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Full Length Research Article

PREVALENCE OF BACTERIA ISOLATES IN WATER AND SOME BIOTA OF LAPAI-AGAIE DAM, NIGERIA

Kabir Mohammed Adamu*[1], Isah Ibrahim Zhigun[1], Isioma Kate Iloba[2], Aliyu Mohammed Babadoko[3], Robert Bamigho Ikomi[2], Rukaiyat Shuaibu[1], Kolawole Olofinsan Ayodapo[4] and Aishat Alhaji Ibrahim[1]

[1]Department of Biological Sciences, Ibrahim Badamasi Babangida University, P.M.B. 11, Lapai, Niger State, Nigeria
[2]Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria
[3]Department of Biological Sciences (Microbiology Unit), The Federal Polytechnic, Bida, Niger State, Nigeria
[4]Department of Biological Sciences, Nile University of Nigeria, Abuja

*Corresponding Author Email Address: kabrmoh@yahoo.com Phone: +2348035826075

ABSTRACT
Lapai-Agaie dam play a pivotal role as primary source of domestic water supply, agricultural (irrigation and fisheries) activities to the host communities. Therefore, this study assessed the prevalence of bacterial isolates in water and on some biota (macrophytes and fishes) in order to provide basic information on bacterial diversity of the dam. The water pH and temperature of the identified sample sites were determined. Water sample for bacteria isolation was collected across water surface at each sites into 500ml sterile container. Macrophytes from the sampling sites were wholly collected with forceps into a sterilized plastic container; while fish samples were obtained from the landing site and placed in sterilized polythene bag for subsequent analyses. The standard procedures for bacteria sampling and identification were employed using phenotypic identification techniques. Water pH was significantly different ($p<0.05$) between sampling sites. Six bacteria isolates were identified from the two water sampling sites. Site A recorded the highest average bacterial colony count of $0.96x22x10^8$ cfu/ml with *Escherichia coli* (12(29 %)) as the most frequent isolate. A total number of seventeen (17) genera of fishes where eleven bacteria were isolated. The fish coded FS3 recorded the highest distribution bacteria isolates. Three species of macrophytes were identified in the dam, with the highest bacteria population of $1.85\pm0.24x10^7$cfu/ml in the stem of *Leerisia havedra* as it had the highest bacterial population of $16.42\pm0.43x10^7$ cfu/ml. Twelve (12) bacteria isolates where identified from the macrophytes with *Klebsiella pneumonia* recording the highest frequency of 8(16 %). However, the distribution of pathogenic bacteria in water was lower than that on the fishes and macrophytes. An indication that the biota may act as causative agent of epidemic disease. Therefore, the existence of these isolates pose challenges to human health, if proper hygiene and implementation of aquatic water policy and regulations are not properly enforced to discourage anthropogenic pollution.

Keywords: Lentic, Biodiversity, Fish, Macrophytes, Bacteria

INTRODUCTION

Lapai-Agaie dam is an example of lentic habitat which is referred to as the most stable and common habitats of the biosphere with multiple functions that are not limited to municipal water supply, domestic, irrigation and aquaculture activities (Radhika *et al.,* 2004) to its host communities. The dam bordered between Paikoro and Lapai Local Government Areas of Niger State, Nigeria. Studies have revealed that water bodies of these multiple functions have abundant fish diversity (Omowumi, 2013) as fishes are known to be the main sources of animal protein for many centuries (Shinkafi and Ukwaja, 2010) due to their high nutritional quality, low fats and cholesterol (Salihu *et al*., 2012). Fishing is one of the main reasons communities settles around water bodies. As these communities settles around the water bodies, they are known to participate in changing the ecology of the water where bacteria that are part of the aquatic biodiversity have received less attention. Therefore, studying the distribution of bacteria isolates in a multipurpose dam such as Lapai-Agaie dam does not only reveal the potential pathogenic bacteria distribution but the possible reduction or improvement of nutritional and healthy nature of the biota as it directly or indirectly affect human.
Studies have shown that bacteria do not only exist in the water but can live on/in aquatic biota like the macrophytes and the fishes. Studies have also shown that fish are host to bacteria species as they are the most causative agents of fish diseases (Mandal *et al.,* 2009, Shinkafi and Ukwaja, 2010, Musefiu, *et al*., 2011, Jimoh *et al.,* 2014; Anyanwu *et al*., 2015, Olugbojo and Ayoola, 2015). Similarly, studies have revealed the distribution of bacteria on water bodies (Adamu *et al*., 2017, Duru and Nwanekwu, 2012, Paulse *et al*., 2012; Mervat *et al*., 2012) and aquatic plants/macrophytes (Adamu *et al*., 2017) an indication that bacterial distribution and ecology are gaining research attention. Ipso-facto, this study seek to determine the prevalence of bacteria isolates in water and on some biota (Fishes and macrophytes) of Lapai-Agaie dam by phenotypically identifying the different bacterial isolates associated with the water and some biota.

MATERIALS AND METHODS

Sampling Site

The study site was Lapai-Agaie Dam, located close to Bakajeba village at latitude $9^O 13^1$ N and Longitude $6^O 35^1$ E (Plate 1). Three (3) samples were collected biweekly during the months of June and July, 2017.

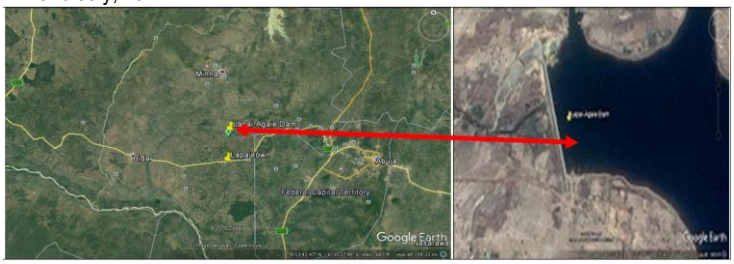

Plate 1: Pictorial representation of Lapai-Agaie Dam source: Google Earth

Sample Collection

Water samples were collected from two identified sites labeled A (characterized by domestic activities such as clothes, plates, bike washings and bathing) and B, (characterized by fishing activities/ landing sites and fish washing) into 500ml sterile plastic container. The container was initially rinsed three times with the sample water before sampling (Ademorati, 1996). Collection was done by dipping the sample bottle at about 20cm below the water surface, projecting the mouth of the bottle against the water current. The samples were preserved in a cool chain and immediately transferred to the laboratory for analyses. The water pH and temperature were monitored using pH meter (Hanna) and mercury glass thermometer (APHA, 2005) respectively before sampling.

Fishes were collected from the landing site and subsequently identified using freshwater fishes key (Olaosebikan and Raji, 2004). The skin of the fishes were swabbed with sterile swab stick, and then placed in a normal saline solution. Macrophytes were sampled in early hours of 800hours, identified and differentiated into the root, stems and leaves using Secateurs into sampling bottles.

Isolation of Bacteria

Following aseptic techniques, ten-fold serial dilution of sample was made; from which 0.1ml was plated into a sterile Petri dish. Nutrient Agar (NA) (Titan Biotech) prepared according to manufacturer's specification was then poured onto sterile Petri-dish containing 0.1ml of the sample using pour plating techniques. The plate was subsequently incubated at 37^{o}C for 24hours to obtain mixed culture. Isolated colonies were recorded and purified to obtain pure culture by repeated sub-culturing on fresh media use for primary isolation as described by Chikozie (2015). Pure stock cultures obtained were inoculated on NA slant and preserved in the refrigerator at 4^{o}C until needed for further characterization and identification. Coliform counts of the water samples were determined by MPN techniques (WHO, 1997).

Characterization and identification of bacterial isolates

Bacterial isolates were characterized by their morphological/macroscopic and microscopic characteristics and identified further by biochemical tests (catalase, coagulase, urease, citrate, oxidase, indole, methylred, glucose, lactose and sucrose) as described by Cheesebrough (2000) and Adeoye, (2007).

RESULTS

Water Temperature and pH

The water temperature and pH are presented in Table 1. Water at Site A recorded the highest water temperature and pH of 27.00±0.32 ^{o}C and 6.90±0.01 respectively. There was significant difference ($p<0.05$) in the monitored pH values.

Table 1: Mean and Standard Error of Water Temperature and pH of sampling sites in Lapai-Agaie Dam

Parameters	Sampling Sites	
	A	B
Temperature (^{o}C)	27.00±0.32^{a}	26.20±0.45^{a}
pH	6.90±0.01^{a}	6.40±0.37^{b}

The same superscript in row =$p>0.05$, different superscript in row = $p<0.05$

Bacteria Isolates in Water Column

The average MPN index/100ml revealed that Site A recorded the highest value of 254(65 %)/100ml (Fig 1). *Salmonella* sp had the highest (9(60 %)) frequency of occurrence compared to *Shigella sp* whilst Site A also recorded the most frequency of the two species (Table 2). The bacteria distributions in water sampled from the two sites are presented in Table 3, where six (6) bacteria were isolated. The most frequent isolate was *E. coli* (12(29 %))

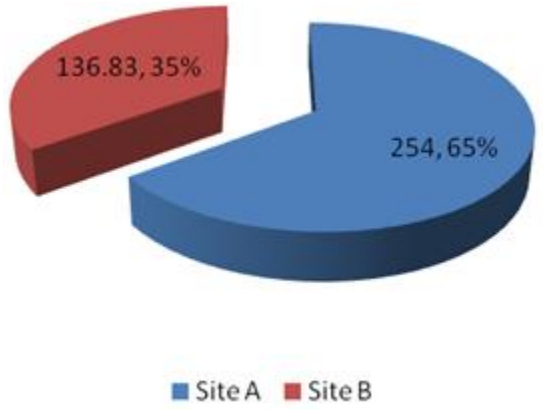

Fig 1: Percentage Frequency of MPN index of bacteria load in Sites of Study at Lapai-Agaie Dam

Table 2: Frequency of occurrence (Percentage (%)) of *Salmonella* and *Shegella* species in water samples of Lapai-Agaie Dam

Sampling Site	*Salmonella/Shigella* sp		Frequency of occurrence
	Salmonella	*Shigella*	
A	4	3	7(46.67)
B	5	3	8 (53.33)
	09 (60)	06 (40)	15 (100)

Table 3: Presence and Frequency of Bacteria species in water sampled from two sites of Lapai-Agaie Dam

Bacteria species	Sites		Frequency (%)
	A	B	
Shigella sp	+	+	8(19.00)
Salmonella typhi	+	+	4(10.00)
Streptococcus faecalis	-	+	4(10.00)
Staphylococcus aureus	+	+	4(10.00)
Escherichia coli	+	+	12(29.00)
Pseudomonas aeruginosa	+	+	9(22.00)

+= Present; - =Absent

Fishes and Bacteria Distribution on Fishes.

The total numbers of 17 fishes comprising of 8 genera were identified and coded as presented in Table 4 with their frequency of occurrence. The FS3, FS6 and FS8 recorded the highest occurrence. The average bacteria colony during the sampling periods were as presented in Fig 2; which revealed FS16>FS9>FS17>FS7>FS10>FS11>FS8>FS2/12>FS4>FS1>FS 5>FS3>FS6/15>FS13>FS14 order of magnitude. The ecto-bacteria distributions from each fish are presented in Table 5 where FS3 recorded the highest bacteria distribution. Eight and three gram negative and positive bacteria were identified

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respectively with *Bacillus subtilis* recording the highest frequency of 8(16%) as presented in Fig 3.

Table 4: Frequency of occurrence and codes of fishes sampled from Lapai-Agaie Dam Landing site

Families	Species	Identity Code	Frequency of occurrence
Cyprinidae	*Labeo senegalensis*	FS1	02
	Barboides gracilis	FS2	02
Cichlidae	*Tylochromis sudanencis*	FS3	03
	Tilapia zilli	FS4	02
	Tilapia dageti	FS5	02
	Pelvicachromis taeniatus	FS6	03
Clariidae	*Clarias gariepinus*	FS7	02
	Clarias anguillaris	FS8	03
Characidae	*Brycinus nurse*	FS9	01
	Bryconaethiops quinquesquamae	FS10	01
Mormyridae	*Marcusenius abadii*	FS11	02
	Petrocephalus soudanensis	FS12	01
	Marcusenius mento	FS13	02
	Petrocephalus bovei	FS14	01
Anabantidae	*Ctenopoma petherici*	FS15	01
Mochokidae	*Brachysynodontis batensoda*	FS16	01
Melapteruridae	*Melapterurus electricus*	FS17	01

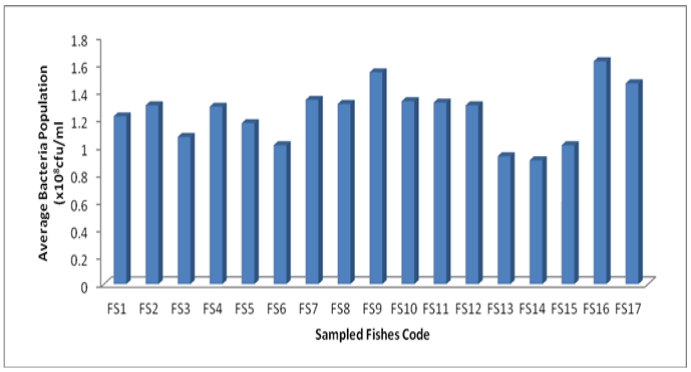

Fig 2: Mean total Bacteria Population counts on sampled fishes from the landing site of Lapai-Agaie Dam

Table 5: Ecto-Bacteria Distribution on fishes from the landing site of Lapai-Agaie Dam

Fish species	Bacteria Isolates										
	Bacillus subtilis	*Salmonella typhi*	*Aeromonas hydrophilia*	*Enterobacter sp*	*Proteus vulgaris*	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Klebsiella ozaenae*	*Escherichia coli*	*Serratia mercescens*	*Staphylococus aureus*
Labeo senegalensis		+		+							+
Barboides gracilis	+				+		+		+		
Tylochromis sudanencis			+				+	+			
Tilapia zilli		+			+					+	
Tilapia dageti			+	+				+			
Pelvicachromis taeniatus	+	+	+				+		+		
Clarias gariepinus	+			+		+			+		
Clarias anguillaris		+			+	+	+	+			
Brycinus nurse	+								+		
Bryconaethiops quinquesquamae	+										
Marcusenius abadii	+				+	+			+		
Petrocephalus soudanensis											+
Marcusenius mento	+						+	+			
Petrocephalus bovei				+							
Ctenopoma petherici	+				+					+	
Brachysynodontis batensoda									+		
Melapterurus electricus					+			+			

+= Present

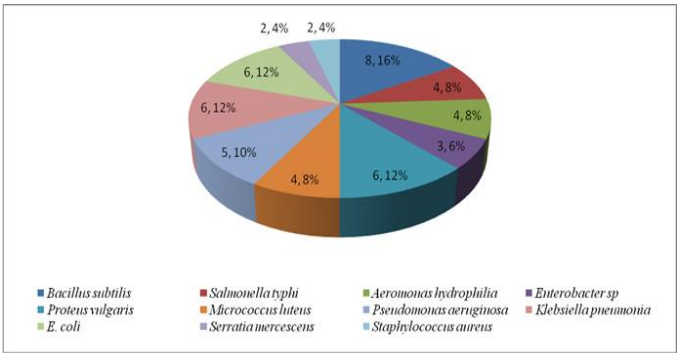

Fig 3: Percentage frequency of bacteria distribution from fishes in Lapai-Agaie Dam

Macrophytes and its Bacteria Isolates

Three species of macrophytes were identified in the dam. The highest average ($1.85\pm0.24x10^7$cfu/ml) bacteria colony count was recorded in the stem of *Leerisia havedra* with the highest average ($16.42\pm0.43x10^7$cfu/ml) bacteria population in whole plant as presented in Table 6. The bacteria isolates identified from the plants are presented in Table 7 with the isolation of *Neisseria gonorrhea*. The total number of twelve (12) bacteria isolates were identified on macrophytes samples (Fig. 4) with *Klebsiella pneumonia* recording the highest frequency of 8(16%). The summary of the different isolates, distribution from the different samples are presented in Table 8.

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Table 6: Mean and Standard deviation of Bacteria Colony Counts in Identified Sampled Macrophytes in Lapai-Agaie Dam

Macrophytes	Plant Part	Colony Count (cfu/ml x10^{-7})	Total/Plant (cfu/ml x10^{-7})
Ludwugia stolonifera	Leaf	1.82±0.07[a]	14.99±0.34
	Stem	1.56±0.10[b]	
	Root	1.62±0.17[c]	
Neptunia. Oleracea	Leaf	1.52±0.12[a]	13.40±0.45
	Stem	1.62±0.22[b]	
	Root	1.33±0.11[c]	
Leerisia havedra	Leaf	1.80±0.07[a]	16.42±0.43
	Stem	1.85±0.24[b]	
	Root	1.83±0.12[c]	

Table 7: Bacteria Distribution on Macrophytes from Lapai-Agaie Dam

Macrophytes	Plant Parts	Bacteria Isolates											
		Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumone*	*Neisseria gonorrhea*	*Streptococcus faecalis*	*Escherichia coli*	*Micrococcus luteus*	*Pseudomonas aeruginosa*	*Micrococcus roseus*	*Bacillus lichiniformis*	*Staphylococcus empidermidis*	*Proteus vulgaris*
Ludwugia stolonifera	Leaf	+		+							+		
	Stem				+	+	+						
	Root	+		+		+							
Neptunia. Oleracea	Leaf			+	+	+							
	Stem							+	+		+		
	Root		+					+				+	
Leerisia havedra	Leaf						+			+			+
	Stem	+					+		+				
	Root		+	+	+								

+= Present

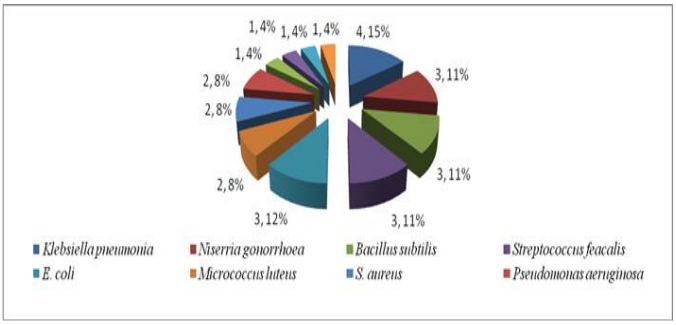

Fig 4: Percentage frequency of bacteria isolates on Macrophytes in Lapai-Agaie Dam

Table 8: Presence of Bacteria distribution in water and biota of Lapai-Agaie Dam

Bacteria Isolates	Fishes	Macrophytes	Water
Escherichia coli	+	+	+
Bacillus subtilis	+	+	-
Bacillus lichiniformis	-	+	-
Salmonella typhii	+	-	+
Pseudomonas aeruginosa	+	+	+
Micrococcus roseus	-	+	-
Micrococcus luteus	+	+	-
Aeromonas hydrophilia	+	-	-
Enterobacter sp	+	-	-
Klebsiella ozaenae	+	-	-
Klebsiella pneumone	-	+	-
Shigella sp	-	-	+
Proteus vulgaris	+	+	-
Streptococcus faecalis	-	+	+
Staphylococcus aureus	+	+	+
Staphylococcus empidermidis	-	+	-
Serratia mercescens	+	-	-
Niserria gonorrhea	-	+	-

-= absent += present

DISCUSSION

The seventeen (17) fish species reported in this study is similar to that reported by Omowumi (2013) in Lake Asejire, an indication that the Dam has good number of fishes. The bacteria colony counts on fish recorded in this study is less than the least of x10^{12} and x10^{11} cfu/ml reported by Adedeji *et al.* (2011) and Tiamiyu *et al.* (2015) in some fish species. However, the colony count reported by Shinkafi and Ukwaja (2010) and Adebayo-tayo *et al.* (2012) of x10^{8} cfu/ml were within the range recorded in this study as Mandal *et al.,* (2009) attributed the maintenance of water quality in fish farm reduces bacteria colony count. Olugbojo and Ayoola (2015) had reported that bacteria colony count above x10^{6} is not suitable for human consumption (ICMSF, 1971). Thus studies in Nigeria had revealed higher counts due to water pollution and non-enforcement and compliance to aquatic ecosystem regulation policies. The study of Raufu *et al.* (2014) alien to this that isolates varies with fish species as high bacteria density on fish skin may be attributed to secondary contamination (Adebayo-tayo *et al*., 2012). The presence of scales may also have been responsible for the higher bacteria isolaes recorded in *M. abadii, B. batensoda, P. sudanensis* and *T. dageti* (Adedeji *et al*., 2011, Ibemenuga and Okeke, 2014 and Tiamiyu *et al.,* 2015). The presence of mostly gram negative bacteria isolates in the Dam is an indication of serious human interference (Panneerselvam and Arumugam 2012 and Olukunle and Oyewumi, 2017) as they are related to pollution (Ikpeme *et al*., 2011, Duru and Nwanekwu, 2012, Mervat *et al*., 2012 and Paulse *et al*., 2012). Mostafa *et al*., (2018) had reported that some of these species bacteria species are causative agents of food poisoning. Food poisoning is considered as one of the common causes of illness and death in developing countries (Sapkota *et al.,* 2012). The presences of non-pathogenic isolates such as *Bacillus subtilis* and *E. coli* in aquatic ecosystem that are not normal flora in fish (Cohen and Shuval, 1973) are related to

faeces from warm blooded animals. This may be an indication of higher risk of other pathogenic isolates (Doyle and Ericson, 2006) and thus may be harmful to man when consumed.

The domestic activities such as washing and bathing in Site A may have contributed to the higher pH and water temperature values recorded in the study area which may be associated with the less bacteria distribution. However, lower pH and water temperature in Site B may have been responsible for higher distribution of bacteria as the site is dominant with the presence of biodegradable components of fish.

Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Bacillus sp and *Klebsiella ozaenae* were amongst the bacteria isolates, identified on water hyacinth in Jabi-Lake, Nigeria (Adamu, *et al*., 2017) and farmed fishes (Salgado-Miranda *et al*., 2013). The presences of these isolates on aquatic macrophytes may be an indication that these macrophytes may be utilized for the production of microbial cellulose (Adamu *et al*., 2017). The presences of *Klebsiella* spp, *Salmonella typhii, N. gonorrhea, Proteus vulgaris,* amongst other pathogenic bacteria may be attributed to the use of the aquatic habitat as toilet and micturition activities during bath. *Salmonella* sp has been reported to cause enteritis and systemic diseases (Shinkafi and Ukwaja, 2010). Therefore, study had revealed that *Streptococcus* spp caused significant economic losses in farm fishes (Hanol Bektas *et al*., 2017). *Staphylococcus aureus* had been isolated on the surface (skin) of fish (Ali, 2014) as the commonest bacteria isolate. They are recognized globally as causative agents of streptococcal infections in several kinds of freshwater fishes (Yuasa *et al*., 2008). While *Serratia mercescens* causes bacteruria in animals (Lateef, 2004)

The study had revealed the presences of pathogenic and non-pathogenic bacteria in the aquatic ecosystem may be potentially harmful to animal/human health. Some bacteria are not host specific. The bacteria load in the water was lower than that on the fish and macrophytes, an indication that the biota may be acting as causative agent of epidemic diseases (Feikin *et al*., 2010). These bacteria are of public health significance thus may be causative agent for zoonotic infections that may develop through handling of aquatic biota and use of the water. Therefore, the existence of these pathogens isolated in aquatic biota may continue to poise challenges to human if proper hygiene and implementation of aquatic water policy and regulations are not enforced to discourage anthropogenic pollution. Adequate measures should be employed during processing of the biota for consumption.

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