PREVALENCE OF *LISTERIA MONOCYTOGENES* IN FRESH RAW MILK AND ABATTOIR EFLUENTS IN NIGERIA

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Abstract

This Study investigated the prevalence of Listeria monocytogenes a foodborne pathogen isolated from fresh raw milk and abattoir effluent from the six geo-political zones of Nigeria namely Southwest, Southeast, Southsouth, Northwest, Northeast and Northcentral. The total samples which consisted of 305 fresh raw milk and 31 abattoir effluents were examined for the presence of Listeria spp. using selective enrichment and isolation protocol. Listeria monocytogenes strains were identified by biochemical tests using Brilliance Listeria chromogenic agar, Oxoid Biochemical Identification System Mono (O.B.I.S. Mono), Rapid Listeria test kit and Polymerase Chain Reaction (PCR) method. The results obtained showed that 54 isolates (8.63%) were identified as Listeria monocytogenes with the milk samples constituting 23 (7.54%) while abattoir effluent had 31 (9.65%). Statistical analysis of the results showed that the prevalence rate of this pathogen across the zones were Southwest (10.6%), Southeast (8.0%), Southsouth(7.9%), Northwest 11.5%), Northeast (7.9%), Northcentral (7.4%) while the national prevalence rate was 8.6%. This indicated the high rate of contamination of this pathogen in dairy and meat products. It also showed the health hazard associated with such food. This study only supported existing knowledge of food contamination in Nigeria.

Keyword: *Listeria monocytogenes, fresh raw milk, abattoir effluents, Nigeria.*

INTRODUCTION

Listeria monocytogenes is widely distributed in the environment and it is the causative agent of listeriosis. The mortality rate for those contracting listeriosis is 30% and may increase up to 75% in high risk groups such as the elderly, pregnant women, neonates and immunocompromised individuals (Vazquez-Boland *et al.*, 2001). Clinical manifestations of invasive human listeriosis include meningitis, encephalitis, late-term spontaneous abortion and septicemia (Nightingale *et al.*, 2005), with a non-invasive febrile gastroenteritis or influenza like symptoms (Lorber, 1990). Epidemiological studies have shown that listeriosis is a foodborne disease (Schlecch *et al.*, 1983; Finlay, 2001) and a number of foodborne outbreaks and sporadic cases of listeriosis have been reported, mostly in North America and Europe (Lovett, 1989). Food implicated in outbreaks of listeriosis

included dairy foods, meat and meat products, vegetable and sea food (Schlech , 2000; Bell and Kyriakides, 2005). The high incidence of *L. monocytogenes* in foods (Faber and Peterkin, 1991) and the high mortality rate associated with listeriosis, have made the pathogen a serious public health concern. The important characteristics of *L. monocytogenes* that favour its foodborne transmission include, the ability to grow as low as -0.40°C (Walker *et al.*, 1990) , withstand osmotic pressure and survive mild preservation treatment (Jalali and Abedi, 2008). The safety challenge of this organism in food control measure have been studied and extensively documented in developed countries. The occurrence in foods in Nigeria remains uncertain and requires scientific determination of potential risk associated with consumption of raw milk, meat and meat products.

MATERIALS AND METHODS

Sample Collection

Samples of fresh raw milk from the teat of lactating cattle and abattoir effluents were randomly and aseptically collected into sterile bottles from the six geo-political zones between May, 2007 and July 2009. The samples which consisted of 305 fresh raw milk and 321 abattoir effluents were transported in an ice pack to the laboratory for microbiological analysis.

Selective Enrichment for *L. monocytogenes*

Strains were isolated in accordance with United States Department of Agriculture and Association of Analytical Chemist/ International Diary Federation (USDA and AOAC/IDF) method 993.12, modified by using Brilliance Listeria Chromogenic Agar (Oxoid, UK) to obtain colonies. Twenty-five millimeter of each sample was aseptically added to 225ml Buffered Listeria Enrichment Broth Base (Oxoid, UK) and incubated at 30°C for 24h. A portion of 1ml of primary enrichment was transferred to 9ml of Buffered *Listeria* Enrichment Broth with *Listeria* Selective Enrichment Supplement (with cycloheximide) (Oxoid, UK) and incubated at 30°C for 24hrs. Secondary enrichment of 0.1ml of each sample was plated on Brilliance Listeria Chromogenic Agar (BLCA) (Oxoid, UK) containing Brilliance *Listeria* Selective Supplement (Oxoid) and Brilliance *Listeria* Differential Supplement (Oxoid, UK) and incubated at 37°C for 24h. The suspected colonies were characterized by Gram-stain and biochemical tests. Gram-positive colonies were tested for haemolysis on 7% sheep blood agar, and species identification were made with Oxoid Biochemical Identification System Mono (O.B.I.S.Mono) and Rapid Listeria Test Kit (Oxoid, UK).

The prevalence rate of each zone was calculated by dividing the number of positive samples with the total number of samples for each zone multiply by hundred while the national prevalence was calculated by dividing the sum of positive samples with the total samples collected from the six zones multiply by hundred

L.monocytogenes specific PCR Typing

All isolates were cultured in buffered Listeria enrichment broth (BLEB) at 37°C for 18 h and their genomic DNA were extracted

using genomic DNA extraction kit (Zymo Research Corp, USA). The procedures as contained in the manual were strictly adhered to during the extraction. Primers target the iap gene which encodes the invasion associated protein .Forward and reverse primers are stated below; forward primer (List-univ 1) 5'- ATG TCA TGG AAT AA-3'; reverse primer (List-univ 2) 5'- GCT TTT CCA AGG TGT TTT T-3' (Inqaba Biotech, South Africa®) . The samples were submitted to an amplification profile of initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 36°C for 2 min and 72°C for 3 min, and a final extension step at 72°C for 7 min (Wiedmann *et al.*, 1997; Harakeh *et al.*, 2009). The primers allowed amplification of 457bp internal fragment of the iap gene. Exactly 8μl of each of the PCR amplified reaction mixture were subjected to horizontal gel electrophoresis in 0.8% agarose gels run in 1x Tris-borate (TBE). PCR products were visualized using ethidium bromide staining (1μl/ml) and photographed under UV light.

RESULTS

Prevalence of *L. monocytogenes* in fresh raw milk and abattoir effluent in Nigeria

In the total of 626 samples examined, *L. monocytogenes* was isolated in 54 (8.63%) of the samples. The organism was isolated from both fresh raw milk and abattoir effluents. The results obtained showed that 23 (7.54%) of fresh raw milk samples (305) were contaminated by the organism while 31 (9.65%) of the abattoir effluents (321) had the organism (Table 1). Statistical analysis of the results on Table 2 shows that the prevalence rate of *L. monocytogenes* across the zones are North Central (7.4%), North East (7.9%), North West (11.5%), South South (7.9%), South East (8.0%), South West (10.6%), while the National prevalence rate is 8.6% (Table3).

Table 1 Percentage occurrence of *L. monocytogenes*in fresh raw milk and abattoir effluent

Sample	Number•	%
Fresh raw milk(n= 305)	23.00	7.54
Abattoir effluent (n= 321)	31.00	9.66

•Number of samples positive for *L. monocytogenes*

Table 2 Rate of prevalence (%) of *L. monocytogenes* in fresh raw milk and abattoir effluents in six geo-political zones of Nigeria

Locations	Fresh raw milk (FRM)	Abattoir effluent (AE)
Southwest	8.75	12.50
Southeast	6.12	9.62
Southsouth	8.11	7.50
Northwest	7.41	12.29
Northeast	5.56	8.62
Northcentral	8.47	6.35

Table 3 Percentage prevalence of *Listeria monocytogenes* across the zones

Location	Number Positive	Percentage
Southwest (n= 160)	**17**	**10.6**
Southeast (n= 101)	**8**	**8.0**
Southsouth (n= 77)	**6**	**7.9**
Northwest (n= 55)	**6**	**11.5**
Northeast (n= 112)	**8**	**7.9**
Northcentral (n = 122)	**9**	**7.4**
Total	**54**	**8.6**

Biochemical and PCR detection of *L. monocytogenes*

Fifty-four strains were isolated from 626 samples with the average prevalence of 8.6% (Table 3). All strains were confirmed to be *L. monocytogenes* by Oxoid biochemical identification system mono, rapid Listeria test kit (Oxoid Ltd, UK) and phosphatidylinositol specific phospholipase-C assay on brilliance Listeria chromogenic agar, They all produced clear zones of â-haemolysis on 7% sheep blood agar. The fifty-four strains were further confirmed by species specific PCR. These results were consistent with the biochemical test results. The specific 457bp product was amplified from all the fifty-four strains (Plate 1) that were confirmed by the above mentioned confirmatory tests.

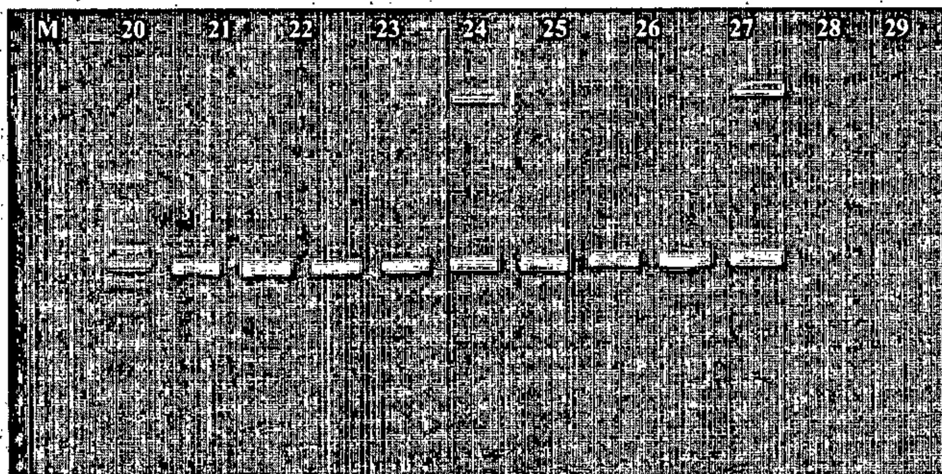

Plate 1 PCR amplification of iap gene from the genome of *L. monocytogenes* strains. Lane M: 100bp molecular weight marker, lanes 20 – 27 represent respective *L. monocytogenes* isolates, lane 28: positive control, lane 29: negative control

DISCUSSION

There is dearth of information on the epidemiology and clinical cases of listeriosis in Nigeria. Although scanty information exists on the prevalence of *L.monocytogenes* in food consumed in the

country. It is also important to note that listeriosis is a poorly reported disease in Nigerian health programme. In addition, there are no criteria or recommendation for *L. monocytogenes* in food in the country. This study is to determine the prevalence of *L. monocytogenes* in abattoirs and fresh raw milk which are the sources from where raw meat, meat products and dairy foods are distributed to the larger population in the country. *Listeria* spp. is widely present in plant, soil, silage and processing environment (Farber and Peterkin, 1991; Beresford *et al*., 2001). Animals may be colonized by *Listeria* due to consumption of contaminated feed and water (Husu *et al*., 1990; Fenlon *et al* ., 1996). Thus, *Listeria* may enter the abattoir via anmals habouring *Listeria* in the intestinal tract or as part of pharyngeal microflora and as a result of improper hygienic practice during processing or slaughter at the abattoirs the raw meat and meat products are contaminated.. The result is that contaminated raw meat and meat products leave the abattoir to the open market where the larger population gets in contact with contaminated products. The butchers and other workers at the abattoirs may also be infected and serve as carriers. The results of this study indicate much lower incidence (9.65%) compared to some previous studies. In Trinidad, Gibbons *et al.* (2006) isolated *Listeria* spp. in 80% of raw meat samples. Yucel *et al* .(2005) found 86.4% of various meat product samples contaminated with *Listeria* spp. in meat processing industries in Turkey. Ryu *et al.* (1992) detected *Listeria* spp. in 56.6% of meat samples in Japan. The occurrence of *Listeria monocytogenes* in fresh raw milk in this study is not surprising as previous workers (Sanaa, 1996; Bhilagonkar *et al*., 1997; Prentice, 1997). Prentice (1997) reported a 5% contamination of fresh milk samples. This is in agreement with the result of this study (7.54%) . Sanaa, (1996) reported that the contamination of milk by *Listeria monocytogenes* could be as high as 10cell/ml. Sanaa (1996) and Prentice (1997) attributed the contamination of raw milk samples to mastitis caused by the organism or of faecal origin. In Nigeria, listeriosis has not been linked to the consumption of any food or dairy products though the organism has been isolated from milk (Onyemelukwe *et* al ., 1983; Oni *et al*., 1989). However, since the transfer of human listeriosis to susceptible individuals has been linked to the consumption of raw milk (Jensen *et al*., 1996) from infected cows, the result of the present work only suggest that raw milk may be a vehicle for human infection with *L. monocytogenes* in the study areas as seen elsewhere in the world. This is very possible as sporadic cases of meningitis of unknown etiology often resurge in rural and urban centres of this country.

CONCLUSION

The results presented in this study show the potential risk of eating raw and undercooked foods.

All the strains of *Listeria monocytogenes* isolated across the six zones of Nigeria are pathogenic as evidenced by their positive â haemolysis on 7% sheep blood agar, a result that constitutes serious public health concern. This result also suggests that the organism is

a common contaminant of foodstuff in the environment.

RECOMMENDATION

The eating habit of Nigerian population is changing towards European, and recommendation for *Listeria monocytogenes* in food needs to be made by government authority.

The concept of HACCP should be introduced and strictly adhere to in food industry, starting from the food processing environment through the distribution chain to the final consumers. This will greatly reduce the presence of *L. monocytogenes* in foods in Nigeria.

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