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Prevalence of *Aeromonas* Species among Patients Attending Five Hospitals in Jos and its Environs

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ABSTRACT

Members of the genus *Aeromonas* are gram negative, non-spore forming, zoonotic bacteria that are widely distributed in aquatic environment. They affect both man and animals causing diseases such as gastroenteritis, peritonitis, endocarditis, pneumonia and ocular infections. The aim of this study was to determine the prevalence of *Aeromonas* species among patients attending five hospitals in parts of plateau state. Eight hundred human stool samples were examined for the presence of *Aeromonas* species using the process of isolation, identification and characterization. Out of the 800 samples 65 (8.12%) were positive for *Aeromonas*. Among the hospitals screened, Jos University Teaching Hospital (Lamingo) has the highest prevalence of 15 (1.87%) followed by Our Lady of Apostle Hospital (Bukuru) with a prevalence of 14 (1.76%). The lowest percentage prevalence was recorded for the specimen collected from Faith Alive Foundation Hospital (Jos) 11 (1. 35%). The percentage prevalence of *Aeromonas* spp. among study population by gender showed that the female had a higher percentage prevalence of 37 (4.63%) of *Aeromonas* infection than the male with a prevalence of 28 (3.47%). The prevalence of *Aeromonas* species among study population by age distribution showed that the highest prevalence was among children below the ages of 1-10 years with a percentage prevalence of 22 (2.75%). There was a significantly higher ($p<0.05$) prevalence of the organism during the wet season (71%) than the dry season (29%). This study indicated that *Aeromonas* is prevalent in the study population, therefore enlightenment programs should be carried out by the Plateau state ministry of health to create awareness on the dangers of *Aeromonas* infections.

KEY WORDS: *Aeromonas*, Hospital, Gastroenteritis, Infection, Population, Patients

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INTRODUCTION

The spectrum of infectious diseases caused by *Aeromonas* species includes gastrointestinal infections as well as extra intestinal infections such as cellulites, wound infections, urinary tract infection, hepatobiliary and ear infections (Vila et al., 2003). *Aeromonas* species are found globally in surface water, groundwater, chlorinated drinking water, bottled mineral water and a broad range of foods (McMahon &Wilson, 2001). These organisms have long been known to cause disease in fishes, reptiles and amphibians. In recent years, however, their role as opportunistic pathogens implicated in human illnesses such as gastroenteritis, wound infections, septicemia, pneumonia and soft tissue conditions, has gained increasing interest (Mukhopadhyay et al., 2008; Janda & Abbott, 2010). The chronic exposure of immune compromised persons to *Aeromonas* via contaminated foods and waters could potentially lead to invasive diseases (Leclerc & Dei-Cas, 2002). *Aeromonas* species are zoonotic affecting both man and animals leading to economic losses (Isoken et al., 2012). Apart from organisms such as *Escherichia coli*, *Salmonella typhi*, *Shigella* species, *Entamoeba histolytica* and *Vibrio cholera* that are known to cause diarrhoea, *Aeromonas* species has emerged as a significant cause of diarrhoea particularly in developing countries where diarrhoeal diseases constitute a very important cause of morbidity and mortality among children and young adults (WHO, 2004).

It has been reported that more than 800 million cases of diarrhoea occur annually in developing countries accounting for an estimated 4.5 million deaths (Oyofo, 2002). Children below the age of five years particularly those in areas without access to safe drinking water and proper sanitation, the elderly and the immune-compromised are most at risk (Ghenghesh et al., 2008). In Nigeria, Obi et al. (1997); Nzeako et al. (2002); Adegoke & Ogunbanwo, (2016) identified *Aeromonas* species as agents

of diarrhoea in urban and rural areas. *Aeromonas* are known to elaborate enterotoxins (haemolysin, adhesin, cytotoxins and lipase) which aid them in their pathogenicity (Uyttendaele et al., 2004). A variety of demographical, cultural, environmental and physiological factors are believed to play critical roles in enhancing the frequency of transmission of the pathogen to hosts. In recent years reports of the isolation of *Aeromonas* species from patients with diarrhoea in different parts of the world have increased. This development has also increased the level of awareness of their potential pathogenic role and geographical spread (Isoken et al., 2012). In Nigeria, as in other West African countries little is known about diarrhoea associated with *Aeromonas* species and since there is no routine screening for the organism in our hospitals, outbreaks of diseases associated with the pathogen may be overlooked or attributed to other sources. Furthermore, poor sanitary conditions, lack of potable water supply and lack of proper sewage disposal system are some of the predisposing factors for the transmission of the organism.

Aeromonas are considered emergent pathogens and its detection in various diarrheal stool samples clearly shows that they are not as far from clinical routine as other enteric microorganisms (Mbuthia et al., 2018). Consequently, interest about the genus *Aeromonas* which comprises of 36 recognized species has risen over the past years (Ahmed et al., 2021; Conte et al., 2021). According to Ahmed et al., 2021 and Meng et al., 2021, several case reports involving *Aeromonas* infections has brought up the diversity of clinical manifestations that these bacteria can evoke to human health. Symptoms range from acute self-limiting diarrhea to lethal sepsis; however, wounds, skin, bones, heart, lungs, eyes, and other organs can be potentially affected. *Aeromonas* possess wide spectra of antibiotic resistance profile and occurrence of multi-resistant strains have already been reported (Galler et al., 2018; Conte et al., 2021). As inhabitants of aquatic environments, these microorganisms can be used as ecological indicators of water pollution since they harbor antibiotic resistance genes obtained from wastewater effluents (Baron et al., 2017; Grilo et al., 2020). Though some research have been carried out on *Aeromonas* species on the Plateau, there is still a dearth of information on their prevalence among patients in attending some hospitals in Jos metropolis and its environs. This study was carried out to determine the prevalence of *Aeromonas* in these patients and generate data to aid in disease surveillance on the Plateau

SAMPLE COLLECTION

Eight hundred (800) Stool samples were collected in wide mouth screw capped bottles from patients (after obtaining ethical clearance) attending the Jos University Teaching Hospital (Lamingo) (160 samples), Our Lady of Apostle Hospital (Bukuru) (160 samples), Faith Alive Foundation Hospital (Jos) (160 samples), Cottage Hospital (Bassa) (160 samples) and Vom Christian Hospital (Vom) (160 samples). 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 using standard bacteriological procedures. The samples were brought to the laboratory in ice boxes containing ice packs held at 4^{o}C to avoid proliferation of the organism and the samples were then analyzed immediately

EXAMINATION OF HUMAN STOOL SAMPLES

Macroscopy: The appearances of all the stool samples were considered to see whether they were formed, semi-formed or watery in nature. They were also examined for the presence of blood, pus, mucus or worms.

Stool Microscopy: Using saline water and iodine with floatation technique, a drop of normal saline was placed on one end of a slide and a drop of iodine on the other end. With the aid of a wire loop, small amount of fresh specimen was mixed with both the normal saline and the iodine. Each preparation was covered with cover slip. The preparations were examined for parasites using ×10 and ×40 objectives with the condenser iris sufficiently closed to give a good contrast (Rogo et al., 2009).

Culture: A loop full of patient's stool was aseptically inoculated into 10 ml of trypticase soya broth (TSB). The mixture was homogenized for about 30 seconds, treated with 0.1 mg/ml ampicillin to reduce the load of normal flora (Islam et al., 2004) and then incubated at 37^{o}C for 2 hours. A 0.5 ml of the enriched suspension was further sub-cultured onto ampicillin dextrin agar, *Aeromonas* ampicillin agar and Mac-Conkey agar in five replicates by streaking and incubating for 24 hours at 37^{o}C in a canister with candle light to provide the micro-aerophilic environment required by *Aeromonas* species.

The second day, the agar plates were checked for grayish raised moist colony which is typical of *Aeromonas* species or a yellowish- green colony on ampicillin dextrin agar. Any colony resembling this was subcultured on a fresh ampicillin dextrin agar (to have pure colonies of the organism) and incubated at 37^{o}C overnight. The ampicillin dextrin agar plates confirmed to have pure growth of a single organism type were used to perform the biochemical tests.

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 data were subjected to statistical analysis of variance (ANOVA) and chi-square (X^2) test. Least significant difference (LSD) was used to test whether there was a significant difference between the means or otherwise. Each value presented represents a means of five values, each consisting of 5 replicates.

RESULTS

Prevalence of *Aeromonas* Spp. isolated from human stool samples in the various hospitals in relation to study area is presented in Table 1. A total of 65 (8.12%) stool samples were positive for *Aeromonas* out of 800 specimens examined. In this study, Lamingo has the highest prevalence of *Aeromonas* in humans with percentage prevalence of 15 (1.87%) followed by Bukuru with a prevalence of 14 (1.76%). The lowest percentage prevalence was recorded for the specimen collected from Jos 11 (1. 35%). The percentage prevalence of *Aeromonas* spp. among study population by gender showed that the female population had a higher percentage prevalence of 37 (4.63%) of *Aeromonas* infection than the male population with a prevalence of 28 (3.47%).

Statistically, there is no significant difference $p>0.05$ between the study population with regard to gender. Table 2 showed the prevalence of *Aeromonas* species among study population by age distribution, *Aeromonas* infection was more prevalent among children below the ages of 1-10 years with a percentage prevalence of 22 (2.75%). There was a high incident of *Aeromonas* in patients aged 51 and above 16 (2.0%). The isolation rate of *Aeromonas* from patients between the ages of 11-20 years, 21 – 30 years, 31 – 40 years and 41 – 50 years were 5 (0.63%), 5 (0.63%), 6 (0.75%) and 11 (1.38%) respectively. The prevalence of *Aeromonas* was higher in human stool in the wet season than the dry season (Figure 1) with a percentage prevalence of 46 (71%) and 19 (29%) respectively. Statistically, p value equal to 0.04 therefore $p<0.05$, which means there is a significant difference in the prevalence of *Aeromonas* species in human stool with regard to season at 95% confidence level. The effect of sources on the percentage prevalence of *Aeromonas* infections among respondents in the various age groups are presented in figure 2, the results showed that patients from the hospitals in Jos had the lowest prevalence while those from Lamingo had the highest.

Table 1 Prevalence of *Aeromonas* Infection among Respondents in Relation to the Study Area

Study Area	Sample Size	Prevalence of *Aeromonas* in Humans			Chi square	P-value
		Male	Female	Total		
Jos	160.00	3.00	8.00	11.00	2.22	0.695
Miango	160.00	7.00	6.00	13.00		
Bukuru	160.00	7.00	7.00	14.00		
Vom	160.00	4.00	8.00	12.00		
Lamingo	160.00	7.00	8.00	15.00		
Total	800.00	28.00	37.00	65.00		

Since $p>0.05$, there is no significant difference between the prevalence of *Aeromonas* spp. with regard to the various study areas and the gender at 95% confidence level

Table 2 Prevalence of *Aeromonas* Species among Study Population by Age Distribution

Age (Years)	Number Examined	Number Positive	Chi-square	P-value
1—10	134	22.00	24.00	0.24
11—20	134	6.00		
21—30	133	5.00		
31—40	133	5.00		
41—50	133	11.00		
>51	133	16.00		
Total	800	65.00		

Since $p>0.05$, there is no significant difference between the prevalence of *Aeromonas* with regard to age distribution at 95% confidence level.

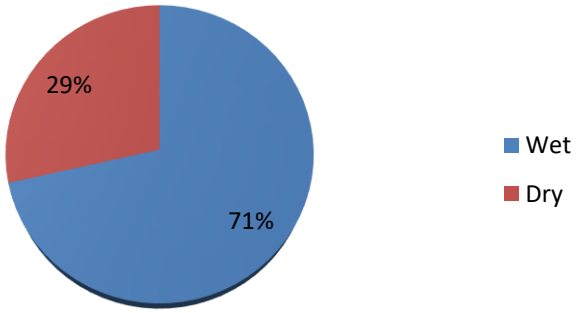

Figure 1: Effect of Season on the Prevalence of *Aeromonas* spp. Isolated from Human Stool Samples

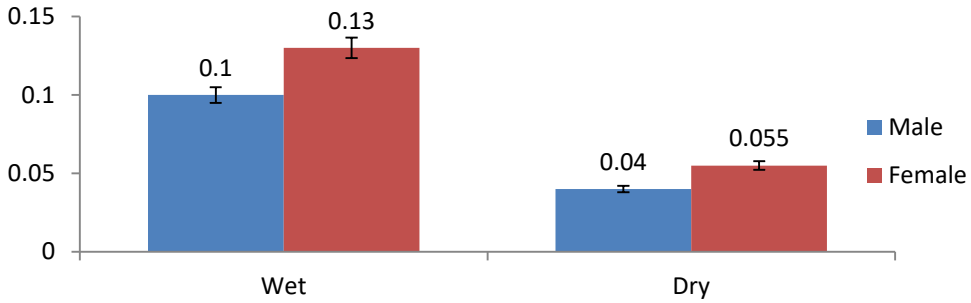

Figure 2 Prevalence of *Aeromonas* spp. Isolated from Male and Female Respondents in Wet and Dry seasons

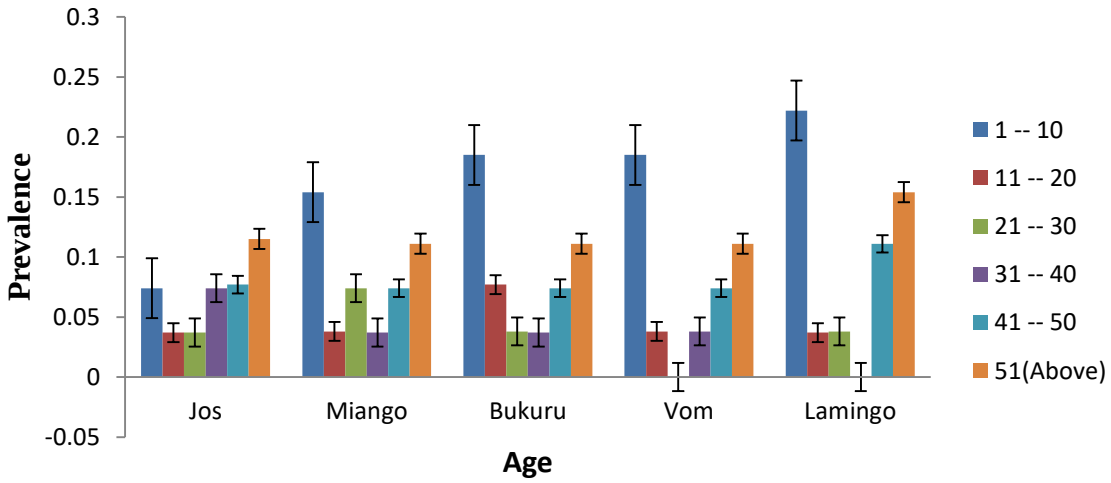

Figure 3 Effect of Locations on the Prevalence of *Aeromonas* species among Respondents in the Various Age Groups

DISCUSSION

A total of 65 (8.13%) human stool samples were found to be positive for *Aeromonas* in this study. This finding supports the report of other workers who isolated *Aeromonas* from clinical samples (Rogo et al., 2009; Figueras et al., 2011; Opara & Nnodim, 2014). Saad et al. (1995) reported an increased rate of *Aeromonas* spp. isolated from human stools during the summer months and linked it with increased incidence of *Aeromonas* spp. in fresh vegetables within the period. This is indicated in the present study where a higher percentage prevalence of the organism was observed in the wet season compared to the dry season. *Aeromonas hydrophila* is the most prevalent species isolated in this study. This result 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 inhabit the aquatic environments. Overall, the percentage prevalence of *Aeromonas* was highest in Lamingo followed by Vom while Jos had the least percentage prevalence. The difference in the percentage prevalence of the organism

among some of the study areas could be attributed to the differences in environmental hygiene and the sanitary practices of the people in these areas.

The findings of the present study indicated that *Aeromonas* species was present in 65 out of 800 stool samples screened. This result is lower than that of Opara and Nnodim (2014) and Rogo et al. (2009) who reported the detection of 64(21.33%) *Aeromonas* out of 100 stool samples and 47(8.30%) out of 566 human stools screened respectively. On the contrary, Jawetz et al. (2007) reported much lower percentage isolation of the pathogen. The prevalence of *Aeromonas* among humans is higher in Lamingo than in Jos metropolis. Statistically, there is no significant difference at $p>0.05$ between the prevalence of the pathogen with respect to the study areas. The prevalence of *Aeromonas* in humans in the current study suggests that the organism may be responsible for some of the diarrhea cases reported in hospitals. Rogo et al. (2009) in their study among respondents that presented with Diarrhea attending ABUTH Zaria and AKIH Kano, Nigeria, expressed similar opinion.

With regards to the season, there is a significant difference ($p<0.05\%$) in the prevalence of *Aeromonas* with the organism having a higher prevalence in the wet season than the dry season. Most of the respondents do not seek medical attention when they are sick, therefore the actual prevalence of the pathogen may not be detected through this type of laboratory based surveillance; hence, the actual rate of disease that occurred among the human population in the study areas could be higher than described. Furthermore, the recovery of *Aeromonas* has been reported to be optimal when stool specimens are obtained within 5 days after the onset of diarrhoea (Nzeako et al., 2002). Thus the rate of isolation of *Aeromonas* also decreases with delay in collection of stool sample. The present report support studies performed in Libya by Ghenghesh et al. (2015), which indicated that enteric infection with *Aeromonas* is a common occurrence. In this study, female respondents have higher percentage prevalence of *Aeromonas* infection than male respondents. These results disagreed with the report of Ghenghesh et al. (2008) who in Libya, found that twenty (56%) of 36 respondents infected with *Aeromonas* were males. The reason why the female subjects would be more vulnerable to *Aeromonas* infection than their male counterparts in this study could most probably be attributed to the fact that the females are occupationally more exposed to the pathogen. This exposure of the females to *Aeromonas* infections may include cloth washing, cooking, cleaning, fetching of water and recreational activities which all has to do with the use of water recalling that *Aeromonas* is mostly an aquatic organism. They can become vulnerable to *Aeromonas* infections by this means as water and food are two very important vehicles for the transmission of the organism (McMohan & Wilson, 2001).

The report of the present study has demonstrated that all age groups were infected by the pathogen, but the highest percentage prevalence of the infection was recorded among the age group of 1-10 years. This was followed by the age group of 51 years and above. The report of this study agrees with that of previous report (Rogo et al., 2009; Opara & Nnodim, 2014), which revealed that children and the elderly had been identified to be more susceptible to the infection with *Aeromonas* than other age groups. The high infection rate among the children could be due to their unguarded eating as well as water drinking habits while the elderly may have weak immune systems and therefore lack the ability to resist most infections especially if they have no access to potable water and live in environments that are hygienically unclean.

The result of the present study has demonstrated that there was a variation in prevalence of *Aeromonas* isolated from respondents during the rainy season and dry season, with *Aeromonas* occurring more in the wet seasons than the dry seasons. The higher isolation rate of *Aeromonas* recorded among the infected respondents during the wet season could be due to the fact that since infection is usually through the oral route, most of the vehicles of infection such as food and water might possibly have been contaminated by waste materials from humans and animals which could be washed by rains into water bodies and thus serve as sources of infection. In like manner, fresh pasture abounds during the wet season which calls for high animal activities in relation to grazing with subsequent soil pollution. Vegetable materials on such polluted soil when consumed could serve as a source of infection. The present report is in consonance with the report of Ghenghesh et al. (2015) which revealed that *Aeromonas* is one of the common bacterial pathogen isolated from diarrhoiec stool in Libya. Diarrhoea, septicaemia, endocarditis and other diseases caused by *Aeromonas* continues to be a problem because people lack enough information about the organism (Daskalov, 2006). The effect of sources on the percentage prevalence of *Aeromonas* infection among patients in the various age groups showed that patients from the hospitals in the Jos area had the lowest prevalence while those from Lamingo had the highest.

In conclusion, the study has emphasized the importance of including *Aeromonas* in the search for the differential diagnosis of diarrhoea alongside other enteric bacteria. Though *Aeromonas* does not pose a big threat like known organisms such as *Vibrio cholera* and *Salmonella*, they are silent threats that can cause a lot of damages by causing serious infections which could be life threatening. It appears that there are no asymptomatic patients of *Aeromonas* infections in the area studied so more work need to be done. The study has also established the fact that *Aeromonas* spp. existed in the various locations where samples were taken for investigation and that they are more prevalent during the wet season than the dry season. Good sanitary measures among individuals, homes, food industries and water treatment plants is recommended to help in curbing the menace of diarrhoea caused by *Aeromonas* infection.

Food processors and consumers must adopt hygienic methods to reduce the danger of food-borne disease transmission. The risk factors for *Aeromonas* infections are much in areas where poor sanitary practices are common and where there is no access to potable water.

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