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Prevalence and Antimicrobial Susceptibility of *Listeria monocytogenes* Isolated from Beef, Pork and Chicken Sold in Makurdi Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AP, EUU and ETA designed the study and wrote the protocol. Authors AP and GAO drafted the first manuscript and literature searches. Author GAO managed the analyses of the study. All authors read and reviewed the first manuscript. Authors AP and GAO read and reviewed the second draft of the manuscript. All authors approved the final manuscript.

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ABSTRACT

Background: The demand for meat and meat products has been on the increase because of its nutritive value. Meat and meat products have been implicated in cases of foodborne diseases in both developed and developing countries. In Nigeria, there exist a dearth of information on the prevalence of *Listeria monocytogenes* which is a known pathogen of man and a major contaminant of meat.
Aim: To investigate the presence of *Listeria monocytogenes* in beef, pork and chicken, determine its distribution in markets within Makurdi, Benue State as well as its antimicrobial susceptibility to antibiotics.
Place and Duration of Study: The study was conducted between June and November 2013, at the Veterinary Pathology and Microbiology Laboratory of Federal University of Agriculture in Makurdi,

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Benue State, Nigeria.
Methodology: Two hundred and five samples of raw beef, pork and chicken were sourced from open markets in Makurdi and analyzed for the presence of *Listeria monocytogenes* following standard microbiological procedures. *L. monocytogenes* isolation and identification involved the use of Listeria Enrichment Broth (LEB), Listeria Selective Agar (LSA), haemolysis, sugar fermentation and Christie Atkins Munch Peterson (CAMP) tests. Antimicrobial susceptibility of *L. monocytogenes* isolates was tested against eight (8) different antibiotics, using the Kirby Bauer disc diffusion method. One Way Analysis of Variance (ANOVA) and Chi-squared test were used to analyze collected data.
Results: Contamination rate of *L. monocytogenes* was found to be 16 (7.8%) indicating that the organism is prevalent in Makurdi markets. *L. monocytogenes* contamination rate in pork, beef and chicken were 8 (11.1%), 6 (6.5%) and 2 (5.0%) respectively. There was however no statistically significant difference between the levels of contamination in the meat samples as well as the markets ($p>0.05$). All the isolates were found to be susceptible to erythromycin, gentamycin, cotrimoxazole and chloramphenicol but resistant to augmentin, amoxicillin, tetracycline and cloxacillin. *L. monocytogenes* contamination in the markets were 6 (37.5%), 5 (31.3%), 2 (12.5%), 2 (12.5%) and 1 (6.3%) for Wurukum, Wadata, North Bank, Modern and High level markets respectively.
Conclusion: There is a high incidence of *L. monocytogenes* contamination in raw beef, pork and chicken sold in Makurdi and hence, an urgent need to curb contamination of raw meat by this pathogen so as to safeguard the health of consumers.

Keywords: Prevalence; listeriosis; contamination; susceptibility; beef; pork; chicken.

1. INTRODUCTION

Listeria monocytogenes is a catalase positive, Gram-positive, facultatively anaerobic and non-spore forming cocco-bacillus flagellate member of the Genus *Listeria,* that grows at optimum temperature of 30-37℃ [1]. *L. monocytogenes* is a ubiquitous organism and pathogenic to man, wild and domestic animals. It is the etiologic agent of listeriosis and causes complications like abortion, miscarriages and still births in man as well as animals [2]. The disease has a high fatality rate ranging between 20 to 30%. Persons who are infected may show signs of meningitis and septicaemia [3], as well as fatigue, headache, gastro-enteritis, muscular and joint pain at the onset of infection [4]. *L. monocytogenes* is a major contaminant of meat, though contamination rate differs from place to place, based on hygiene, food content and environmental factors. It has been reported as a contaminant of fresh meat, minced beef, meat and poultry products [5-6] and has also been detected in vacuum processed meat and on the hands of meat processors [7-9]. Its transmission in food is favoured by its ability to grow at temperature as low as -0.40℃ [10] and to withstand osmotic pressure and mild preservation treatments [11].

Outbreaks caused by the pathogen leading to series of deaths in places such as Europe and the USA have been reported [12]. Genera consist of *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welchimeri*, *L. seeligeri*, *L. innocua*, *L. grayi* [13] and the recently discovered *L. rocourtie, L. marthii, L. weihenstephanensis* and *L. fleischmannii* [14-16]. However, only *L. monocytogenes* and *Listeria ivanovii* are known to be pathogenic to humans and animals. *Listeria ivanovii, Listeria seeligeri*, *Listeria innocua* and *Listeria welchimeri* have however, been reported as agents of sporadic cases of human infections [17-18]. In most African countries, there are only few reports on *Listeria* and listeriosis, when compared to Europe and USA [2] as there has been a dearth of information on the epidemiology of listeriosis in the region, including Nigeria [19]. This is because the organism seems not to have been given attention as required. Antibiotic resistance has been reported severally in literature with clinical isolates from human beings. Recent evidences however, suggest that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them [20]. Thus, the food microflora is not separated from its human counterpart in cases of antibiotic resistance. The occurrence of antibiotic resistance complicates therapy and lengthens convalescence from illness [21]. This trend has been worsened by prophylactic use of common broad spectrum antibiotics, indiscriminate usage in humans and in animal feed as growth promoters, particularly in developing nations [21-23].

Meat and meat products have increasingly become part of daily human diet because of its rich and nutritive composition. Slaughtering of livestock continues to increase as a result of the increase in demand for meat and its products [24]. The highly nutritious nature of meat provides a suitable environment for the growth of pathogenic, nonpathogenic as well as spoilage organisms [25]. Its high consumption rate and popularity hence, makes contamination and its consequences an issue of concern, especially when pathogens are involved. *L. monocytogenes* accounted for 26% (186 out of 713 food products) cases of food recall between the years 2000 to 2003 in the United States [26]. Despite the foregoing, there is currently limited or no documented information regarding the prevalence and antimicrobial susceptibility pattern of *L. monocytogenes* in beef, pork and chicken sold in Makurdi, Benue State, though these food sources are consumed on a daily basis by a large population of the inhabitants. This exposes the inhabitants to potential *L. monocytogenes* infection. Hence, the purpose of the study was to investigate the presence of *Listeria monocytogenes* in beef, pork and chicken, determine its distribution in Makurdi, Benue State as well as its antimicrobial susceptibility to antibiotics.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted between June and November 2013, in Makurdi, Benue State, Nigeria. Makurdi is the capital of Benue State, located on latitude 7°38' and 7°50' North and longitude 8°24' and 8°38' East [27]. Majority of the inhabitants are civil servants and farmers, hence rearing of animals make good business in Makurdi and its environs. Meat is a major delicacy of the inhabitants and large number of animals are slaughtered, processed and consumed on a daily basis. These are sold in the open markets, streets and meat shops within the metropolis.

2.2 Sample Collection

A total of 205 raw meat samples comprising 93 beef, 72 pork and 40 chicken were randomly collected in the morning hours and weekly between June and November 2013 and examined for the presence of *L. monocytogenes.* The samples were collected from five markets in Makurdi metropolis, namely Wadata, Wurukum, North- Bank, High-Level and Modern markets respectively. Samples were purchased from open market sites into polythene bags the same way they are sold to other customers and transported to the laboratory for microbiological examination. Processing and analysis of the samples were performed immediately on arrival at the laboratory.

2.3 Inoculation and Isolation

The procedure recommended by the International Organization for Standardization and the United States Department of Agriculture (USDA) were followed with slight modification. Five grams of each sample was weighed and transferred into 45 ml of Listeria Enrichment Broth (LEB) (Oxoid, CM0862) in a conical flask. The conical flask and its content was incubated for at least 24 hours at 37℃. A loopful of the broth culture was streaked on Listeria Selective Agar (Oxoid CM0856) and incubated for 48 hours at 37℃, after which the plates were examined for the presence of listerial-like growths. Portions from suspected colonies were Gram stained and also tested for motility and presence of catalase enzyme. CAMP test was also performed on isolates. Hemolysis reaction was tested on sheep blood agar, as well as reaction of the isolates to the presence of glucose, sucrose and lactose.

2.4 Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was performed for *L. monocytogenes* isolates against eight (8) different antibiotics (Abtek Biologicals London) using Kirby Bauer disc diffusion method. The antibiotics used were augmentin (30 µg), amoxicillin (25 µg), erythromycin (5 µg), tetracycline (10 µg), cloxacillin (5 µg), gentamycin (10 µg), cotrimoxazole (25 µg) and chloramphenicol (30 µg). The zone of inhibition around each disc was measured and the results were interpreted as sensitive, intermediate and resistant using interpretative criteria of Clinical Laboratory Standards Institute [28-29] with *Staphylococcus aureus* ATCC 29213 as reference control strain.

2.5 Statistical Analysis

Statistical analyses (both inferential and descriptive) were done using Statistical Package for Social Sciences (SPSS) version 17. One Way Analysis of Variance (ANOVA) was

used to compare means, and Chi-square was used to determine associations.

3. RESULTS AND DISCUSSION

The results of this study showed that *Listeria monocytogenes* is a contaminant of beef, pork and chicken sold in Makurdi, with a prevalence rate of 7.8% (Table 1). Out of the 205 raw meat samples collected, 16 (7.8%) were contaminated with *L. monocytogenes*. Pork recorded the highest contamination of 8 (11.1%) out of 72 samples analyzed. *L. monocytogenes* contamination in raw beef and chicken were 6 (6.5%) and 2 (5.0%) out of 93 and 40 samples respectively. There was however no statistically significant difference in the levels of contamination ($p > 0.05$) between the meat types sampled. These findings are consistent with earlier reports of slightly lower prevalence rate in raw meat [2,30]. On the other hand, a much higher prevalence rate was reported by a similar work in Bangkok [31]. Presence of *L. monocytogenes* in milk and abattoir effluents in the geopolitical zones of Nigeria with prevalence rate of 8.6% as reported by [19] also corroborate our finding. *L. monocytogenes* contamination may be attributable to lack of adequate hygiene and sanitation in the slaughtering methods and evisceration process adopted by meat vendors. Cross contamination and recontamination can occur from the use of slaughtering tools in meat processing without any form of sterilization. People handling meat at different stages can also be sources of contamination. Considering the ability of this organism to grow and reproduce even at refrigeration temperatures, its presence in raw meat is totally undesirable and calls for urgent steps to control or better still, totally eliminate it. Although proper cooking of meat before consumption could kill the bacterium, there still exist the possibility of the populace consuming inadequately heat-treated meat products which could pose serious health risks to especially infants, pregnant women and the immunocompromised fractions of the populace.

Table 1. Rates of *Listeria monocytogenes* contamination according to meat type

Meat type	Negative (%)	Positive (%)	Total (%)
Beef	87(93.5)	6(6.5)	93(100)
Pork	64(88.9)	8(11.1)	72(100)
Chicken	38(95.0)	2(5.0)	40(100)
Total	189(92.2)	16(7.8)	205(100)

Out of the 16 (7.8%) samples that were positive for *L. monocytogenes*, 6 (37.5%) were from Wurukum market and 5 (31.3%) were from Wadata market (Table 2). North Bank and Modern markets both recorded contamination in 2 (12.5%) of the samples collected, while the least frequent was from High level market 1 (6.3%). Over all, beef recorded 37.5% of *L. monocytogenes* contamination in the raw meats, while pork and chicken had 50.0% and 12.5% respectively. The differences in frequency of *L. monocytogenes* in the markets were not statistically significant ($p > 0.05$). The high contamination rate of *L. monocytogenes* noted in our findings corroborate the works of different researchers in other places [32-34] who reported high contamination rates of *Listeria monocytogenes* in pork. The high contamination rate observed in pork may not be unconnected with the feeding habit of pigs which exposes them to ingesting feed contaminated with *Listeria monocytogenes*. This organism has been reported to be abundant in soil and contaminated water, from where it gets into the food chain. Pigs scavenge for food in dirty and muddy environments. The high contamination rate observed in pork is worrisome, considering that it is a popular delicacy among the locals in Makurdi. The prevalence in beef and chicken also calls for concern as pork, chicken and beef are popular meat types mostly prepared as peppered meat in restaurants and bars within the town through grilling, a process which may not supply enough heat to kill the bacterium if present in the meat before it is consumed.

The incidence of *L. monocytogenes* contamination according to market location was also determined (Table 3). Incidence of *L. monocytogenes* contamination was highest in Wurukum market (11.3%); Wadata market had an incidence rate of 12.2%, while Modern market and North-bank markets had incidence rates of 4.9% and 5.0% respectively. Incidence rate was least in High-level market (3.3%). Statistical analysis however, did not reveal any significant differences ($p > 0.05$) in the contamination rates between markets. It is possible that *Listeria monocytogenes* contaminated water may have been used in the washing of the meat before they are transported to the markets for sale. The bank of the River Benue which is the major source of water in Wurukum and Wadata markets harbours waste from the surrounding community. A number of dump sites were also observed to be located close to the very point where water is collected for washing slaughtered

Table 2. Frequency of *Listeria monocytogenes* contamination in beef, pork and chicken in five open markets

Meat type	Wurukum market	Wadata market	High level market	Northbank market	Modern market	Total (%)
Beef	2 (12.5)	3 (18.8)	0 (0.0)	1 (6.3)	0 (0.0)	6 (37.5)
Pork	3 (18.8)	2 (12.5)	1 (6.3)	1 (6.3)	1 (6.3)	8 (50.0)
Chicken	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)	2 (12.5)
Total	6 (37.5)	5 (31.3)	1 (6.3)	2 (12.5)	2 (12.5)	16 (100)

animals in the two markets. Modern market and High level market were observed to have healthier water sources than the other markets. The lower prevalence rates observed in samples from these two markets may not be unconnected with the state of their water sources. This underscores the need to make healthy water sources available in market places for meat processing so as to reduce contamination and its related problems. Inadequate hygiene by butchers in these markets could also have contributed to the high rate of contamination observed. Butcher tables have been reported to harbor a large population of *Listeria* species [35].

Table 3. *Listeria monocytogenes* contamination in five open markets sampled

Market	Negative (%)	Positive (%)	Total
Wurukum	47(88.7)	6(11.3)	53
Wadata	36(87.8)	5(12.2)	41
Highlevel	29(96.7)	1(3.3)	30
Northbank	38(95.0)	2(5.0)	40
Modern market	39(95.1)	2(4.9)	41
Total	189(92.2)	16(7.8)	205

All *L. monocytogenes* isolates were found to be susceptible to cotrimaxazole, erythromycin, chloramphenicol and gentamicin (Table 4). The isolates were however resistant to augmentin, amoxacillin, tetracycline and cloxacillin. These findings are consistent with that of an earlier report that *Listeria monocytogenes* was highly susceptible to gentamycin, erythromycin, chloramphenicol and tetracycline [36]. However, in this study, the susceptibility of *L. monocytogenes* to tetracycline was not established as reported. Resistance to tetracycline as observed in this study agrees with the report [37]. Similarly, in this study, *Listeria monocytogenes* was found to be susceptible to chloramphenicol, in contrast to the findings of [37]. These results further reveal that food items such as fresh meat are also contaminated with antibiotic resistant strains of *L. monocytogenes*. Evidence in literature suggests that resistance to antibiotics by food-borne pathogens can be transferred from one bacteria to another by conjugation [20]. The resulting mutant strains can contaminate raw materials and be ingested through contaminated food [38]. Also, the indiscriminate use of antibiotics by veterinarians on the farm and by humans, have been fingered as possible reasons for antibiotic resistance in food borne pathogens [39].

Table 4. Antibiotic susceptibility pattern of *Listeria monocytogenes* isolated from raw beef, pork and chicken

Antibiotic (µg)	Resistant (%)	Sensitive (%)
Augmentin (30)	16 (100)	0 (0.0)
Amoxicillin (25)	16 (100)	0 (0.0)
Erythromycin (5)	1 (6.3)	15 (93.7)
Tetracycline (10)	16 (100)	0 (0.0)
Cloxacillin (5)	16 (100)	0 (0.0)
Gentamycin (10)	2 (12.5)	14 (87.5)
Cotrimoxazole (25)	1 (6.3)	15 (93.7)
Chloramphenicol (30)	0 (0.0)	16 (100)

4. CONCLUSION

This present study has observed a high incidence of *L. monocytogenes* contamination in raw beef, pork and chicken sold in Makurdi, indicating poor hygiene, handling and environmental sanitation. The implication of this finding is that a large population of the inhabitants may be exposed to *L. monocytogenes* infection from consumption pork, beef and chicken especially when not properly cooked. There exist the possible existence of antibiotic resistant *L. monocytogenes* in meat, which could pose a great challenge in the treatment of listeria infections. There is urgent need to curb contamination of raw meat by this pathogen so as to safeguard the health of consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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