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The Prevalence of *Aeromonas* Species in Salad Vegetables Sourced from Four Local Government Areas of Plateau State

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ABSTRACT

The current study was conducted to determine the prevalence of *Aeromonas* species in five salad vegetables, namely lettuce, cabbage, carrot, cucumber, and green pepper, in four local government areas of Plateau state. *Aeromonas* species have been linked to a number of gastroenteritis outbreaks connected to the consumption of contaminated fresh vegetables and fruits. In Nigeria, the use of untreated wastewater in irrigation is often regarded as an unavoidable alternative for compensating for water scarcity. This study was conducted to determine the prevalence of *Aeromonas* species in salad vegetables from four local government areas of Plateau State. 800 lettuce samples, including 160 lettuces, 160 cabbages, 160 carrots, 160 cucumbers and 160 green peppers, were analyzed for the presence of *Aeromonas* species using standard isolation, identification and characterization methods. Of the 800 samples, 202 samples were positive. *Aeromonas* spp. was isolated from 60(7.50%) lettuce samples, 52(6.50%) cabbage samples, 35(4.38%) carrot samples, 30(3.77%) cucumber samples and 25(3.12%) green pepper samples making lettuce the vegetable with the highest prevalence of the organism. According to the findings, samples of salad vegetables from Lamingo had the highest percentage prevalence of *Aeromonas* species, at 45 (6.25%), followed by samples from Miango, at 42 (5.25%), and samples from Vom, at 37 (4.64%) had the lowest percentage prevalence of the organism. According to the statistical analysis's findings, the prevalence of the organism in various vegetable types with relation to the study locations did not differ significantly (p>0.05) at the 95% confidence level. The incidence of *Aeromonas* species isolated from the various study regions varied significantly depending on the season, with the organism being more prevalent during the wet season (3.7%) than during the dry season (1.35%).

KEYWORDS: Aeromonas, Prevalence, Gastroenteritis, Vegetables, Salad, Samples

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INTRODUCTION

Aeromonas species are waterborne microorganisms that have been recovered from animal and plant-based food products and are frequently implicated as the causative agent of clinical illnesses (Aarestrup et al., 2008). These are gram-negative, facultative anaerobes, non-spore producing rod-shaped bacteria with polar flagella that move. *Aeromonas* species can cause disease in humans and aquatic organisms, as well as contribute to food spoiling (Rahman et al., 2007). *Aeromonas* is an enterobacteriaceae member with the ability to create toxins that allow it to cling to the intestinal wall, resulting in diarrhoea and haemorrhage symptoms (Carnahan et al., 1991). They are known to cause gastroenteritis, meningitis, septicemia, pneumonia, peritonitis, cellulitis, wound sepsis with necrosis, gangrene, urinary tract and ocular infections (Janda & Abbott, 2010).

Aeromonas species are zoonotic, harming both humans and animals, resulting in massive economic losses (Isoken et al., 2012). *Aeromonas* species have been isolated from diarrhoea patients all across the world in recent years, according to reports. This advancement has also raised awareness of their potential pathogenic role and geographical spread (Igbinosa et al., 2006). Little is known about diarrhoea caused by *Aeromonas* species in Nigeria and other West

African nations, and because there is no routine screening for the organism in our hospitals, outbreaks of infections caused by the pathogen may be neglected or attributed to other causes. *Aeromonas* species are currently regarded as emerging food-borne diseases of significant relevance and have been included to the food Contaminant Candidate List (Pablos et al., 2010).

There is evidence that the prevalence of *Aeromonas* infections in developing countries may be underestimated, and that routine endemic exposure to waterborne and food-borne pathogens may occur more frequently than previously thought (Ghenghesh et al., 2008). A number of demographical, cultural, environmental, and physiological emerging factors are anticipated to play important roles in increasing the frequency of pathogen transmission to hosts (Isoken et al., 2012). *Aeromonas* have garnered attention due to their capacity to grow at refrigerated temperatures, relationship with salad vegetables, acquisition of antibiotic resistance, and ability to persist in food safety procedures (Berger et al., 2010).

"Despite the nutritional and health benefits of fresh food, salad vegetables are susceptible to contamination by soil and water microorganisms, resulting in increased gastroenteritis outbreaks" (Ghenghesh et al., 2015). Salad vegetables, which are strong in energy and high in minerals, vitamins, fibres, and phenolics, are an important dietary group that is linked to individual health and well-being and helps to minimize the incidence of chronic diseases (Donkor et al., 2010). There are numerous sources of contamination of fresh vegetables due to interaction with disease-causing bacteria in the farm to table process, including both on and off the farm sources (Buckley & Howard, 1999).

Vegetables contaminated with pathogenic bacteria can cause human sickness while still on the plant at the farm, or during harvesting, transportation, processing, distribution, and marketing, or at home (Saad et al., 1995). Because minimally processed vegetables have a physical structure that is vulnerable to microbiological invasion, both microbiological and physiological activities may contribute to quality degradation and the spread of microbial contamination and illnesses (Gil, 2015). Animal faeces transport enteric bacterial pathogens into the agricultural environment. Water, soil, and compost/seeds are the most likely sources of crop contamination from excrement. Water can come into direct contact with crops in two ways: irrigation and the often overlooked pesticide or fertilizer diluents. Pathogens associated with these faeces can be mobilized during rain or aerosolized by high winds. Once mobilized in water, these pathogens can enter surface water, which is commonly used for irrigation, pesticide and fertilizer dilution in some growing regions (Kamapreet et al., 2017).

Antibiotic resistance in prevalent and harmful bacteria in vegetables helps to increase horizontal resistance propagation within diverse isolates. *Aeromonas* antibiotic resistance is on the rise, necessitating in-depth research on the organism. Additionally, poor hygienic conditions, a lack of drinkable water supply, and a lack of a competent sewage disposal system are some of the risk factors for the organism's transmission (Adegoke & Ogunbanwo, 2016). The goal of this study was to find out how common *Aeromonas* species were in salad veggies.

MATERIALS AND METHODS

Eight hundred (800) samples of salad vegetables, including 160 carrots (*Daucus carota* spp. *sativa*), 160 cabbages (*Brassica oleracea* var. *capitata*), 160 lettuces (*Lactuca sativa* var. *capitata*), 160 green peppers (*Capsicum annuum*), and 160 cucumbers (*Cucumis sativus*), were obtained from producers and merchants in the various markets located within the study areas. Each sampling period included the collection of three samples. The samples were appropriately labeled and taken right away to the lab for a microbiological study that included isolating, identifying, and describing *Aeromonas* species. The samples were brought to the lab in ice boxes with ice packs kept at 4 °C and were evaluated right away. Those that were not immediately examined were stored in the refrigerator for a maximum of three days in accordance with Cheesebrough's (2005) technique to reduce microbial development.

Examination of Salad Vegetable Samples

Weighed at 10 grams each, 90 ml of sterile distilled water was used to wash the samples of carrot, cabbage, lettuce, green pepper, and cucumber salad veggies. A loop-full of the deposit was inoculated into sterile alkaline peptone water and cultured at 37°C for 2 hours using aliquots of the washed water after centrifuging them for 10 minutes. In order to identify *Aeromonas* colonies, the inoculum was then sub-cultured onto the proper selective agar media (Ampicillin dextrin agar and Mac-Conkey agar) in five duplicates. This incubation period lasted 24 hours at 37 degrees Celsius. Picked up and re-streaked onto Ampicillin dextrin agar plates for an additional 24 hours at 37°C were raised convex yellow colonies (2–3 nm in diameter), which were ostensibly identified as *Aeromonas*. After the incubation time, the number of colony forming units (cfu/ml) was counted. All isolates that were Gram negative and oxidase positive were kept on nutrient agar slants for future biochemical and phenotypic analysis.

Statistical Analysis

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

RESULTS

202 of the 800 salad vegetables tested positive for *Aeromonas* species. *Aeromonas* spp. was identified from 60(7.50%)

lettuce samples, 52(6.50%) cabbage samples, 35(4.38%) carrot samples, 30(3.77%) cucumber samples, and 25(3.12%) green pepper samples (Table 1). Salad vegetables taken from Lamingo exhibited the greatest percentage prevalence of *Aeromonas* species 45(6.25%), followed by samples from Miango 42(5.25%), Bukuru 40(5.00%), Jos 38(4.75%), and Vom 37(4.64%) (Table 2).

At the 95% confidence level, statistical analysis revealed that there was no significant difference ($p>0.05$) in the prevalence of the organism in the different vegetable kinds with reference to the locations. The results showed that among the crops tested, lettuce from the farm had the highest prevalence rate, while green pepper from the market had the lowest number of positive samples. According to the findings (Table 3), salad vegetable samples collected from farms had the highest percentage prevalence (10.13%) of the pathogen, followed by those obtained from hawkers (8.50%), and those obtained from market places had the lowest (6.63%). Figure 1 shows a significant difference ($p<0.05$%) in the incidence of *Aeromonas* species in salad vegetables by season, with the prevalence rate during the rainy season being 148 (3.7%) compared to 54 (1.35%) during the dry season.

Table 1. Prevalence of *Aeromonas* Species Isolated from Salad Vegetables obtained from the Study Areas

Study Area	Cabbage	Carrot	Lettuce	Green Pepper	Cucumber
Jos	8.00^c	6.00^b	10.00^b	7.00^a	7.00^a
Bukuru	10.00^b	6.00^b	12.00^a	4.00^b	8.00^a
Vom	11.00^b	6.00^b	13.00^a	4.00^b	3.00^c
Miango	13.00^a	7.00^b	13.00^a	4.00^b	5.00^b
Lamingo	10.00^b	10.00^a	12.00^a	6.00^a	7.00^a
SEM	0.58	0.47	0.37	0.4	0.53

a-b-c: Means along the same row with different superscripts are significantly ($p<0.05$) different.

S.E.M= Standard Error of Mean

Table 2. Prevalence of *Aeromonas* Species Isolated from Salad Vegetables obtained from Various Study Areas in Percentages

Study Area	Cabbage	Carrot	Lettuce	Green Pepper	Cucumber	Total
Jos	8/32(1.00)	6/32(0.75)	10/32(1.25)	7/32(0.88)	7/32(0.88)	38/160 (4.76)
Bukuru	10/32(1.25)	6/32(0.75)	12/32(1.50)	4/32(0.50)	8/32(1.00)	40/160(5.00)
Vom	11/32(1.38)	6/32(0.75)	13/32(1.63)	4/32(0.50)	3/32(0.38)	37/160(4.64)
Miango	13/32(1.63)	7/32(0.88)	13/32(1.63)	4/32(0.50)	5/32(0.63)	42/160(5.25)
Lamingo	10/32(1.25)	10/32(1.25)	12/32(1.50)	6/32(0.75)	7/32(0.88)	45/160(6.25)
Total	52/160(6.50)	35/160(4.38)	60/160(7.50)	25/160(3.12)	30/160(3.77)	202/800(25.25)

Since $p>0.05$, there is no significant difference between the percentage prevalence of *Aeromonas* isolated from salad vegetables with respect to the various study areas at 95% confidence level.

Table 3. Effect of Sources on the Percentage Prevalence of *Aeromonas* Species Isolated from Salad Vegetables.

Sources	Cabbage	Carrot	Lettuce	Green Pepper	Cucumber	Total
Farm	$18/54(2.25)^b$	11/53(1.38)	$31/53(3.88)^a$	9/54(1.13)	$12/53(1.50)^a$	81/270(10.13)
Market	$14/53(1.63)^c$	12/54(1.50)	$12/53(1.50)^c$	7/53(0.87)	$8/54(1.00)^c$	53/265(6.63)
Hawker	$20/53(2.50)^a$	12/53(1.50)	$17/54(2.13)^b$	9/53(1.13)	$10/53(1.25)^b$	68/265(8.50)
Total	52/160(6.50)	35/160(4.38)	60/160(7.50)	25/160(3.12)	30/160(3.77)	202/800(25.25)
SEM	0.93	0.33	2.86	0.44	0.65	

a-b-c: Means along the same row with different superscripts are significantly ($p<0.05$) different.

S.E.M= Standard Error of Mean

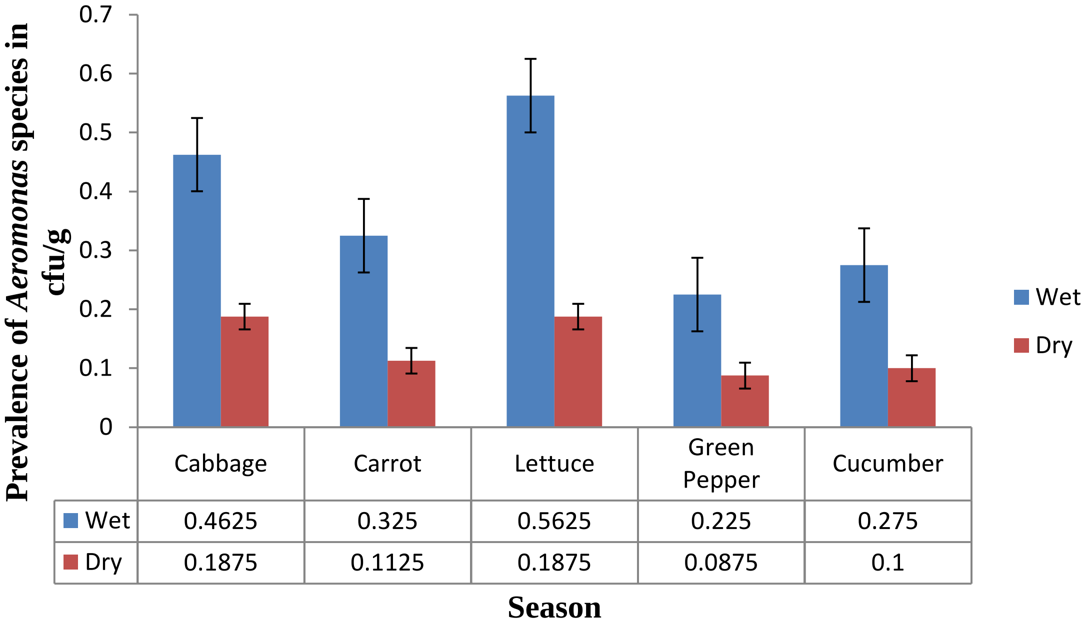

Figure 1. Prevalence of *Aeromonas* Species Isolated from Salad Vegetables in Wet and Dry Season

DISCUSSION

According to the findings of this study, some of the salad vegetables grown and sold in Jos city and its environs are infected with *Aeromonas* spp. at a prevalent rate of (25.25%). This finding is consistent with the findings of Qian (2012), who identified *Aeromonas* spp. from salad veggies provided to students at a college in Xingyi City, Guizhou, China, and that of Kamalpreet et al. (2017) who isolated *Aeromonas* from salad vegetables marketed in Punjab, India. Alcides et al. (2003) investigated the prevalence of *Aeromonas* in parsley and watercress samples obtained from a street market in Rio de Janeiro, Brazil. Among the 39 isolates of *Aeromonas* strains, 51.5% were enterotoxigenic.

Most pathogenic *Aeromonas* species displayed protease activity, with 48.5% being hemolytic and 100% showing protease activity. Proteases are enzymes capable of cleaving peptide bonds produced by gram-negative bacteria, which are thought to provide bacterial resistance (Sou et al., 2012). When compared to the other salad vegetables studied in this study, lettuce had the highest prevalence. This finding contrasts with the findings of Adegoke and Ogunbanwo (2016), who found a greater incidence in cabbage. "Since vegetables are carried from the farm before they reach the consumer, there are various risks of contamination of the vegetables owing to interaction with disease-causing microbes, both on and off the farm" (Beuchat, 2002). Given the significant prevalence of the organism in the salad vegetables studied, the findings of this investigation support the preceding conclusion.

According to Table 2, of the five salad vegetables analyzed, cabbage samples exhibited the second highest percentage prevalence of *Aeromonas*, followed by carrot. The increased percentage predominance of *Aeromonas* in lettuce could be attributed to the plant's near proximity to the ground, with the leaves exposed to soil microbes. The leaves also have a huge surface area, which allows for increased microbial infections. Sinha et al. (2004) reported that lettuce leaves protects microorganisms on it from external variables and increases the organism's survivability. *Aeromonas* prevalence at different sampling sites is not significantly different (p>0.05%), showing that the organism is present in all of them. These locations could be a source of microbial contamination.

Salad vegetable contamination can arise in numerous ways: Farmers irrigate their farms using water tainted with *Aeromonas* from dams and streams. 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). The high level of contamination of the cucumber in this study may have resulted through irrigation of the crop with contaminated water or direct contact of the fruit with the soil. The pH range of 4.9 to 6.0 found in cabbage and carrots creates an ideal environment for the growth and spread of bacteria. The survival of germs on vegetables is increased by other factors such as contaminated water, cross contamination, and improper management after harvest. Cucumbers grow on soil that can contain dangerous bacteria; their water activity and pH ensure the survival of the bacteria on them (Kersters et al., 1996). The current study's findings supported this assertion because the cucumber utilized in the study spoiled after a few days of storage.

Jeddi and Zare (2014) found that vegetables that come into contact with soil and animals during growing and harvesting are more likely to be contaminated by bacteria. Lettuce, cabbage, carrot, green pepper, and cucumber samples obtained from farms had greater log10 cfu/ml values than those obtained from markets. Hawked salad veggies had a slightly higher incidence of *Aeromonas* species than market salad vegetables. This could be as a result of dust exposure and inappropriate handling. According to McMohan and Wilson (2001), 34% of organic vegetables examined in their study named “The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables” were contaminated with *Aeromonas* spp. Callister and Agger (1987) discovered *Aeromonas hydrophila* on vegetables and concluded that retail vegetable produce could be a significant source of *A. hydrophila* gastroenteritis.

In their investigation on motile *Aeromonas* spp. in retail veggies from Sao-Paulo, Brazil, Saad et al. (1995) found *Aeromonas* in 47.8% of the vegetables studied. *Aeromonas* has also been found in restaurant lettuce (Garcia-Gimeno et al., 1996), pre-made salads (Gómez-López et al., 2007), and commercial vegetable salads (Sechi et al., 2002). Green pepper had the lowest incidence of *Aeromonas* among the five salad vegetable sample groups studied. The presence of *Aeromonas* species on these vegetables poses a significant risk to human safety, as some of these vegetables are not heated before ingestion. The first known outbreak of *Aeromonas* infection employed Parsley as the mode of transmission (Ghenghesh et al., 2008). *Aeromonas* has also been isolated from the following vegetables: radish, tomato, long melon, spinach, cauliflower, and broccoli (Mukhopadhyay et al., 2008). Vegetable contamination with *Aeromonas* species is assumed to occur on farms primarily through contaminated washing water, while soil and animal manure may also play a role (Ibenyassine et al., 2006).

The effect of seasons on the percentage prevalence of *Aeromonas* species isolated from various types of samples revealed that the bacterium was more common during the wet season than during the dry season. This result is consistent with the findings of Rogo et al., (2009), who discovered a higher incidence of the organism during the wet season than during the dry season. The findings also supported the work of Bartz (1982) on the penetration of tomatoes immersed at different temperatures and depths in Erwinia carotovora suspensions. The effect of source on the percentage prevalence of *Aeromonas* isolated from various sample categories revealed that the organism was more common in rural areas than in urban areas.

In conclusion, the study has established that *Aeromonas* spp. existed in the various study regions where samples were taken for investigation, indicating that the pathogen has the potential for public health concerns. According to the findings of this study, lettuce and cabbage represent a greater public health risk than the other salad vegetables. To lessen their chances of being engaged in the transmission of *Aeromonas*, they should be washed more completely before ingestion. There is a need to adopt food safety assurance systems in the salad vegetable manufacturing chain in order to improve their microbiological quality. Food processors and consumers must also adopt hygienic methods to reduce the danger of food-borne disease transmission. The findings of this study are important for public health because *Aeromonas* can cause diarrhoea and other disorders such as hemolytic uremic syndrome in both animals and humans. Most developed countries, including the United States, the United Kingdom, and Australia, have declared *Aeromonas* infection in humans and animals a reportable disease (Newell et al., 2010). It is suggested that Nigeria follow suit in order to raise awareness of the organism's presence in our environment and the hazards it represents. This study uncovered the possible dangers of ready-to-eat salads and underscores the need of surveillance studies in resolving public health issues.

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