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Isolation of *Listeria monocytogenes* from Raw Meat Sold in Nasarawa State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Listeriosis is an emerging infection with major public health concerns worldwide because of occurrence of associated food-borne outbreak and significant risk of mortality and morbidity. This study aimed to isolate *Listeria monocytogenes* in raw meat samples in selected markets in Nasarawa State, determine the level of contamination, as well as the antibiogram of the isolates. A total of 60 samples of raw meat were collected from different animals, including 4 samples of cow meat, 4 samples of goat meat and 4 samples of chicken meat, from each market. In all, 60 raw meat samples were collected and analyzed microbiologically using the method of the Clinical and Laboratory Standards Institute (CLSI, 2009). Out of the 60 samples analyzed *Listeria monocytogenes* was detected in 32 samples, giving an overall prevalence of 53.5%. Sixteen out of 20 (80%) of cow meat samples, 10 out of 20 (50%) of goat meat samples, and 6 out of 20 (30%) of chicken meat samples were infected with *Listeria monocytogenes*. All the isolates were further subjected to biochemical analysis for the confirmation of the isolates. The results revealed the presence of *Listeria monocytogenes* in over 50% of the raw meat samples analyzed. The 53.5% prevalence was considered high and indicated the hazard linked to the consumption of the raw meats sold in Nasarawa State if not properly cooked. The susceptibility tests were also conducted using disc diffusion method. The results revealed that most of the isolates were resistant to most of the commonly used antibiotics such as Septrin, Ampiclox, Erythromycin, Zithromycin, Amoxillin, and Pefloxacin. However, some of the isolates were relatively sensitive to Ciprofloxacin, Sreptomycin, Gentamycin, and Rifampicin. The results signal a chemotherapeutic problem in case of any outbreak of the infection.

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1. INTRODUCTION

Listeriosis is an infectious disease of humans and animals caused in 99% of cases by consumption of food contaminated by *L. monocytogenes*, and rarely from the environment [1]. Some cases of listeriosis are caused by consumption of dairy products (such as milk, cheese, butter); cabbages, and meat. In clinical picture in humans and animals the disease manifests in similar way. In humans it can lead to the following diseases: meningitis, encephalitis, septicemia, diarrhoea, skin infection etc. The vulnerable group includes pregnant woman (causing miscarriage or still born children), infants, older person and person with weakened immune system [1]. The presence of listeriosis in humans is low in the percentage (1%), but with high percentage of fatal outcome (30%). It is estimated that listeriosis annually causes with approximately 500 deaths [2]. Also the presence of this bacterium in intestinal tract of 5-10% of healthy humans without any obvious symptoms of the disease was established [1]. However, in healthy adult individuals it can be totally unnoticed.

Listeria monocytogenes is a small gram positive, oxidase-negative, non-spore forming, motile and facultative anaerobic bacterium. It grows at temperature range of -0.4 to 50°C. *Listeria monocytogenes* can be found ubiquitous in different environments including dust, water, soils, animal feeds, silage, human and animal faeces, where it enters the food chain [3,4]. It has also been identified to be a major pathogen that contributes to domestically acquire food-borne illnesses after non typhodial *Salmonella*, and *Toxoplasma gondii* [5]. In humans the average case-fatality rate of listeriosis is between 20% and 30% even with adequate antimicrobial treatments [6] but can be a serious invasive disease with mortality rates ranging between 80% and 99% primarily in neonates, immunocompromised adults and pregnant women causing encephalitis, septicemia and abortion [7].

Large food-borne outbreaks of listeriosis have occurred during the last decade in Europe and USA.Between 1991 and 2002, 19 outbreaks of invasive listeriosis were reported in nine (9) European countries, with total of 526 related cases [4]. In 1997, a large outbreak resulting in 1566 cases of listeria Gastro-enteritis was reported in Italy and traced to the consumption contaminated corn salad [7]. A recent nationwide outbreak linked to contaminated package meat product occurred in Canada in 2008 resulting 56 patients including 20 deaths [8]. In Europe, listeriosis surveillance data is available at the national level in 16 countries. Recent report on this national surveillance system indicated an increase in the incidence of listeriosis in many European countries including England, Wales, Denmark, and Germany. The other countries include Netherlands, Switzerland and Pineland among others during 2000-2006 [9]. In United States, the CDC (Center for Disease Control and prevention) food-borne disease active surveillance network (Foodnet) monitors trends in listeriosis overtime. In contrast to the EU countries, there has been a continued reduction in incidence in recent years from 1996 through 2003. The incidence decreased significantly by 24% from 4.1millions to 3.1 cases per million people in 2003. In 2007, listeriosis cases have decreased by 42% with rates reaching 0.27 per 100,000 compared to the baseline period, (1996-1998) [10]. In the Asia-pacific region, Australia national notifiable disease surveillance system reported an annual number of listeriosis cases that ranged from 35-73 during the period 1991-2007. In Singapore, are the annual numbers of administratively-required (Non statutory) notification to the Ministry of Health ranged from 1-9 cases from 2001-2007 [11].

Diverse environments in Nigeria provide favourable conditions for *Listeria* to thrive and contaminate food sold in open places, especially ready to eat meat. The tropical weather is warm and humid all year round and many rural places are not very hygienic and have poor meat sanitation. The abattoirs where fresh meat is sold have also being implicated as a vehicle for *Listeria*. Five surface swabs from butchers table taken in Nsukka, South eastern Nigeria showed occurrence of *Listeria* in all samples [4]. Adetunji and Ishola who enumerated *Listeria* on meat tables before and after sales of meat in Ibadan municipal abattoirs in Nigeria, found an increase in *Listeria* counts after meat sales [12].

In Africa recent studies have confirmed the presence of *Listeria monocytogenes* in a wide variety of food-stuffs. Meat, milk product, raw vegetable are considered to be most frequently contaminated with *Listeria* [11]. Listeriosis incidence in Africa is significantly increasing in

high percentage due to the poor hygiene in the region, compared to the EU, USA and Asian countries.

Meat is a popular and important source of protein. Poultry meat consumption has increased since 2001 [13]. Contamination of poultry with *Listeria monocytogenes*is unpleasant. *L. monocytogenes* is able to survive during processing techniques and increase chance of cross contamination for other foods [14,15].

In view of the popularity of meat as a very important source of protein for human and the high fatal consequences of listeriosis, this work was set out to investigate the prevalence of *L. monocytogenes* infection in meat samples sold in some selected markets in Nasarawa State of Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Five markets in Nasarawa State were randomLy selected for this study and they included Mararaba, Masaka, Nyanya, Keffi and Lafia Markets.

2.2 Study Population

This consists of 60 meat samples collected from the 5 selected markets. The meat samples included 4 cow meat samples, 4 goat meat samples, and 4 chicken meat samples from each of the markets.

2.3 Sample Collection

Sixty samples of raw meat selected from five markets were collected in sterile plastic bag and transferred to Bingham university microbiology laboratory. Samples were kept in the refrigerator (temperature of -4 to -8°C for laboratory investigations).

2.4 Microbiological Analysis

The media used in this work included Xylose Lysine Deoxycholate (XLD) agar, Blood agar (BA), Peptone water (Enrichment medium), and Mueller Hinton agar for susceptibility determination. The media were prepared according to manufacturers' instructions on the packages of the media.

The samples were analyzed microbiologically and biochemically according to the National Council for Clinical Laboratory Standard (NCCLS). About 100g of each meat sample was chopped using sterile knife, and homogenized using a sterile laboratory blender at high speed for 2 min. The homogenized samples were then transferred into sterile bottles for later use.

2.4.1 Enrichment method

The initial procedure of isolation involved the use of selective enrichment broth medium (peptone water) to enhance the growth of *Listeria* species (buffered method). The peptone water powder was weighed using weighing balance following the manufacturer's instruction on the medium pack. The powder was then mixed in appropriate amount of distilled water contained in clean conical flask. It was allowed to dissolve completely. Finally nine mL (9) were dispensed into each bijou bottle and sterilized by autoclaving at 121°C for 15min. Approximately 1mL of each homogenized sample was added into the 9 mL of peptone water contained in bijou bottle and incubated at 37°C for 24hr.

2.4.2 Plate Inoculation

This was done using plate streaking method [16]. A loopful from the overnight culture (from the enrichment broth) was sub-cultured onto XLD agar, which is a medium of choice to isolate *Listeria monocytogenes,* and blood agar plates to detect haemolysis. The inoculated plates were incubated at 37°C for 24hrs.

2.4.3 Colony identification and comfirmation

Listeria monocytogenes colonies were identified by the colony characteristics on the XLD agar plate, which appeared blackish, and β-haemolysis on the blood agar plate. It was further identified by running Gram staining and the biochemical tests.

2.4.4 Biochemical Tests

Following the cultivation of *L. monocytogenes* on XLD, the following biochemical tests were conducted for the confirmation of the organism: Catalase test, Oxidase test, and Indole test. Motility Test was carried out according to [17].

2.4.5 Susceptibility Test

Antimicrobial drugs for sensitivity test were selected considering the antibiotics used most

frequently for the treatment of food pathogens, which included Pefloxacin 10mcg (PEF), Streptomycin 30 mcg (S), Gentimycin 10 mcg (CN), Ciprofloxacin 10 mcg (CPX), Rifampicin 20 mcg (RD), Erythromycin 30 mcg (E), Ampiclox 20 mcg (APX), Septrin 30 mcg (SXT), Zithromycin 20 mcg (Z), and Amoxacillin 30 mcg (AM). The colonies of the isolates were plated separately on Mueller Hinton agar by spreading over the surface of the medium. Antibiotic discs as stated above were aseptically placed on each seeded plate. The plates were then incubated at 37°c for 24hrs. The antibiogram was reported as Resistant or Sensitive.

3. RESULTS

A total of 60 samples were collected from five randomLy selected markets in Nasarawa State, which included Lafia, Keffi, Nnyanya, Mararaba and Masaka markets. Twelve samples were collected from each market. The organism isolated in many of the samples (over 50%) was found to be small Gram positive, oxidase-negative, indole negative, and catalase positive, motile rods. The organism also showed β-haemolysison on blood agar plates, which are features of *Listeria monocytogenes*. In all the 60 samples collected and investigated, 32 were positive and 28 samples were negative. The overall prevalence was 53.3(%). In the results, the markets that had the highest rate of contamination were Lafia and Nyanya, with 8 out of 12 samples (66.7%) being positive for *Listeria*. This was followed by Keffi and Mararaba markets where 6 out of 12 samples (50%) were positive for *Listeria*. Masaka market had the lowest rate of contamination having 4 out of 12 samples (33.3%) being positive for *Listeria* (Table 1).

Table 2 shows the prevalence of *Listeria monocytogenes* in the five market locations in Nasarawa State.

The Table 2 shows the general prevalence for each market site studied. In Lafia market 8 out of 12 samples were positive, giving a general prevalence of 13.3%. In Keffi, 6 out of 12 samples were positive, giving a general prevalence of 10.0%. In Masaka market, 4 out of 12 samples were positive, giving a general prevalence of 6.7%. In Mararaba market, 6 out of 12 samples were positive, giving a general prevalence of 10.0%. In Nyanya market, 8 out of 12 samples were positive, also giving a general prevalence of 13.3%. The overall general prevalence was 53.3%.

Among the meat types, cow meat had the highest rate of contamination with 16 out of 20 samples (80%) being positive for *Listeria monocytogenes. This was* followed by goat meat, which had 10 out of 20 samples (50%) being positive for *Listeria monocytogenes*. Chicken meat had the least rate of contamination with 6 out of 20 samples (30%) being positive for *Listeria monocytogenes*.

The general prevalences for the meat types were 26.7% for cow meat, 16.7% for goat meat, and 10.0 for chicken meat, giving an average general prevalence of 53.33% (Tables 3 and 4).

Table 1. Source distribution of samples from 5 different markets

Source of sample	No. of positive	No. of Negative	Total
Lafia	8 (66.7%)	4 (33.3%)	12
Keffi	6 (50.0%)	6 (50.0%)	12
Masaka	4 (33.3%)	8 (66.7%)	12
Mararaba	6 (50.0%)	6 (50.0%)	12
Nyanya	8 (66.7%)	4 (33.3%)	12
Total	**32**	**28**	**60**

X^2=3.8039, CL=0.95, df =4 (P>0.05)

Table 2. Overall general prevalence of *L. monocytogenes* in 5 locations

Sample site	No positive	No negative	Prevalence (%)
Lafia	8	4	13.3
Keffi	6	6	10.0
Masaka	4	8	6.7
Mararaba	6	6	10.0
Nyanya	8	4	13.3
Total	**32**	**28**	**53.3**

Table 3. Distribution of *L. monocytogenes* infection among different meat types

Meat type	No. of positive	No. of negative	Total
Cow meat	16 (80%)	4 (20%)	20
Goat meat	10 (50%)	10 (50%)	20
Chicken	6 (30%)	14 (70%)	20
Total	**32**	**28**	**60**

X^2=10.1192, CL=0.95, df =2 (P>0.05)

Table 4: Overall General Prevalence of *L. monocytogenes* according to meat type

Meat type	No. of positive	No. of negative	Prevalence (%)
Cow meat	16	4	26.7
Goat meat	10	10	16.7
Chicken	6	14	10.0
Total	**32**	**28**	**53.3**

Table 5. Sensitivity test

S/N	Sample	PEF	SXT	S	CPX	APX	E	CN	R	Z	AM
1	COW	R	R	R	S	R	R	R	R	R	R
2	COW	R	R	S	R	R	R	R	R	R	R
3	COW	R	R	R	R	R	R	S	R	R	R
4	COW	R	R	R	S	R	R	R	R	R	R
5	COW	R	R	R	R	R	R	S	R	R	R
6	COW	R	R	R	R	R	R	R	S	R	R
7	COW	R	R	R	S	R	R	R	R	R	R
8	COW	-	-	-	-	-	-	-	-	-	-
9	COW	-	-	-	-	-	-	-	-	-	-
10	COW	R	R	R	R	R	R	S	R	R	R
11	COW	R	R	R	S	R	R	R	R	R	R
12	COW	R	R	R	S	R	R	R	R	R	R
13	COW	R	R	R	R	R	R	R	S	R	R
14	COW	R	R	S	R	R	R	R	R	R	R
15	COW	R	R	S	R	R	R	R	R	R	R
16	COW	-	-	-	-	-	-	-	-	-	-
17	COW	-	-	-	-	-	-	-	-	-	-
18	COW	R	R	R	R	R	R	S	R	R	R
19	COW	R	R	R	S	R	R	R	R	R	R
20	COW	R	R	R	S	R	R	R	R	R	R
21	GOAT	R	R	S	R	R	R	R	S	R	R
22	GOAT	R	R	R	S	R	R	R	R	R	R
23	GOAT	S	R	R	R	R	R	R	R	R	R
24	GOAT	-	-	-	-	-	-	-	-	-	-
25	GOAT	-	-	-	-	-	-	-	-	-	-
26	GOAT	-	-	-	-	-	-	-	-	-	-
27	GOAT	R	R	R	S	R	R	R	R	R	R
28	GOAT	R	R	R	S	R	R	R	R	R	R
29	GOAT	-	-	-	-	-	-	-	-	-	-
30	GOAT	-	-	-	-	-	-	-	-	-	-
31	GOAT	R	R	R	S	R	R	R	R	R	R
32	GOAT	R	R	S	R	R	R	R	S	R	R
33	GOAT	-	-	-	-	-	-	-	-	-	-
34	GOAT	-	-	-	-	-	-	-	-	-	-
35	GOAT	R	R	R	S	R	R	R	R	R	R
36	GOAT	S	R	R	R	R	R	R	R	R	R
37	GOAT	-	-	-	-	-	-	-	-	-	-

S/N	Sample	PEF	SXT	S	CPX	APX	E	CN	R	Z	AM
38	GOAT	R	R	R	R	R	R	R	R	R	R
39	GOAT	-	-	-	-	-	-	-	-	-	-
40	GOAT	-	-	-	-	-	-	-	-	-	-
41	CHICKEN	-	-	-	-	-	-	-	-	-	-
42	CHICKEN	-	-	-	-	-	-	-	-	-	-
43	CHICKEN	R	R	R	S	R	R	R	R	R	R
44	CHICKEN	R	R	R	S	R	R	R	R	R	R
45	CHICKEN	-	-	-	-	-	-	-	-	-	-
46	CHICKEN	-	-	-	-	-	-	-	-	-	-
47	CHICKEN	-	-	-	-	-	-	-	-	-	-
48	CHICKEN	-	-	-	-	-	-	-	-	-	-
49	CHICKEN	R	R	S	R	R	R	R	R	R	R
50	CHICKEN	-	-	-	-	-	-	-	-	-	-
51	CHICKEN	-	-	-	-	-	-	-	-	-	-
52	CHICKEN	R	R	R	S	R	R	R	R	R	R
53	CHICKEN	-	-	-	-	-	-	-	-	-	-
54	CHICKEN	-	-	-	-	-	-	-	-	-	-
55	CHICKEN	R	R	R	S	R	R	R	R	R	R
56	CHICKEN	-	-	-	-	-	-	-	-	-	-
57	CHICKEN	-	-	-	-	-	-	-	-	-	-
58	CHICKEN	R	R	S	R	R	R	R	R	R	R
59	CHICKEN	-	-	-	-	-	-	-	-	-	-
60	CHICKEN	-	-	-	-	-	-	-	-	-	-
Total no of isolate sensitive		2	0	6	16	0	0	4	4	0	0

Key: S = Sensitive; PEF-Pefloxacin, SXT-Septrin, S-Streptomycin, CPX-Ciprofloxacin, R = Resistant; APX-Ampiclox, E-Erythromycin, CN-Gentimycin, R-Rifampicin, - = No growth; Z-Zithromycin, AM-Amoxillin

Table 5 shows the sensitivity analysis for all of the samples from the five market location in Nasarawa State.

4. DISCUSSION

The results of this work indicated a high prevalence of *Listeria monocytogenes* in raw meats processed and sold in Nasarawa State markets. The overall prevalence of *Listeria monocytogenes* obtained in this work was 53.3%. This prevalence in meat is considered quite high in view of the devastating consequences of the infection, coupled with its multiple modes of transmission. However, the prevalence compares favourably with the 43.5% calculated mean average prevalence reported by [18]. The prevalence here is higher than 7% reported in raw meat in Rivers State, Nigeria by [19], and 4% prevalence reported in Zaria by [20]. The prevalence is still higher than the overall calculated average of 22.2% reported from different parts of Africa by [18].

Considering the source distribution of samples, Lafia and Nyanya samples had the highest level of contamination with *Listeria monocytogenes,* the two sources having 8 out of 12 (66.7%) samples contaminated with *Listeria monocytogenes*, followed by Keffi and Maraba markets with 6 out of 12 (50%) meat samples each being contaminated and lastly with Masaka market having 4 out of 12 (33.3%) meat samples being contaminated. Although our results were not statistically significant (P>0.05), the distribution of the infection could be attributed to market size. Lafia is the state capital town with a large market. Nyanya is a densely populated town with a large market too. Keffi and Maraba are not as large in size as Lafia and Nyanya, hence they have medium size markets. Masaka is a small satellite settlement with a small market. The volumes of meats entering these markets could, therefore, determine the level of meat contamination.

Considering the meat types, cow (beef) meat was most contaminated with 80% of the samples being contaminated with *Listeria monocytogenes,* giving 26.7% overall prevalence. This is similar to the finding reported from Taiwan by [21] with the prevalence of 24%, and 27.5% prevalence reported from Bulgaria by [22]. Goat meat was the next contaminated meat type with 50% of the goat meat samples being contaminated with *Listeria monocytogenes* and giving 16.7% overall

prevalence. The prevalence is similar to the 14% prevalence reported from Cenral India by [23], but higher than the 4-8% prevalence reported from Egypt by [24]. The 16.7% prevalence is, however, lower than the 29.9% reported from China by [25]. Chicken meat was the least contaminated meat type, with only 30% of the chicken meat being contaminated, giving an overall prevalence of 10%. This prevalence rate is however lower than the 35% prevalence reported for chicken meat in Greece by [22]. The prevalence is also less when compared to the 95.8% reported from Oyo State, Nigeria by [26].

Though our results concerning meat types were not statistically significant (P>0.05). The level of contamination in the meat types could be attributable to animal size and amount of processing involved in the meat types. Cow meat presents a large surface area for contamination since *Listeria monocytogenes* is present in every environment. Goat meat presents lesser surface area for contamination, while chicken meat presents the least surface area for contamination. Handling and processing are minimal with chicken meat compared to goat and cow meats.

Considering the antibiogram profile in this work, most of the *Listeria monocytogenes* isolates were resistant to most of the antibiotics used. Only Ciprofloxacin was effective on 16 of the 60 isolates. This general resistance of *Listeria monocytogenes* to commonly used antibiotics was also reported in the work of [26]. This scenario is considered a chemotherapeutic challenge in case of any outbreak of the infection and should be viewed very significant in public health.

5. CONCLUSION

The present study demonstrates that raw meats sold in Nasarawa State markets are generally contaminated with *Listeria monocytogenes,* with averagely more than 50% of the meats sold in the state yielding the pathogen. The general overall prevalence of 53.33% infection is quite alarming. Likewise the general resistance of *Listeria monocytogenes* isolates to the common antibiotics in use is equally worrisome and of great public health concern. Therefore, adoption of strict hygienic practice is recommended in the handling, processing and marketing of meat and meat products. Strict control of antibiotic use in human listeriosis treatment and livestock management is highly recommended.

6. RECOMMENDATIONS

- It is important to limit the presence of this organism in raw meat by taking all the necessary preventive measures such as good hygiene practice in the production, processing, distribution and marketing of meat and meat products.
- Public sensitization about the disease listeriosis, which is lacking in the state studied, and precautory measures to be taken is highly recommended.
- Better diagnostic methodsthat will demonstrate the organism down to the specie level, such as polymerase chain reaction (PCR), nucleic acid amplication, virulence determination, could be employed in order to enhance quicker and accurate diagnosis and effective treatment.
- In view of the high level of contamination by this notorious pathogen, it is recommended that raw meats be properly and sufficiently cooked before consumption.
- Furthermore, prompt and effective treatment of the infected patients is highly recommended upon laboratory diagnosis.
- Specific health advice should be given to the vulnerable group e.g. pregnant woman, old age, and HIV/AIDS patient to prevent themselves from getting infected with *Listeria monocytogenes.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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