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DISTRIBUTION AND MOLECULAR DETECTION OF *LISTERIA MONOCYTOGENES* FROM FRESH AND FERMENTED MILK (KINDRIMU) IN MAIDUGURI METROPOLIS, BORNO STATE, NIGERIA

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Abstract: *This study was carried out to isolate Listeria species and molecularly detect virulence genes associated with Listeria monocytogenes from fresh and locally fermented milk (kindirmu) in Maiduguri metropolis, Borno State, Nigeria. A total of 280 samples of fresh (120) and locally fermented (kindirmu) (160) milk were collected from three dairy farms and four points of sales. The samples were analysed using standard bacteriological techniques and molecular protocols. The detection of Listeria species using conventional biochemical test showed only 12% β-hemolysis on sheep blood agar, Catalase positive, Gram positive rods and Oxidase negative which is suggestive of L. monocytogenes. Of all the 12% of presumptive L. monocytogenes subjected to multiplex polymerase chain reaction, only 67% of L. monocytogenes were found to be associated with one or more virulence genes of L. monocytogenes; Iap, InA, ActA, and hlyA at molecular weights of 131 bp, 255 bp, 268 bp and 702 bp respectively. This gives a detection rate of 2.9% of L. monocytogenes. The detection of L. monocytogenes associated virulence genes in milk is an indication that the isolates are pathogenic and therefore may pose a risk to public health. It is therefore recommended that adequate hygienic measures be observed during milking, preparation, retail and consumption of fresh and locally fermented milk in Maiduguri Metropolis, Borno State, Nigeria.*

Keyword: *Listeria monocytogenes, Listeriosis, Virulence genes, pasteurization.*

*Int*roduction

Listeria monocytogenes is a gram positive, non-spore forming rods, facultative anaerobic bacteria of the family Listeriaceae and catalase positive that are sometimes arranged in short chains widely distributed and abundant in nature (Farber and Peterkin, 1991; Alzubaidy *et al*., 2013, Kayode and Okoh, 2022) *Listeria monocytogenes* is an emerging food-borne pathogen that poses a threat to global food safety and it is the etiological agent responsible for animals and human listeriosis . Its occurrence in milk and milk products constitute a negative impact to the dairy industry (Kayode and Okon, 2022; Usman *et al.*, 2016a).). *Listeria monocytogenes* is of major significance in human and veterinary medicine. Most human *Listeria* infections are food-borne and the association of contaminated milk and dairy product consumption with human listeriosis is noteworthy. *Listeria* is commonly present in nature, found widely in such places like water, soil, infected animals, humans and animal feaces, raw and treated sewage, leafy vegetables, effluent from

poultry and meat processing facilities, decaying corn and soybeans, improperly fermented silage and raw unpasteurized milk (Marler, 2017; Lee *et al*., 2019).

Foods commonly identified as source of *Listeria* infection include improper pasteurized milk, cheese (particularly soft-ripened varieties, ice-cream, raw vegetables, fermented raw-meat, sausages, raw and cooked poultry and cooked ready to eat sliced meats often referred to as deli meats (Meldrum *et al*., 2010; Marler, 2017).Therefore, the quality of milk and milk products continues to attract global attention in the dairy industry and public health sectors (Maduka *et al.,* 2013). Production of good and high quality milk and milk products is an important aspect of standard dairy practice. High quality milk contains a low bacterial count, lower number of somatic cells, and is free of human pathogens (Maduka, *et al.,* 2014; Usman *et al*., 2016a). In dairy industry, occasional presence of *L. monocytogenes* in milk, dairy products and dairy facilities/plant is considered as a major obstacle to quality production (Szczawinski *et al*., 2016). The organism grows over a wide range of temperature from 1^{o}C-45^{o}C, with an optimum temperature around 30^{o}C to 37^{o}C. *Listeria monocytogenes* can grow at pH values between 4.4 and 9.4 and become more sensitive to acidic condition at higher temperature (FDA, 2013; Alzubaidy *et al.,* 2013; Bertrand *et al*., 2016). Like most bacterial species, *L. monocytogenes* grows optimally at a water activity of 0.90 and 0.97 with sodium chloride as solute. The bacterium is resistant to various environmental stresses, such as highly salty or acidic solutions which allows it to survive longer under stressful condition than non-spore forming bacteria of food borne disease. It has the ability to form biofilms which contribute to its ability to colonize food processing facilities (FDA, 2013; Lakicevic *et al.,* 2015; Kayode and Okoh, 2022). The organism also has a multi factorial virulence traits (ActA,hly, iap, plcA,plc, prfA, and mpl) re gulated *Listeria* pathogenic inlands (Lipi-1) , with the thiol activated haemolysin, listeriolysin O (LLO) located on the LIPI-2 practically contribute to the severity of the infection and being identified as a crucial role in the organisms' ability to multiply within host phagocytic cells and to spread from cell to cell (Disson *et al*, 2021; Markey *et al*., 2013; chen *et al*, 2018). Extensive sanitation policies and procedure should be strictly observed as preventive measures to avoid *Listeria* contamination (Todar, 2012). Listeriosis being an emerging infection worldwide associated with food borne outbreaks and significant risk of mortality and morbidity, prompted the Centre for Disease Control and Prevention (CDC) to evaluate the case of 2,500 and more than 500 deaths related to listeriosis in the United States of America (Beumer and Hazelzer., 2013; Gohar *et al.*, 2017). Although there were no reported cases of listeriosis in Nigeria, the prevalence case of 6.6% detection rate of *L.monocytogenes* in milk from Kaduna, Nigeria was reported by Usman *et al* (2016a), similarly, Faeji *et al* (2016) also isolated 6.9% *L.monocytogenes* in fresh milk samples in Northern, Nigeria and 11% detection rate in milk from Jos, Nigeria. . More recently, in South Africa, there was an outbreak of listeriosis from January 2017 through March 2018, 879 laboratory confirmed cases were reported to National Institute of communicable Diseases (NICO) from all provinces. The outcome of illness is known for 674 patients of whom 183 (27%) of them died (WHO, 2018). Most of the cases are persons who have higher risks from severe disease outcome such as neonates, pregnant women, the elderly and immune-compromised persons (WHO, 2018). Another confirmed case of listeriosis was reported in Namibia, in this outbreak 42% of the cases were neonates who are infected during pregnancy or delivery (WHO, 2018). A multi-state outbreak of *L. monocytogenes* affected nine States in the U.S.A, in 2016, nineteen of the infected persons were hospitalized and one person died of listeriosis from Michigan.

Symptoms may begin a few days after eating contaminated food, but it may take as long as 30 days or more before the onset of first signs and symptoms of infection begin, which include; headache, fever, muscle aches, nausea and diarrhoea (Mayo Clinic, 2017). If *Listeria* infection spreads to the nervous system, signs and symptoms may include headache, stiff neck, confusion or changes in alertness, loss of balance and convulsions in pregnancy. *Listeria* infection is likely to cause only mild signs in the mother and

the consequences may lead to the death of baby prior to parturition (Mayo Clinic, 2017, Zhu *et al*., 2017). It is unique among food borne pathogens, since the incubation time from ingestion of *Listeria* cells to illness is at least seven days ((Jones, 2010; Kayode and Okoh, 2022). Listeriosis is a rare disease with high mortality rate causing about 43% of food poisoning associated with death in the U. S (Zhu *et al*., 2017).

The common route of transmission of *L. monocytogenes* to human is via consumption of contaminated food. However *L. monocytogenes* can be transmitted directly from mother to child or from contact with animals and through hospital acquired infection (Nosocomial infection). Healthy individuals can be asymptomatic carriers of *L. monocytogenes* (Nwachukwu and Orji, 2012; FDA, 2013). Human to human transmission of *Listeria* infection is caused by ingestion of bacteria, most often through the consumption of contaminated food (Momtaz and Yadollahi, 2013; Marler, 2017).

Despite an increasing rate of outbreaks of listeriosis in recent years in the United States, Canada, China and some Afrian coutries, the occurrence and prevalence of the organism in food borne diseases in Nigeria is scarcely reported. There is limited information on the status of food borne listeriosis caused by *L. monocytogenes* in Nigeria especially in Maiduguri metropolis associated with fresh and locally fermented milk. Therefore, this study will provide information on distribution of *Listeria* species and Virulence genes associated with *L. monocytogenes* from fresh and locally fermented milk (*kindirmu*) in Maiduguri metropolis.

MATERIALS AND METHODS

The Study Area

The study was conducted in Maiduguri the capital city of Borno State in North-eastern Nigeria. Maiduguri lies between Latitude 11.46^{o} N to 11.54^{o} N and longitude 13.04^{o} E to 13.14^{o} E and situated at an elevation of 300 meters above sea level. Maiduguri has a population of 1,112,449 making it the biggest city in Borno State. The climate of Maiduguri is generally hot, dry, windy, and dusty for most part of the year with an average rainfall of 613 mm per annum (that starts in June and ends in September), and temperature range of 25.8^{o}C to 40^{o}C (NIMET, 2018). The major occupation of the people is farming, livestock rearing, trading and fishing

Sampling Technique

A convenience sampling technique was employed in this study, where samples were collected based on availability. Fresh and locally fermented milk samples were obtained directly from farms and different sales points. Samples were collected on a weekly basis for the period of two months (May to June 2019). The samples were analyzed using standard bacteriological techniques and molecular protocols

Sample Collection, Packaging and Transportation

A total of 280 samples of fresh and locally fermented milk (*kindirmo*) were collected from three cattle dairy farms and four points of sales in Maiduguri Metropolis. Forty samples of fresh milk each from three different farms and forty samples of locally fermented milk (*kindirmo*) from four selected points of sales were collected. All samples were labeled accordingly, placed in cool box containing ice packs, and

transported to the Veterinary Microbiology Laboratory, Department of Veterinary Microbiology, University of Maiduguri for processing.

Isolation of *Listeria* species on Laboratory Media

Listeria species were isolated according to the procedure described by Food and Drug Administration; Bacteriological Analytical Manual (FDA-BAM, 1997). One millilitre of milk sample was aseptically added to 9 ml of *Listeria* enrichment broth (LEB) containing selective *Listeria* enrichment supplement in a conical flask then incubated at 37^{o}C for 24 hours. A loop full of this enrichment culture was streaked on to *Listeria* selective agar (Oxoid SR 141E) and incubated at 37^{o}C for 24 hours. The culture plates were examined for grayish colonies with black halos and sunken centre as described by Ribeiro and Carminati (1996).

Haemolysis on Blood Agar

The suspected *Listeria* isolates were grown on 5% sheep blood at 37^{o}C for 24 hour. *Listeria* colonies were identified as clear zone around the colonies. The β-haemolysis was observed by producing narrow zones of haemolysis.

Phenotypic Identification of *Listeria* species

Gram Staining

Presumptive colonies based on colony morphology were subjected to gram staining based on the method of Cheesbrough (2000). A smear was made on a slide from the isolates and heat-fixed, crytal violate stain was then poured on the smear. The smear was allowed to stand for 1 minute and rinsed with tap water. Lugols iodine solution was added to the smear for 1 minute and rinsed with tap water before it was decolourized with 95% alcohol for 2 seconds and then counterstained with safranin for one minute. The slide was rinsed with water and allowed to air dry and viewed under the microscope using the oil immersion objectives lens.

Biochemical Tests

Conventional biochemical tests were carried out according to the method described by Alzudaidy *et al*. (2013**)**. Catalase, oxidase and sugar fermentation tests (Glucose, sucrose and maltose) were performed to identify *Listeria* species.

Catalase Test

Suspected isolates were tested for their ability to produce catalase enzyme. A drop of 3% hydrogen peroxide was placed on a slide and a colony of the suspected isolates was picked with a sterile wire loop and then suspended in the hydrogen peroxide. A positive result showed a formation of bubbles.

Oxidase Test

Suspected *Listeria isolates* were tested for their ability to produce cytochrome oxidase. A sterile wire loop was used to aseptically transfer a colony of the suspected isolates unto the surface of the oxidase detection strip and observed for 3 minutes. The appearance of purple colour within 20-30 seconds indicates positive result and no colour change showered oxidase negative

Sugar fermentation tests

All suspected *Listeria* like organisms were tested for their ability to ferment glucose, lactose and sucrose. The suspected isolates were inoculated into phenol red-peptone broth containing 1% of any the sugars for their fermentation tests and incubated at 37^{o}C for 24 hours. A positive result was indicated by changing from red to yellow colour.

Molecular Detection

Extraction of Genomic DNA of *Listeria* isolates

The *Listeria* isolates were grown on *Listeria* selective media and incubated at 37^{o}C for 24-48 hours. Three colonies of each isolates were picked, inoculated into 5 ml of Tryptone soya broth and incubated at 37^{o}C for 24 hours and the cells were suspend in 600 µl of nucleic lysis solution. The solution was gently pipetted to mix and then incubated for 5 min at 80^{o}C, then cooled to room temperature. Additionally, 200 µl of protein precipitation solution was added and vortexed followed by incubation on ice for 5 minutes, then centrifuged at 16000 g (gyra) for 3 minutes. The supernatant was transferred to a clean tube containing 600 µl of isopropanol at room temperature. The solution was centrifuge to pellet the cells and the supernatants was discarded. Six hundred microliter of 70% ethanol at room temperature was mixed and centrifuged for 2 minutes 16000 g. The ethanol was aspirated and the pellet was air-dried for 10-15 minutes, the DNA pellet was rehydrate in 100 µl of rehydration solution for 1 hour at 65^{o}C or overnight at 4^{o}C. The mixture was centrifuged at 16000 g for 2 seconds and then allowed to cool at room temperature. The DNA obtained from the *Listeria* isolates using the Quick-g DNA Miniprep-uncapped column kit from Zymo®-Research, was used for the detection of virulence-associated genes using a multiplex PCR (mPCR).

Agarose Gel Electrophoresis and Imaging

The resultant PCR products were separated by electrophoresis in 1.5% agarose gel for 45 minutes at 100 V in Tris-acetate EDTA buffer, stained with ethidium bromide (0.5 µg/ml) visualized with UV transilluminator (Syngene Fredericka, MD). The gel image was documented by gel documentation apparatus and DNA size was determined by 100 bp DNA ladder (Promega®) (Usman *et al*., 2016b).

Data Analyses

The data obtained from this study were analysed using statistical packaged for solution service software (SPSS) version 16.0. The data were presented using chi square and descriptive statistics such as tables and figures.

Results

Distribution of *Listeria* species isolated from milk in Maiduguri, Borno state

The distribution of *Listeria* species isolated from milk on *Listeria* selective agar showed that the total of 93 (33.2%) positive samples for *Listeria* species, only 12% were positive for presumptive *L. monocytogenes* after biochemical test. When the association between the isolation rate for *Listeria* species and the location of the sample were tested, there was no significant association between the two ($p< 0.082$). The distribution of *Listeria* species base on sample type and positive *Listeria* species showed that 2 (50.0%) were from fermented milk and 10(11.2%) were fresh milk. When the association between the positive *Listeria* species and the sample type were tested. There was significant association between the two ($p< 0.0024$)

Table2: Distribution of *Listeria* species isolated from Fresh and Locally Fermented milk (*kindirmo*) in Maiduguri Metropolis, Borno state

Variable	No. of sample (%)	No. of positive (%)	X^2	P value
Location				
Bornostate dairy Farm	31	2 (6.5)	6.695	0.082
Collegeof Agric.	36	4 (11.1)		
University farm	22	4 (18.2)		
Bulunkutu fermented milk	4	2 (50.0)		
Total	93	12(85.8)		
Milk type				
Fermented milk	4	2 (50.0)	5.118	0.0024
Fresh milk	89	10 (11.2)		

Occurence of *Listeria monocytogenes* Virulence genes in milk in Maiduguri.

The occurrence of *Listeria monocytogenes* virulence genes in milk based on sample locations showed that *iap was* found in the following sampling locations (BSDF, CAF, UMF AND BLF), when subjected to statistical analysis showed a statistical significant association ($p<0.002$) and there was no significant difference for the rest of the three virulence genes (*inlA*, *HlyA* and *ActA*). There is significant association between *iap* gene and sample type (<p0.00) and no significant differences between sample type and the following virulence genes (*InlA*, *HlyA* and *ActA*).

Table 3: Occurrence of *Listeria monocytogenes* Virulence genes in milk in Maiduguri

Variable		Virulece Genes						
Locations	Iap		InlA		HlyA		ActA	
	No. (%)	X^2 (P-value)	No. (%)	X^2(Pvalue)	No. (%)	X^2(P-value)	No. (%)	X^2(P-value)
BSDF	0 (0.0)	15.0511 (0.002)	1 (3.2)	3.116 (0.374)	0(0.0)	1.436 (0.697)	1(3.2)	0.89(0.847)
CAF	3 (8.3)		2 (5.6)		1(2.8)		1(2.8)	
UMF	1 (4.5)		2 (9.1)		1(4.5)		0(0.0)	
BLF	2(50.0)		1(25.0)		0(0.0)		0(0.0)	
Milk type								
Fermented milk	2(50.0)	13.134 (0.00)	1(25.0)	2.383 (0.123)	0(0.0)	0.092 (0.762)	0(0.0)	0.092 (0.762)
Fresh milk	4 (4.5)		5 (5.6)		2(2.2)		2(2.2)	

Detection of *L. monocytogenes* virulence associated genes

The detection of *L. monocytogenes* associated virulence genes with mPCR on 1.5% agarose gel revealed 67% of the *Listeria* isolates. The *L. monocytogenes* possessed at least one or more of the virulence genes. Out of the five *L. monocytogenes* associated virulence genes screened for, 4 (*HlyA,* Iap, *ActA* and *InlA*) genes were detected. The detection of the virulence genes positive isolates showed that *Iap* (molecular weight of 131 bp) were detected in six isolates;(BLF34, BLF36 both in locally Fermented Milk and CAF1, UMF1, CAF28, BSDF1 were detected in Fresh Milk) and *InlA* (molecular weight of 255 bp) were detected in six isolates; (UMF16, CAF9, CAF28, BSDF1 and UMF1 were from Fresh Milk and BLF34 from Fermented Mik), while *ActA* (molecular weight of 268 bp) were detected in two isolates; (CAF28 and BSDF1, from Fresh Milk) and *HlyA* (molecular weight of 702 bp) were detected in two isolates;(UMF16 and CAF9 from Fresh Milk) as shown in (Fig 1).

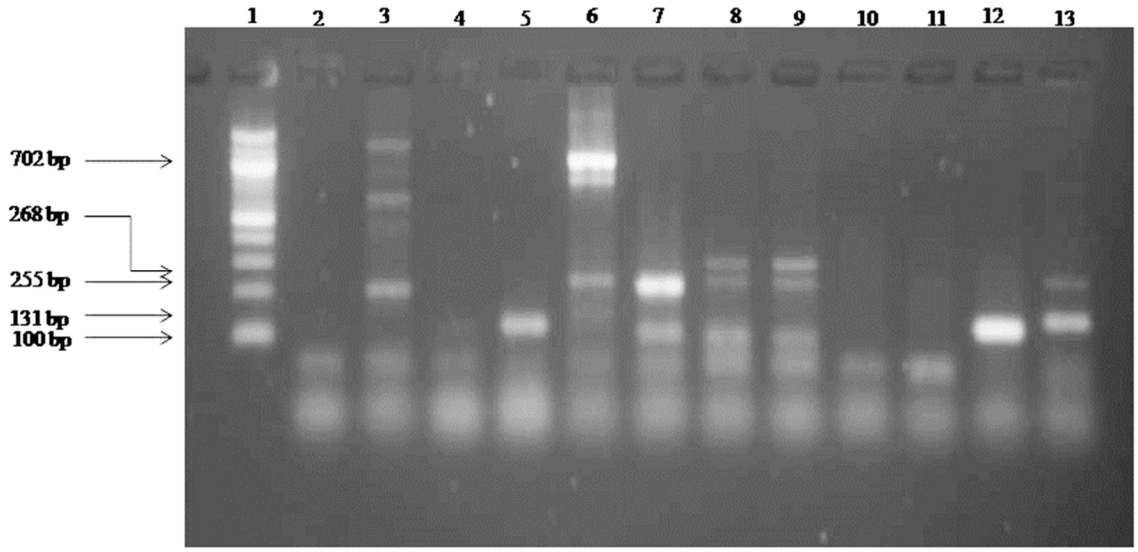

Figure 1: Agarose gel electrophoresis with multiplex PCR amplification of virulence genes associated with *L. monocytogenes*

L1: 100 bp molecular marker, lanes 2 -13: test samples.

Lanes 5, 7, 8, 9, 12 and 13 (positive for *Iap*), lanes 3, 6, 7, 8, 9 and 13 (positive for *InlA*), lanes 3 and 6 (positive for *HlyA*) and lanes 8 and 9 (positive for *ActA*).(promega)

DISCUSSION

The quality of milk and milk products continues to attract global attention in the dairy industry and public health sectors (Maduka *et al.,* 2013). Production of good and high quality milk and milk products is an important aspect of standard dairy practice. High quality milk contains a low bacterial count, lower number of somatic cells, and is free of human pathogens (Maduka, *et al.,* 2014; Usman *et al*., 2016a). In dairy

industry, occasional presence of *L. monocytogenes* in milk, dairy products and dairy facilities/plant is considered as a major obstacle to quality production (Szczawinski *et al*., 2016.

The distribution of *Listeria* species isolates from fresh and locally fermented milk (*kindirmo*) samples in Maiduguri Metropolis showed that only 2(6.5%) were from Borno State Dairy Farm, 4(11.2%) from College of Agriculture. 4(18.2%) from University of Maiduguri and 2 (50.0) from Bulumkutu which gave a total of 12% isolation in this study which is similar to 11% *Listeria* species in milk from Jos, Nigeria by Chukwu *et al*. (2004), but lower than 35.10% isolation rate in milk reported by Usman *et al*. (2016b) in Kaduna, Nigeria. When the association between the isolation rate for *Listeria* species and the location of the sample were tested, there was no significant association between the two ($p< 0.082$). The distribution of *Listeria* isolate based on sample type showed 2 (50.0%) were from fermented milk and 10 (11.2%) fresh milk which gave an overall isolation rate of 12% in this study. When the association between the positive *Listeria* species and the sample type were tested. It was found to be significant. This is not in agreement with Usman *et al*. (2016a), who showed that there was no significant different between the sample type and positive *Listeria* species in milk sample collected from Kaduna. The differences in the isolation rates may likely be due to locations, identification methods, the health care given to the cows as well as precautions taken by milk handlers (Chukwu *et al*., 2004; Faeji *et al*., 2016). Also, the cattle rearing practice by the herdsmen in the study area that are likely to increase or decrease the isolation rate may be that the herdsmen have been health and hygiene conscious and have been consulting veterinary doctors for adequate health services and the use of new technologies, another risk factor could be inadequate frequency of cleaning the exercise area, poor cow cleanliness and incorrect disinfection of towels between milking might be some contributing factors (Faeji *et al*, 2016). The *Listeria* detection rate in milk observed in this study is lower than the 60% detection rate in milk reported by Mugampoza *et al*. (2011) in Uganda. Such differences might be due to poor hygiene and sanitation activities in the milk production; processing and supply chains which may be mainly associated with faecal contamination of milk (Uhitil *et al.*, 2004).

The isolation of *Listeria* species from milk observed in this study could be due to faecal or environmental contamination during milking, storage and/or infected cows in dairy farms (Faeji *et al*., 2016). The low detection rate of *Listeria* species in fermented milk (*kindrimu*) observed in this study may be due to conversion of lactose during fermentation by lactic acid bacteria present in the locally fermented milk (*kindrimu*) leading to the development of low pH in the milk and hence the inhibition of *Listeria* species (Jamali *et al*., 2013; Usman *et al.,* 2016b). Although, 12% of all the isolates in this study gave colonial appearance typical of *Listeria* species, only 12% were found to be Gram positive rods, catalase positive, oxidase negative and showed β-haemolysis on sheep blood agar. This finding is suggestive of *Listeria monocytogenes* isolates. Haemolytic activity on blood agar has been used as a marker to distinguish *L. monocytogenes* from other *Listeria* species.

The 12% isolates of *Listeria* species observed in this study were found to ferment glucose, sucrose and maltose. Some *Listeria* species are known to harbour enzymes that are capable of fermenting sucrose, maltose, lactose and glucose (Alzubaidy *et al*., 2013; Jamshidi and Zeinali, 2019).

The result of the multiplex PCR has revealed 2.9% of the *L. monocytogenes* isolates to be carrying one or more *L. monocytogenes* virulence associated genes; this gives a detection rate of the total milk samples examined. This finding is similar to 3.2% *L. monocytogenes* isolation rate from yohurt and raw milk reported by Gabriel. (2015) in Kaduna, Nigeria. The detection rate of *L. monocytogenes* observed in this

study is higher than the 1.5% detection rate from Australia (Deutz *et al*., 1999), 1.95% from Canada (Fedio and Jackson, 1990) but lower than the 6.6% from raw milk reported by Usman *et al*. (2016a) in Kaduna, Nigeria.

The occurrence of *L*. *monocytogenes* virulence genes in the milk samples which are characterized by the presence of four virulence genes (*iap*, *inlA*, *HlyA* and *ActA*) were subjected to statistical analysis and showed that *iap* gene has significant difference between the association of samples location and virulence genes (<p0.002), while three (3) virulence genes has no significant association between sample location and virulence gene present in *L*. *monocytogenes*.The occurrence of *L*. *monocytogenes* in milk based on sample type and virulence genes showed that *iap* in both fermented milk and fresh display significant association between sample type and virulence genes (p<0.000) but there was no statistical differences between milk samples and three virulence gene (*inlA*, *HlyA* and *ActA*).

About 70% of the *L. monoytogenes* detected in this study harbour virulence associated genes namely; *iap*, *InlA, actA* and *hlyA* amplified according to their respective molecular weight 131 bp, 255 bp, 268 bp and 702 bp but no *PrfA* gene was detected. The detection of these four virulence genes in this study does not agree with the report of Usman *et al*. (2016b), who detected only three (*prfA*, *hlyA*, and *iap)* virulence associated genes of *L. monocytogenes* from milk samples in Kaduna, Nigeria. The detection of these virulence genes is important because *hlyA* (*Listeriolysin O*) and *ActA* are associated with the bacterium's capability of passing the intestinal barrier cell to cell spread and motility cell invasion and cellular parasitism (Rawool *et al*., 2007; Usman *et al*., 2016b; Ezeonu *et al.*, 2019, Enurah and Nwonsu, 2019). It may be possible that some *L. monocytogenes* strains may lack one or more virulence determinant because of spontaneous mutations (Momtaz and Yadollahi, 2013). This difference could have arisen as a result of variation in the sampling methods, isolation; molecular methods sample location and dynamics of the disease.

Conclusions and Recommendations

In this study, 12% of *Listeria* species was isolated from fresh and fermented milk in Maiduguri metropolis, Nigeria. Only twelve (12%) of the isolates were suggestive of *L. monocytogenes* based on colonial appearance, microscopy, biochemical and sugar fermentation tests. Eight (2.9%) of these isolates were found to be carrying one or more virulence associated genes (*InlA, ActA, HlyA* and *Iap*). Two of the isolates harbored only one virulence gene, 4 isolates harbored 2 virulence genes each and 2 isolates harbored 3 virulence genes each. The detection of these virulence associated genes suggests that the *L. monocytogenes* isolates from milk in this study are pathogenic and is of significant public health concern.

Fresh milk should be pasteurized properly before consumption so as to reduce the bacteria load High risk communities such as those living in the study area should be enlightened on how to maintain good hygiene, preparation and storage practices when handling milk.

It is recommended that cold storage facility and alternative power supply be made mandatory for fresh and locally fermented milk sellers by national food safety to ensure wholesome milk supply, fit for human consumption.

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