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Detection Of Aeromonas Species From Water Sources In North Eastern Nigeria

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ABSTRACT

The genus *Aeromonas* contains gram negative non spore forming rods that are ubiquitous to aquatic environments worldwide. They are emerging pathogens that are frequently isolated from aquatic environments, including ground water, lakes, drinking water and wastewater. A total of 274 samples from boreholes (124) and lakes (150) were collected over a one year period (January to December, 2011) in Maiduguri, at Lakes Alau and Chad; and analysed for the presence of *Aeromonas* species based on Aerokey II group of tests for the identification and speciation of *Aeromonas*. Overall, 54 (36%) *Aeromonas* spp were isolated with 38 (38%) and 16 (32%) from lakes Chad and Alau respectively. There was no statistically significant difference ($P > 0.05$) in the occurrence of the isolates among various sampling points. *A. hydrophila* was the most common species with an overall prevalence of 91% followed by *A. sobria* with 9% prevalence. Water samples from boreholes located at Gwange ward had the highest rate of isolation (87.5%) followed in descending order by Galadima shuwarin (45%), London ciki (30%), Bulabulin (25%), Custom (15.8%) and University of Maiduguri staff quarters (10%). The water samples from Mairi and Bulabulin wards did not yield any *Aeromonas* isolate. The findings of this study confirmed the presence of potentially pathogenic *Aeromonas* species in the studied lakes and water samples from boreholes in Maiduguri metropolis. These sources of water are used for fishing, drinking and household chores. As *Aeromonas* is considered an emerging pathogen and can cause health problems, proper surveillance of water sources is strongly recommended to protect susceptible individuals from exposure to potentially pathogenic strains of *Aeromonas*.

Keywords: Aeromonas, Boreholes, Lakes, Water, Northeast

INTRODUCTION

The genus *Aeromonas* are gram negative non spore forming rods that are ubiquitous to aquatic environments worldwide. They are frequently isolated from aquatic environments, including ground water, lakes, estuarine, marine, wastewater and even drinking water [1, 2]. *Aeromonas* species have been isolated from chlorinated drinking water supplies in several countries [3, 4]. Aeromonas species have been recovered in abundance from lakes particularly in warmer temperatures [1, 2]. Some researchers have attributed human infection to ingestion of food and water harboring these organisms [5]; while Majeed [6] suggested that fish and other aquatic

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animals that live in contaminated water may serve as means of transmission of the *Aeromonas* pathogens. Emerging pathogens such as *Aeromonas,* pathogenic *Escherichia coli*, *Campylobacter*, *Legionella*, and *Pseudomonas* are associated with various gastrointestinal and extra intestinal infections [7]. According to USEPA [8], *Aeromonas hydrophila*, a member of the genus *Aeromonas*, is listed on the first and second Contaminant Candidate List of potential waterborne pathogens. Children, the elderly and immunocompromised individuals, are mostly vulnerable to these emerging pathogens. *Aeromonas* species are considered as opportunistic waterborne pathogens responsible for acute gastroenteritis and wound infections in humans and aquatic animals. Although several studies have been carried out with regards to the virulence factors of *Aeromonas*, many aspects are yet to be properly clarified. Pathogenicity of *Aeromonas* cannot be ascribed to individual factors because of the wide variety of extracellular enzymes produced by the organism [7]. Aeromonads produce various putative virulence factors such as cytotoxic and cytotonic enterotoxins, proteases, leukocidin, phospholipase, endotoxin, and outer membrane proteins [9, 10, 11].

This study aims to determine the prevalence of *Aeromonas* species from boreholes used for drinking water and major lakes used for fishing in Maiduguri, North eastern Nigeria. This is with a view to investigate the occurrence of the organisms in the said water samples so as to highlight the impending health problem posed by the organism.

MATERIALS AND METHODS

Study Area

Borno State occupies the greater part of the Chad Basin and is located in the North Eastern part of Nigeria. The state comprises of 27 local government areas and shares boundaries with

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Republic of Niger, Republic of Chad and Cameroon. The Climate of the region is dry between November and April with the hottest months being April and May with a temperature range of about 31- 44°C, during which water consumption is very high. It is cold from November to February with the dusty harmattan peak period in December and January. The wet season begins between late April and May and last for about 5 months with rainfall of about 25 inches. Lakes Chad and Alau however, are the major water bodies in the region proving thousands of peoples with livelihood through fishing and farming.

Sample Collection

A total of 274 samples from boreholes (124) and lakes (150) collected over a one year period (Jan-Dec, 2011) in and around Maiduguri were analysed for the presence of *Aeromonas*. Water samples from lakes included water from Lakes Alau and Chad. Lake water samples were collected using scoop bottle as described by [12]. One liter of water was collected from several sites of the lakes about 1m below the water surface by immersion of 1 liter sterile plastic container; stored in a cold box and transported to the laboratory within 2- 8 hours of collection. Drinking water from boreholes within various wards in the Maiduguri Metropolis were collected aseptically in 100mls sterile containers according to standard procedures [13] and processed within 2 hours of collection.

Bacterial Isolation and Phenotypic Identification

The water samples from boreholes were analysed according to protocol used by Ghengesh *et al*. [14] with slight modification. Aliquot, 2.5 ml of the water samples were added to 25 ml of alkaline peptone water (APW, pH 8.6) for enrichment. After an overnight incubation at 37°C, a loopful from the APW was plated onto Ampicillin Dextrin Agar supplemented with 10 mg/l

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ampicillin and the plates were incubated at 37°C overnight. Raised convex yellow colonies (2-3 nm diameter) considered presumptive *Aeromonas* were picked up, re-streaked onto Rimlershotts plates for another 24hrs at 37°C. Yellow colonies were picked and subcultured onto trypticase soy agar (TSA). All Gram negative, oxidase positive isolates were stored on slants for further biochemical characterization.

Biochemical Characterization (Speciation)

Preliminary aeromonads were identified to genus and species levels after conducting some conventional biochemical tests [15] and speciation based on Aerokey II group of tests for the identification and speciation of *Aeromonas* [16]. These included bile esculin hydrolysis, gas from glucose, acid from arabinose, mannitol and sucrose, indole production, Voges-Proskauer reaction and resistance to cephalothin (30 ug).

Haemolysis Assay: Hemolysis was assayed on tryptone soy agar plates with 5% whole sheep blood according to Benson [17]. Plates were incubated at 37°C and were checked for the type (*a* or *b*) of hemolytic activity after 24 hours by detecting a clear zone formed around colonies.

Chi-square test was used for comparison of the different variables. P value of <0.05 was considered to be statistically significant**.**

RESULTS

Prevalence of Aeromonas

A total, 85 *Aeromonas* spp were isolated from the total number of samples collected. For the boreholes, 31 *Aeromonas* pp were isolated from 124 samples whereas, 54 species were isolated from 150 lake water samples.

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Table 1 shows the species identification and distribution of the *Aeromonas* isolates among the different samples form lakes. Overall, 54 (36%) *Aeromonas* spp were isolated with 38 (38%) and 16 (32%) from lakes Chad and Alau respectively. There was no statistically significant difference in the number of isolates between the two water sources. *A. hydrophila* was the most common specie with an overall prevalence of 91% followed by *A. sobria* with 9%.

Table 2 presents the location of boreholes and distribution of *Aeromonas* in the various wards. Overall, 31(25%) *Aeromonas* spp were isolated from the boreholes. Water samples from boreholes located at Gwange ward had the highest rate of occurrence of the organism (87.5%) followed in descending order by Galadimashuwarin (45%), London ciki (30%), Bulabulin (25%), Custom (15.8%), and University of Maiduguri staff quarters (10%). The water samples from Mairi and Maduganari wards did not yield any *Aeromonas* isolate.

Table 1: Prevalence and species distribution of *Aeromonas* in Lakes Alau and Chad

Sampling site	Number examined	Species			
		A. Hydrophila(%)	*A. sobria(%)*	*A. caviae(%)*	*Total No.(%)*
Lake Chad	100	34 (69.4)	4 (10.5)	0	38 (38)
Lake Alau	50	15 (30.6)	1 (6.3)	0	16 (32)
Total	**150**	**49 (90.7)**	**5 (9.3)**	**0**	**54 (36)**

X^2= 0.521, df = 1, P= 0.470, P>0.05 (Not significant)

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Table 2: Frequency of isolation of Aeromonas species according to location of Boreholes

Sampling site	Number of samples	Number positive	Percentage (%)
Mairi	7	0	0
Gwange	8	7	87.5
Custom	19	3	15.8
Unimaid staff qtrs.	10	1	10
Bulabulin	20	5	25
Galadimashuwarin	20	9	45
Maduganari	20	0	0
London ciki	20	6	30
TOTAL	**124**	**31**	**25**

DISCUSSION

Results from this study shows that fifty four (54) *Aeromonas* isolates were detected in the water samples from the Lakes with 38% and 16% occurrence from Lakes Chad and Alau respectively. Occurrence of these organisms at this rate is substantial considering the socio-economic significance of the water sources. The people living near the bank of the lake not only use it for fishing but also dry season farming and domestic purposes such as washing. The abundance of *Aeromonas* spp in these Lakes, particularly Lake Alau could be due to some environmental factors, such as Ammonia and nitrates from fertilizers and cleaning agents. This result corroborates well with Parveen *et al*. [1] and Zaky *et al*. [2] who reported abundance of Aeromonads in rivers and lakes. *Aeromonas* species are commonly isolated from lakes, rivers, ground water, drinking water as well as variety of foods [1, 2, 14].

A. hydrophila and *A. sobria* were the only species isolated from the lakes in the present study, with *A. hydrophila* as the most prevalent (91%) species. The result agrees with the findings of Hayes [18] who reported that *Aeromonas hydrophila* was the most well-known of the species belonging to the genus *Aeromonas* that inhabits aquatic environments.

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Water samples from shallow aquifers commonly referred to as boreholes collected from various wards within Maiduguri metropolis revealed that boreholes water from Gwange ward had the highest prevalence of *Aeromonas* (88%), while no *Aeromonas* was isolated from Mairi and Maduganari wards of the metropolis. The high isolation rate in Gwange ward could be attributed to the fact that the wash boreholes that supply the area with water are very shallow with depth of about 20 metres or less and the area is located near the bank of river Alau which is highly contaminated with human and animal wastes. In addition, the ward is densely populated with majority of houses using pit latrines that are almost of the same depth with the boreholes. This scenario could create potentials for the contamination of underground water resulting from seepage. *Aeromonas* spp are frequently found in faecal polluted water and their presence in drinking water is well documented [19, 20].

In Borno State and other parts of Nigeria where less than 30% of the population has access to potable drinking water, it is common to use water obtained from shallow wells and other untreated sources for drinking, bathing and other purposes. This may be hazardous to individuals with wounds, lacerations or abrasions. *A. hydrophila* and *A. sobria* were the 2 species isolated from these samples with *A. hydrophila* as the most dominant specie. These results are consistent with the findings of others [21, 22] who reported that *A. hydrophila* was the predominant species in freshwater and municipal drinking water supplies.

The findings of this study confirmed the presence of potentially pathogenic *Aeromonas* species in the studied lakes and water samples from commercial boreholes in Maiduguri metropolis. These sources of water are used for fishing, drinking and household chores. As *Aeromonas* is considered an emerging pathogen and can cause health problems, proper surveillance of water

sources is strongly recommended to protect susceptible individuals from exposure to potentially pathogenic *Aeromonas* strains.

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