampling periods. Location D had the highest bacterial count and Location C had the lowest bacterial count, although there was no significant difference ( $p = 0.370$ ) among locations (Figure 3). Bacterial counts at locations were not significantly different from each other. Seventy-nine bacteria belonging to 16 genera and 23 species were isolated (Table 2). The genus *Bacillus* was the most diverse and most represented with eight species and 31 isolates. Other genera isolated are *Aeromonas*, *Citrobacter*, *Listeria*, *Proteus*, *Escherichia*, *Klebsiella*, *Alcaligenes*, *Enterobacter*, *Corynebacterium*, *Lactobacillus*, *Edwardsiella*, *Flavobacterium*, *Staphylococcus*, *Streptococcus* and *Acinetobacter*. Of the 23 genera isolated 10 (43.5%) were Gram negative bacteria, 60% of which are coliform bacteria. Eight (61.5%) of the Gram positive bacteria belonged to the Genus *Bacillus* (Table 2). *Aeromonas hydrophilla* was isolated only at Location A while *Bacillus* sp, *Flavobacterium johnsoniae* and *Staphylococcus epidermidis* were the only three isolated from all the four sampling locations (Table 3).

Table 1: Most probable number (MPN) of bacteria at the different sampling locations during the three sampling months.

Sampling Locations	October (MPN100/ml)	March (MPN100/ml)	June (MPN100/ml)
A	23	93	43
B	93	4	93
C	28	4	43
D	43	4	1100 <sup>+</sup>

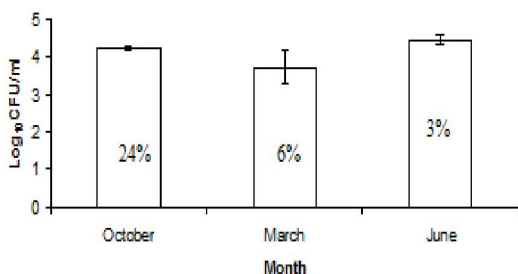


Figure 2: Total bacterial counts during the three sampling periods (Each bar represents standard error).

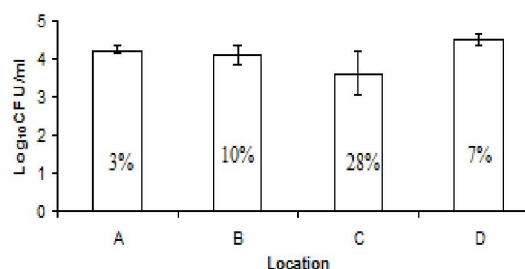


Figure 3: Total bacterial counts at the sampling locations (Each bar represents standard error).

Table 2: Distribution of isolated bacteria from Aiba Reservoir.

Identified bacteria	Gram reaction	Number isolated
1 <i>Acinetobacter</i> sp.	-	2
2 <i>Aeromonas hydrophila</i>	-	4
3 <i>Alcaligenes</i> sp.	-	3
4 <i>Bacillus azotoformans</i>	+	2
5 <i>Bacillus badius</i>	+	2
6 <i>Bacillus brevis</i>	+	2
7 <i>Bacillus cereus</i> Subsp. <i>Mycoides</i>	+	7
8 <i>Bacillus circulans</i>	+	4
9 <i>Bacillus larvae</i>	+	2
10 <i>Bacillus megaterium</i>	+	7
11 <i>Bacillus</i> sp.	+	5
12 <i>Citrobacter diversus</i>	-	3
13 <i>Corynebacterium kutscheri</i>	+	3
14 <i>Edwardsiella tarda</i>	-	2
15 <i>Enterobacter aerogenes</i>	-	5
16 <i>Escherichia coli</i>	-	5
17 <i>Flavobacterium johnsoniae</i>	-	4
18 <i>Klebsiella</i> sp.	-	3
19 <i>Lactobacillus delbrueckii</i>	+	3
20 <i>Listeria</i> sp.	+	2
21 <i>Proteus</i> sp.	-	2
22 <i>Staphylococcus epidermidis</i>	+	4
23 <i>Streptococcus agalactiae</i>	+	3
Total		79

Table 3: Prevalence of bacteria at the different sampling points/periods

Isolated bacteria	A	B	C	D
1 <i>Acinetobacter</i> sp.	-	-	-	+
2 <i>Aeromonas hydrophila</i>	+	-	-	-
3 <i>Alcaligenes</i> sp.	-	-	+	-
4 <i>Bacillus azotoformans</i>	-	-	+	-
5 <i>Bacillus badius</i>	-	+	-	-
6 <i>Bacillus brevis</i>	-	-	+	-
7 <i>Bacillus cereus</i> Subsp. <i>Mycoides</i>	-	+	+	+
8 <i>Bacillus circulans</i>	+	-	-	-
9 <i>Bacillus larvae</i>	-	-	+	+
10 <i>Bacillus megaterium</i>	+	+	+	-

11	<i>Bacillus</i> sp.	+	+	+	+
12	<i>Citrobacter diversus</i>	-	-	-	+
13	<i>Corynebacterium kutscheri</i>	-	+	-	-
14	<i>Edwardsiella tarda</i>	+	-	-	-
15	<i>Enterobacter aerogenes</i>	-	-	-	+
16	<i>Escherichia coli</i>	+	+	-	+
17	<i>Flavobacterium johnsoniae</i>	+	+	+	+
18	<i>Klebsiella</i> sp.	+	+	-	-
19	<i>Lactobacillus delbrueckii</i>	-	+	+	+
20	<i>Listeria</i> sp.	+	-	-	-
21	<i>Proteus</i> sp.	+	-	-	-
22	<i>Staphylococcus epidermidis</i>	+	+	+	+
23	<i>Streptococcus agalactiae</i>	-	-	+	-

KEY: + isolated from sampling location; - not isolated from sampling location.

#### 4.0. DISCUSSION

The high populations of viable heterotrophic bacteria encountered are typical of water bodies that receive organic pollutants. Similar high populations of heterotrophic bacteria have been reported by Olayemi (1994). Many freshwater clades appear to have widespread geographic distributions; this suggests that freshwater habitats, despite their geographic isolation harbor bacterial species drawn from a consistent pool of potential colonists (Yannarell and Kent, 2009). The Actinobacteria are a group of bacteria that are commonly found in lakes with a wide range of water chemistries (Warnecke et al., 2004). The phylum Bacteroidetes contains members of the genera *Cytophaga*, *Bacteroides* and *Flavobacterium*. In freshwaters, Bacteroidetes comprise a major proportion of particle-associated bacterial communities and they can also be found at depths in lakes where they may degrade recalcitrant macromolecules (Van der Gucht et al., 2005).

Overall, sampling Location D was most contaminated over the three sampling months followed by Location B while Location C had the lowest contamination count. Location D happens to be the landing site for fishermen and the most visited part of the reservoir where human activities is highest. Fish processing especially removal of fish intestines could contribute greatly to the high bacteria count in this sampling site. The highest number of bacteria was isolated in the month of June, with low variations (3%) in the bacterial count at the different points. This is likely due to mixing of reservoir water during rainy season. Highest level of variation in isolated bacteria genera was in October from all the four sampling points. This may be due to high water activity and lesser mixing of reservoir water. There were significant differences in the bacterial counts isolated during the sampling periods. Location C had the highest variation of bacteria (28%) during the 3 sampling seasons. This shows that the level of

contamination is very high. This location is where there is encroachment of houses and food vendors. Domestic sewage effluents and wastes from the food vendors and workshops can contribute greatly to the organic pollution hence microbial bloom of this area. Location A had the lowest coefficient of variation in bacterial load (3%) because there is little human activity at this location. Bacterial count at Location D was higher than for other locations, this could be due to the fact that there is high level of human activity, cleaning of fish intestines will also introduce bacteria which normally would not have been present. Different bacteria associated with fish and their intestines have been documented e.g *Flavobacterium columnare* in fish kidney (Bernardet, 1989); *Streptococcus* (Eldar et al., 1994); *Aeromonas* (Huys et al., 1997); *Carnobacter* from fish intestines (Ringø, 2008). The isolation of various genera of bacteria from this water body indicates the success of these bacteria in adapting to that environment (Baghat et al., 2010). The Genus *Bacillus* accounted for 61.5% of the Gram positive bacteria. Representatives of the genus *Bacillus* have been found to be dominant in several hypersaline aquatic systems e.g. Buzzers Bay, Nigeria (Oguntoyinbo, 2007), Savuzavu hot springs, Fiji (Narayan et al., 2008) and Lake Howz-Sultan, Iran (Amoozeger et al., 2009). The capacity of these bacteria to produce spores contributes to their resistance to a broad range of physiological stresses (Tiquia et al., 2007, Baghbat et al., 2010). The remaining genera of Gram positive bacteria have been known to be pathogenic to man and different species of fish (Buller, 2004; Nzeh and Udeze, 2011).

The Department of Water Affairs and Forestry, South Africa DWAF (1996) set a range of guidelines for drinking water for domestic use. The bacterial counts at all sampling points (>100 bacteria/100ml) from this study indicate that this water body has a significant and increasing risk of infectious disease transmission. If used for drinking or for bathing and washing of laundry, there are risks of clinical infections because of the unacceptable quality (WRC, 2001). In addition these bacteria can be termed opportunistic pathogens when they begin to cause diseases in the fish and people that consume them. The Aiba reservoir is said to serve as a daily source of fish protein and livelihood to the Iwo community. The high bacteria count and the type/genera of bacteria isolated is indicative of contamination of the system with soil from agricultural runoff, domestic sewage and fish remains. This on the long run could lead to spread of infective diseases to the populace.

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