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The Prevalence of *Aeromonas* Species Isolated from Raw and Locally Fermented Cow Milk from Four Local Government Areas of Plateau State, Nigeria

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ABSTRACT

Aeromonas species are non sporulating gram negative bacteria that are ubiquitous to the aquatic environment. Species such as Aeromonas hydrophila are pathogenic both to humans and animals causing gastroenteritis, peritonitis, endocarditis and meningitis. Milk with its high nutritional composition and neutral pH is a haven for microbial growth especially if not properly processed. Eight hundred milk samples comprising of raw milk (400) and locally fermented cow milk ("nono") (400) were collected from Jos, Bukuru, Vom, Miango and Lamingo located within four local government areas of Plateau state. Raw milk samples were obtained from the farms and retailers while the locally fermented cow milk ("nono") was purchased only from retailers. Each milk sample was collected in sterile well corked plastic containers, to prevent contamination in the course of transportation. The samples were examined for the presence of Aeromonas species using standard procedures. The isolates were subjected to cultural, biochemical, serological and phenotypic tests for the identification of Aeromonas species. The influence of seasonal variation on the prevalence of Aeromonas species in the milk samples was determined. Out of the 800 milk samples examined, 78 (9.75%) were positive for Aeromonas species. Among the two milk types investigated, raw milk had higher percentage prevalence, 53 (6.62%) than "nono" 25 (3.25%). The result of the effect of season on the prevalence of Aeromonas species isolated from milk, showed that a higher percentage prevalence of 59 (1.48%) of Aeromonas spp. was recorded in the two forms of milk during the wet season than during the dry season 19 (0.47%). Statistical analysis revealed a significant difference ($p<0.05$) ($p=0.002$) in the prevalence of the organism in both the raw and the fermented milk samples.

Keywords: *Aeromonas, species, gastrointestinal, milk, prevalence, fermented*

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INTRODUCTION

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). Several case reports involving Aeromonads infections brought up the diversity of clinical manifestations that these bacteria can provoke 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 (Ahmed *et al*., 2021; Meng *et al*., 2021). *Aeromonas* possess wide spectra of antibiotic resistance profile and occurrence of multi-resistant strains have already been reported (Conte *et al*., 2021; Galler *et al*., 2018). *Aeromonas* species are found in aquatic habitats and in a host of foods including meat, milk, salad vegetables and ready to eat foods such as ice-cream, meat pies, yoghurt etc. Milk is an excellent medium for the growth of numerous microbes which cause spoilage of food. *Aeromonas* species related to human health disorders grow well in higher temperatures and, consequently, lead to an increase in the number of infections under warm seasons due to the high bacterial cell count in the environment, especially in water (Bhowmick & Bhattacharjee, 2018). New syndromes attributed to this genus include hemolytic uremic syndrome, burns associated sepsis and a variety of respiratory tract infections (Janda & Abbott, 2010). Haemolytic uraemic syndrome a life-threatening condition normally associated with infections due to *Escherichia coli* 0157:H7, have also been associated with *Aeromonas hydrophila* infection (Bogdanovic *et al*., 1999; Robson *et al*., 1993). The role of aeromonads in water-borne infections has been established with *Aeromonas caviae* and *Aeromonas media* being the most prevalent (Khajanchi *et al*., 2010; Pablos *et al*., 2010). *Aeromonas hydrophila*, *Aeromonas caviae, Aeromonas veronii, Aeromonas sobria*, *Aeromonas jandaei* and *Aeromonas schubertii* are associated with peritonitis, meningitis, and various infections of the eye, joint and bone in humans (Janda & Abbott, 2010). *Aeromonas caviae, A. hydrophila,* and *A. veronii* biotype *sobria* have been associated with opportunistic infections of humans such as food-borne gastroenteritis, diarrhea and wound infections (Naviner *et al*., 2011). These infections are usually treated with antimicrobial agents, in both humans and animals, although increasing frequency of occurrences of antimicrobial resistance had been reported (Janda & Abbott, 2010).

Aeromonas species are resistant to 0/129 vibriostatic agents and are chemo organotrophic (Parker & Shaw, 2011; Igbinosa *et al*., 2012). These microorganisms produce different extracellular hydrolytic enzymes such as arylamidases, esterases, elastase, amylases, deoxyribonucleases, chitinases, peptidases and lipases (Igbinosa *et al*., 2012). Members of the genus *Aeromonas* have the ability to utilize urease, pectinase, ornithine decarboxylase, tryptophan and phenylalanine deaminase (Parker & Shaw, 2011). The genus in question exhibits several distinguishing characteristics compared to other genera such as *Plesiomonas*. These include its inability to grow in the presence of 6.5% sodium chloride, its inability to ferment inositol, and its inability to grow on thiosulfate citrate bile salts sucrose agar. Additionally, it demonstrates variability in the presence of ornithine decarboxylase activity and possesses the ability to liquefy gelatin. These traits serve as critical identifiers for differentiation, as outlined by the United States Environmental Protection Agency (USEPA, 2006).

Aeromonas species isolated from raw milk sample was used for this experiment. The inoculum was prepared by growing the organism in peptone water at 37^{O}C for 2 hours. The culture was centrifuged and the sediment suspended in phosphate buffer. Ten milliliter of the inoculum was then added to 90ml of sterile milk samples and further serial dilutions were made to obtain cell concentration of approximately 10^{3}cfu/ml using alkaline peptone water (Isoken *et al*., 2012). Though some research works have been done on *Aeromonas* species on the Plateau, there is still a dearth of information on their prevalence and pathogenicity. This study was carried out to address this gap and to generate data to aid in disease surveillance on the Plateau and Nigeria in general.

MATERIALS AND METHODS

A total of 800 milk samples were collected, consisting of 400 raw milk samples and 400 samples of a locally processed milk product known as "nono." These samples were sourced from various locations within the Jos area, including Bukuru, Vom, Miango, and Lamingo, which are situated in four local government areas of Plateau State. Raw milk samples were obtained directly from farms and retailers, while "nono" samples were acquired from local retailers. All samples were collected in sterile plastic containers with secure closures to minimize the risk of contamination during transportation.

Examination of milk samples

The milk samples were analyzed according to the method described by Korashy (2006). Isolation and enumeration of *Aeromonas* species from milk samples was performed using serial dilution (10^{-3}ml) of milk samples in peptone water. The decimal dilutions were prepared in series in sterile stoppered bottles with sterile peptone water in five replicates. The bottles were then incubated at 37^{o}C for 24 hours after which they were examined for acid and gas production through a colour change in the Durham's tubes inserted in the medium. A 2.5 ml of the positive samples were added to 25 ml of alkaline peptone water (APW, pH 8.6) for enrichment (Jatau & Yakubu, 2004).
After an overnight incubation at 37°C, a loopful from the APW was plated onto ampicillin dextrin agar, *Aeromonas* ampicillin agar and MacConkey agar supplemented with 10 mg/l ampicillin and incubated at 37^{o}C for 24 hours. Raised convex yellow colonies (2-3nm diameter) presumptively considered *Aeromonas* were picked up, re-streaked onto Ampicillin dextrin agar plates for another 24 hours at 37°C. The number of colony forming unit

(cfu/ml) were recorded after the incubation period. All Gram negative, oxidase positive isolates were stored on nutrient agar slants for further biochemical characterization. Evaluation of the effect of seasons and sources on the prevalence of *Aeromonas* species in all the samples examined above was carried out. To this end, samples were collected and examined for the isolation of *Aeromonas* species both in the wet season and the dry season. In the same vein, samples were collected from the rural and urban areas and analyzed to determine the prevalence of *Aeromonas* species in the two areas.

RESULTS

Prevalence of *Aeromonas* species in milk

The result of the prevalence of *Aeromonas* in raw cow milk and locally fermented cow milk ("nono") samples obtained from the various sampling sites is shown in (Table 1).

Table 1: Prevalence of *Aeromonas* Species Isolated from Raw and Fermented Cow Milk (Nono) obtained from the Study Area

Sampling sites	Raw Milk	"Nono"	P-value
Jos	11.00^{a}	5.00^{b}	0.002
Bukuru	14.00^{a}	7.00^{b}	0.001
Vom	10.00^{a}	2.00^{b}	0.008
Miango	9.00^{a}	5.00^{b}	0.021
Lamingo	9.00^{a}	6.00^{b}	0.021

Since $p<0.05$, there is a significant difference between the percentage prevalence of *Aeromonas* species isolated from the two milk types at 95% confidence level.

Out of the 800 milk samples examined, 78 (9.75%) were positive for *Aeromonas*. Among the two milk types investigated, raw milk had higher percentage prevalence, 53 (6.62%) while "nono" had 25 (3.25%). Statistical analysis showed a significant difference ($p<0.05$) ($p=0.002$) between the prevalence of the organism in the raw milk and the fermented milk. The result of the effect of season on the prevalence of *Aeromonas* species isolated from milk, showed that a higher percentage prevalence of 59 (1.48%) of *Aeromonas* spp. was recorded in the two forms of milk during the wet season than during the dry season 19 (0.47%), (Figure1). The result of the effect of sources on the percentage prevalence of *Aeromonas* in raw cow milk samples is presented (Table 2). The result indicated that higher percentage prevalence of the organism was obtained from the samples purchased from the retailers 31 (7.75%) as compared to those purchased from the farms 22 (5. 50%). This result showed that there was no significant difference, $p=0.23$ ($P>0.05$) between the prevalence of *Aeromonas* in raw milk with regard to the sources at 95% confidence level.

DISCUSSION

Prevalence of *Aeromonas* Species in Milk

The ability of *Aeromonas* to grow at low temperature is of great concern particularly in dairy foods, which are stored under refrigeration. This explains why milk may represent an important vehicle of transmission for *Aeromonas* species, some of which could be potential food borne pathogens (Enquebaher *et al*., 2015). Of the 800 samples of milk examined in this study 78(9.75%) was positive. The result of this study indicates that *Aeromonas* spp. is higher in raw milk (6.63%) than locally fermented milk product ("nono") (3.12%) sold in some parts of Plateau State. This result is similar to that reported by Laba & Udonsek (2013) where lower microbial load was isolated from pasteurized milk than raw milk. Milk has been documented as a vehicle for the transmission of infection associated with *Aeromonas* species (Akineden *et al*., 2011; Singh & Praskash, 2010).

The relatively high concentration of *Aeromonas* in raw milk samples obtained in this study may be attributed to lack of effective sanitary practice and less precaution in handling processes during milk production. Milk contains lipids, proteins (casein, whey), carbohydrates (lactose), amino acids, vitamins and minerals (calcium), essential for growth (Haug *et al*., 2007; Javaid *et al*., 2009). The fact that milk contains a lot of nutrients made it a haven for growth and development of a host of microorganisms including the pathogenic ones (Saeed *et al*., 2009). Pareke & Subhash, (2008) asserted that; animal health, milking utensils and the environment are contributors to contamination of fresh raw milk. Also, the unclean or unsterilized teat can introduce a lot of microorganism into the raw milk sample.

The teats and udders of cows are most times not disinfected prior to milking despite the fact that the cows lie in muddy barnyard and dirty environment which inevitably contaminate the milk and increase the microbial load. The bacterial count of milk (4.6×10^{5}cfu/ml) in this study far exceed the EC Regulation (No. 853, 2004) of the European Parliament and of the Council (EC) which sets down the hygienic limit ≤ 100,000 (cfu/ml) for the total bacteria count (TBC) in milk. De-Buyser *et al*. (2001) reported that *Salmonella* is one of the most etiological agents responsible for several outbreaks associated with the consumption of raw milk and milk products. All *salmonellae* are of public health concern having the ability to produce infection ranging from a mild self-limiting form of gastroenteritis to septicemia and life threatening typhoid fever (Oliver *et al*.,

Table 2: Effect of sources on the prevalence of *aeromonas* species isolated from raw cow milk samples

Source	Number Positive (%)	Number Negative (%)	Total (%)	Chi-square	P-value
Retailers	31.000 (7.75%)	169.000	200	2.00	0.16
Farms	22.000 (5.50%).	178.000	200		
Total	53	347	400		

Statistical analysis showed no significant difference (P>0.05) at 95% confidence level.

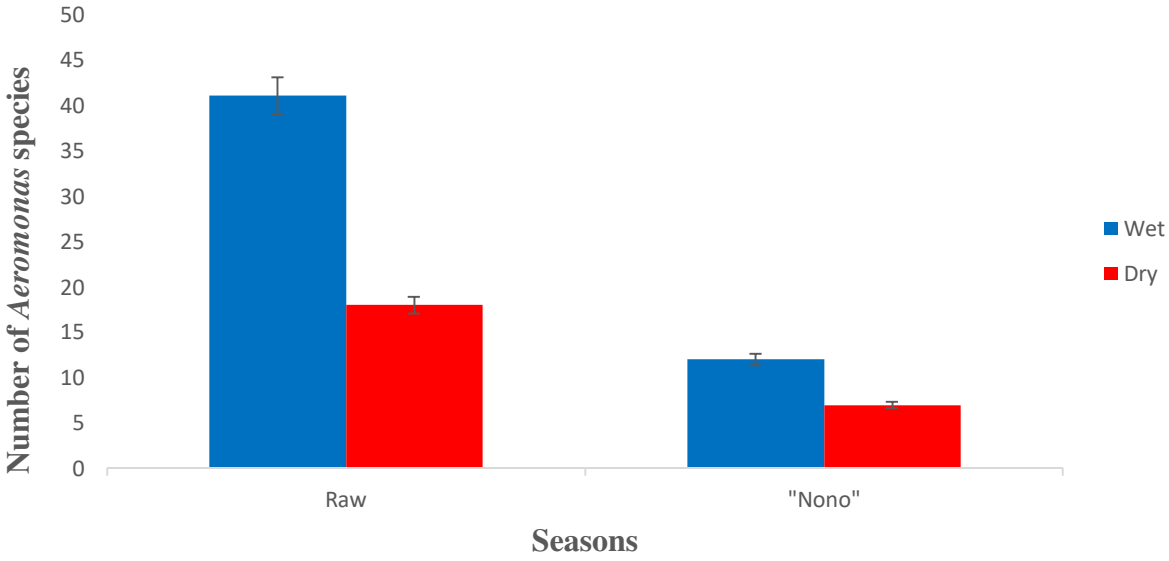

Figure1: Seasonal effect on the percentage prevalence of *aeromonas* species isolated from raw milk and "nono"

2005). Thus, their presence in raw milk, pose a health risk to consumer that consume them without any heat treatment.

The presence of *Escherichia coli* in the raw milk is an indication of fecal contamination and could be dangerous as the strain isolated may be either toxigenic or enteropathogenic, causing major public health hazard (FAO/WHO, 2004). *E. coli* 0157:H7 strain has been associated with a number of food-borne outbreaks and is the cause of bloody diarrhoea, frequently associated with dairy cattle. The ability of *Aeromonas* spp. to survive laboratory pasteurization is probably why some strains of this organism are found in the refrigerated "nono" samples. From this study, it is revealed that milk samples from Bukuru have the highest percentage prevalence of 2.62% while Vom has the lowest percentage (1.5%).

Statistical analysis showed no significant difference in the prevalence of *Aeromonas* spp. in milk in relation to the study areas as p>0.05%. Different measures can be taken to reduce microbial contamination of raw milk, these includes taking care of animal health which may be a vehicle for infecting the consumer. The environment should also be worked on to reduce contamination of the animals. The sanitary state of the milk handler is of great importance and the milking bowl should be washed with detergent and disinfected after use. Before milking, the teat and other breast area should be disinfected. This research along with previous work on consumption of raw cow milk indicated that raw milk consumer stand a high risk of exposure to food borne pathogens. According to U.S. Food and Drug Administration (FDA, 2006), EEC directive 92/46 (EC 2001), Beuvier & Buchin, (2004) the principal pathogens of concern associated with milk and processed milk products are *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, pathogenic *E. coli* and *Aeromonas hydrophila*. Many of the common

enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats (Murphy & Boor, 2000). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic-Prodaova & Necev, 1991). In recent years *Aeromonas* spp. has become very important milk-borne pathogen and even though water is considered to be its main reservoir, cross contamination from water used in milk preparation can occur (Mazurek *et al*., 2004 and Karmali, 1989). The results of this study showed that the higher the initial population of *Aeromonas* in milk subjected to heat treatments, the higher the level of contamination of the milk by the organism during cold storage at refrigeration temperature

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