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Original paper

Prevalence of *Aeromonas* Species in Treated and Untreated Waters in Parts of Plateau State, Nigeria

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ABSTRACT: Emerging infectious diseases are a significant burden on global economy and *Aeromonas* infections remain among those infectious diseases of potentially serious threat to public health. For this reason, the present study was aimed at determining the prevalence of *Aeromonas* species in treated and untreated waters in parts of Plateau state, Nigeria. The study was carried out in four local government areas of Plateau state comprising of Jos North, Jos South, Jos East and Bassa local government areas. Eight hundred (800) water samples including Tap water (160), well water (160), dam water (160), sachet water (160) and bottled water (160) were randomly collected in 300 ml sterile storage bottles from different sites while the bottled water and sachet ("pure water") were purchased from different vendors within the sampling area. Isolation, enumeration and identification of *Aeromonas* species were carried out according to standard procedures. Out of the 800 water samples examined for *Aeromonas* species, 386 (48.25%) were positive. Among the water samples examined, dam water yielded the highest percentage prevalence of *Aeromonas* with a percentage of 19.25%, while the lowest was bottled water with a percentage of 1.37%. Statistically, there was a significant difference ($p<0.05$) in the frequency of isolation of the organism with regard to treated and untreated water at 95% confidence level. There was also a significant difference ($P<0.05$) between the prevalence of *Aeromonas* species in water with regard to the sources at 95% confidence level. This study revealed that there is a high prevalence of *Aeromonas* species in the various water samples examined and this may pose a health risk especially for immune compromised individuals, therefore, proper personal hygiene and the provision of potable water is highly advocated.

Keywords: *Aeromonas*, prevalence, water, public health, infectious diseases

INTRODUCTION

The genus *Aeromonas* comprises of gram-negative, non-spore forming rod-shaped, facultative anaerobes that are widely distributed in aquatic environments (Daskalov, 2006). Aeromonads are straight, cocco-bacillary to bacillary bacteria with rounded ends measuring 0.3 - 1.0 × 1.0 - 3.5 µm (Parker and Shaw, 2011). *Aeromonas* spp. are found in freshwater, groundwater, brackish water, seawater and a host of foods including meat, milk and salad vegetables (Ansari et al., 2011). They are also found in sinks, drain pipes and household effluent and have been isolated from ice-cream, poultry and soil (Janda and Abbott, 2010; Araujo et al., 1991). Thus, food and water are the probable sources of human infections (Khajanchi, et al., 2010). Some *Aeromonas* species have the intrinsic ability to grow in water distribution systems, especially in biofilms, where they are unaffected by chlorination (Chauret et al., 2001). The occurrence of *Aeromonas* species in the aquatic environment has been

recognized as a potential health risk and some countries have embraced aeromonad counts as indicator of water quality (Borchardt et al., 2003). Biochemical analysis proved that *Aeromonas* species are oxidase and catalase positive bacteria that are motile by polar flagellum. They belong to the family *Aeromonadaceae* alongside other genera such as *Oceanimonas*, *Oceanisphaera* and *Tolumonas* (Igbinosa et al., 2012). The pathogenesis of infection of this emerging pathogen is complex and multifactorial (Janda and Abbott, 1998; Chopra et al., 2000). Due to the fact that *Aeromonas* are commonly isolated from the aquatic environment and are associated with gastroenteritis and wound infections in humans, studies concerning the presence of these organisms in raw and drinking water distribution systems are very important to ensure the good quality of public water, resulting in protection and promotion of public health (WHO, 2006).

Aeromonas species have been associated with natural disasters (hurricanes, tsunamics and earthquakes) and had been linked to emerging illnesses including near drowning events, prostatitis and haemolytic- uraemic syndrome (Janda and Abbott, 2010). Thus, some authors have suggested that the quantitative search of *Aeromonas* spp. should be included among the indices of the bacteriological quality of water (Araujo et al., 1989). Drinking water should be free of pathogenic microorganisms for the sake of public health. In many epidemiological investigations, it has been shown clearly that there is a link between water sources and *Aeromonas*-mediated infections (Edberg et al., 2007). Transmission of the outbreak of *Aeromonas* is through the consumption of contaminated water and food. Poor sanitization and lack of proper access to potable water are well documented routes of infection (Ghenghesh et al., 2008). Recently other vehicles of transmission such as acidic foods, sea foods, yoghurts and fruits have been documented (Daniel et al., 2015). The prevalence and distribution of *Aeromonas* in aquatic environments, its role as a contaminant for drinking water supplies and potential for pathogenicity mediated by mesophilic *Aeromonas* are all of great public health concern (Dumontet et al., 2000). Therefore, the present work was aimed at determining the prevalence of *Aeromonas* species in treated and untreated waters from different parts of Plateau State.

MATERIALS AND METHODS

Study area

The study area included: Jos, Bukuru, Vom, Miango and Lamingo towns all within Jos North, Jos South, Bassa and Jos East Local Government Areas of Plateau State.

Sample collection

Eight hundred (800) water samples from different sources were randomly collected in 300 ml sterile storage bottles from different locations within the study area while the bottled water and sachet (“pure water”) were purchased from different vendors within the sampling areas. Samples were collected in triplicates during each sampling period. The samples were clearly labeled and immediately taken to the laboratory for microbiological analysis which included isolation, identification and characterization of *Aeromonas* species. The samples were brought to the laboratory in ice boxes containing ice packs held at 4^{O}C and analyzed immediately according to the procedure described by Cheesebrough (2005).

Isolation of *Aeromonas* species

Examination of water

The microbiological quality of the collected water samples was determined by the multiple tube technique of WHO (1996) and the membrane filter technique. The multiple tube technique detects the total faecal coliforms as a measure of the sanitary quality of water. This method was used for the detection of *Aeromonas* species from untreated water such as wells and dams. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) of the water samples were made using alkaline peptone water and 0.5 ml. of each dilution was spread onto Ampicillin Dextrin Agar (ADA), *Aeromonas* Ampicillin Agar (AAA) and Mac-Conkey Agar (MCA) in five replicates and incubated for 24 hours after which the plates were examined for growth.

The membrane filter technique described by Environmental Protection Agency [EPA], (2001) was used for the detection of *Aeromonas* in treated water (sachet water, tap water and bottled water). This method uses a highly porous cellulose membrane with pore space of 0.45 µm. A known volume of water sample (100 ml) was allowed to pass through the membrane filter under pressure, bacteria that were present in the water samples were retained on the surface of the filter which was then placed aseptically on petri-dishes containing ampicillin dextrin agar, *Aeromonas* ampicillin agar and Mac-Conkey agar.

The membrane filters were first incubated in alkaline peptone water for 4 hours before transferring onto the agar plates. The petri-dishes were then incubated at 37^{O}C for 24 hours after which the plates were examined for the presence of *Aeromonas* species. Grayish raised moist colony which is typical of *Aeromonas* species or a yellowish- green colony on ampicillin dextrin agar were identified and sub-cultured onto nutrient agar to obtain pure cultures.

Identification and characterization of *Aeromonas* species

The identification of *Aeromonas* spp. was carried out using both bacteriological and biochemical tests (Martin-Carnahan and Joseph, 2005).

Microbiological examination of isolates

The isolates were differentiated on the basis of their cultural and cellular morphology such as growth type, shape, elevation, size, pigmentation and consistency by employing both macroscopic and microscopic processes. After this they were subjected to various biochemical tests using oxidase, catalase, esculin hydrolysis, the Voges-Proskauer (VP) reaction, gas from glucose, growth on mannitol and sorbitol etc. *Aeromonas* isolates were further characterized phenotypically using API 20E**.**

Statistical analysis

The Pearson's chi-square test and the analysis of variance (ANOVA) test were used for comparison of the different variables, p value of <0.05 was considered to be statistically significant.

RESULTS

The results of the studies indicated that out of the 800 water samples examined for the presence of *Aeromonas* species, 386 were positive making a total of 48.25% prevalence. The results of the prevalence of *Aeromonas* spp. isolated from different water sources (Figure 1), showed that water from the dam had the highest rate of prevalence of 154 (19.25%) followed by water from well with a prevalence of 116 (14.5%). The water sample that had the lowest percentage prevalence was bottled water with a prevalence rate of 11 (1.37%) while Sachet water had 48 (6.0%) and tap water had 57 (7.13%).

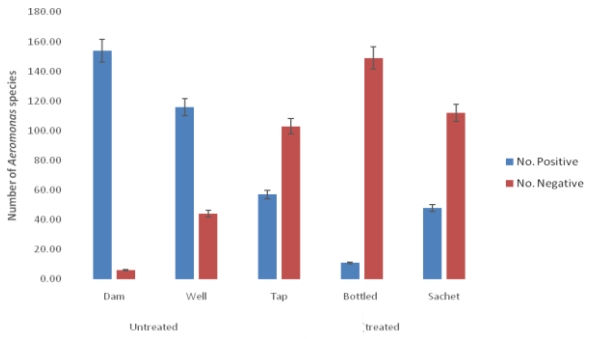

Figure 1: Percentage Prevalence of *Aeromonas* species isolated from different water sources

Statistical analysis showed that there was a significant difference (P<0.05) between the prevalence of *Aeromonas* species in water with regard to the sources at 95% confidence level. Similarly, there was a significantly higher (p<0.05) prevalence of the organism during the wet season 257 (32.1%) than the dry season 129(16.12%). Water samples were examined according to locations and it was observed that the water samples (Table 1) obtained from Lamingo dam located at Jos East Local Government Area of Plateau State had the highest percentage growth with a prevalence of 102 (12.75%) while the lowest was obtained from Jos town with a percentage prevalence of 61 (7.63%).

Table 1: Percentage prevalence of *Aeromonas* species isolated from water in the different locations

Locations	Sample Size	No. +%	No. -%
Jos	160	61(7.63)	99(12.38)
Bukuru	160	67(8.37)	93(11.62)
Miango	160	70(8.75)	90(11.25)
Vom	160	86(10.75)	74(9.25)
Lamingo	160	102(12.75)	58(7.25)
Total	800	386(48.25)	414(51.75)

Miango, Bukuru and Vom had percentage prevalence of 70 (8.75%), 67(8.37%) and 86 (10.75%) respectively. Out of the 386 isolates of *Aeromonas* species which were isolated from treated and untreated water samples (Table 2), 112 (29.01%) isolates were presumptively identified phenotypically as *Aeromonas hydrophila*, *Aeromonas veronii* was 48(12.43%), *Aeromonas caviae* 70(18.14%), *Aeromonas salmonicida* 15 (3.89%) and other species of *Aeromonas*, 141(36.53%). The result of the chi-square test conducted on the data of the different species of *Aeromonas* isolated from water indicated that there was a significant difference (p<0.05) in the prevalence of the organism with regard to the different species at 95% confidence level. This is presented in (Table 3).

Table 2: Prevalence of *Aeromonas* species isolated from treated and untreated water in percentages.

***Aeromonas* species**	**No. of isolates**	**(%) Prevalence**	
		Treated	**Untreated**
Aeromonas hydrophila	112	30(7.77)	82(21.24)
Aeromonas veronii	48	17(4.4)	31(8.04)
Aeromonas salmonicida	15	0(0)	15(3.89)
Aeromonas caviae	70	21(5.44)	49(12.69)
Aeromonas species	141	48(12.44)	93(24.09)
Total	386	116(30.05)	270(69.95)

Table 3: Prevalence of *Aeromonas* Species Isolated from Treated and Untreated Water.

Organisms	Treated	Untreated	p-value
Aeromonas hydrophila	30.00[b]	82.00[a]	0.003
Aeromonas veronii	17.00	31.00	0.162
Aeromonas salmonicida	0.00[b]	15.00[a]	0.140
Aeromonas caviae	21.00[b]	49.00[a]	0.027
Aeromonas species	48.00[b]	93.00[a]	0.005
Total	116.00[b]	270.00[a]	0.000

Means having the same superscript letter are significantly different ($p<0.05$) at 95% confidence level using Duncan Multiple Range Test (DMRT).

DISCUSSION

The potential of water to harbor microbial pathogens and cause subsequent illness is well documented for both developed and developing countries (Okonko et al., 2009). The recovery percentages of all *Aeromonas* species identified in this study were found to be higher in untreated water (dam and well) compared to the treated water (sachet, tap and bottled water). These results are similar to those reported by Bello et al. (2016) in his work on the Detection of *Aeromonas* species from water sources in North Eastern Nigeria where the incidence of *Aeromonas* was as high as 38%. The occurrence of *Aeromonas* spp. in water in the present study is substantially high especially in dams and wells and considering the socio-economic significance of water, this could pose a threat for public health as *Aeromonas* sp. is known to be responsible for a wide variety of human illnesses (Janda and Abbott, 2010). The presence of *Aeromonas* in these water bodies in large quantities could be due to some environmental factors such as ammonia and nitrates washed into the water bodies from fertilizers (Bello et al., 2016). In like manner, animal dung used as organic fertilizers could also be washed from the soil during the raining season into the water body causing proliferation of the organism. This may account for the reason why the organism is more prevalent in the wet season as compared to the dry season. This observation is in consonance with the report of Kamalpreet et al. (2017) who reported the isolation of more *Aeromonas* species from salad vegetables in the wet season than the dry season in Punjab, India.

The findings of this study showed that Lamingo village had the highest percentage prevalence of *Aeromonas* followed by Vom while Jos had the least percentage prevalence. The difference in the percentage prevalence of the organism among some of these study area could be attributed to the differences in environmental hygiene and the sanitary practices of the people. In Iraq, Al-Khalidi, (2006) reported that the most frequent species which had been isolated from river water was *Aeromonas hydrophila* (58.8%). While Evangelista-Barretol et al. (2010) reported that *Aeromonas caviae* were the predominant species, the report of this study agrees with the report of the first author but disagrees with the report of the second author because *A. hydrophila* was the most predominant species isolated with a prevalent rate of 29.01%. It also agrees with the findings of Hayes (2003) who reported that *Aeromonas hydrophila* was the most well known of the species belonging to the genus *Aeromonas* that inhabits aquatic environments.

The findings of this study also corroborated the findings of Parveen et al. (1995) and Zaky et al. (2011) that reported the abundance of *Aeromonas* species in Dams and streams and indicated that water is a major reservoir of *Aeromonas* species. Studies had shown that *Aeromonas* species isolated from water have different virulence factors that can cause diseases in man as well as in other animals (Nam and Joh, 2007). *Aeromonas* species were able to survive in some of the bottled and sachet water examined in this study, these water samples are known to be treated with chlorine which is supposedly able to destroy the organism.

This observation agrees with the research work of Massa et al. (1999) who reported that certain strains of *Aeromonas* sp. are resistant to the usual chlorine concentrations used for purified drinking water. The same observation was also reported by Mete et al. (2011) who isolated *Aeromonas* from tap water samples in the city of Denizli. Similarly, Adegoke and Ogunbanwo, (2016) also isolated *Aeromonas* from drinking water in Ibadan, Nigeria. Moreover, Ghanem, et al. (1993) reported that 90% of domestic water supplies in areas of Cairo contained *Aeromonas*.

From the present study, it has been shown that *Aeromonas* spp. is found in water samples in parts of Plateau state. As transmission of the pathogen occurs through the oral route via contaminated water and food or by contact with muddy waters, the potentials for its transmission are enormous especially in areas where poor sanitary practices are common and where there is no access to potable water. Since water-related diseases continue to be one of the major health problems globally, one of the strategies for tackling the problem of water-borne diseases caused by *Aeromonas* species or it possible transmission from person to person is the provision of protected sources of water such as boreholes, taps and spring. Secondly, it was observed from this study that the presence and the pathogenic effect of some species of *Aeromonas* (*A. hydrophila*, *A. caviae* and *A. veronii*) in the study area seem to go unnoticed. However, the findings of this study so far have not only revealed the existence of this pathogen but it's possible impact on the public health of people of Plateau state in particular and the Nigeria nation in general.

In view of this, the organism should be given adequate recognition as a potential pathogen and be included in the routine screening for pathogenic organism in our hospitals.

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