Prevalence and Antibiogram of *Listeria Monocytogenes* Contamination of Liver, Spleen, Ruminal Content and Effluent in Jos, Nigeria

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Abstract

**Objective:** This study was designed to determine the prevalence and antibiogram of *L. monocytogenes* from the liver, spleen, ruminal content of cattle and goats and effluent from abattoir/slaughter slabs in Jos, Nigeria.

**Methods:** A cross-sectional study was conducted and 360 samples were collected which include raw liver, spleen, ruminal content and effluent. Statistical test using Pearson’s chi-square test for independence was done to determine the frequency of isolation of *L. monocytogenes*.

**Results:** Confirmation of the isolates using conventional biochemical characterization showed that 9 (2.5%) were positive for *L. monocytogenes*. Distribution of *L. monocytogenes* by sample type showed effluent (6.7%) had the highest prevalence compared to ruminal content (2.2%) and liver (1.1%) and the association was statistically significant (p < 0.05). Distribution of *L. monocytogenes* based on location showed Yan-Shanu (6.9%) had the highest prevalence although, the association was not statistically significant (p > 0.05). Antibiotic susceptibility testing revealed all (100%) of the *L. monocytogenes* isolates were resistant to ampicillin and colistin sulphate while none of the isolates showed resistant to ciprofloxacin. The sensitivity regimens were 8 (88.9%) for chloramphenicol as the most effective while the least sensitive were 1 (11.1%) for nalidixic acid and penicillin G each. Antimicrobial resistant profile of the isolates revealed that they were all resistant to at least four antimicrobial agents and the mean multiple antibiotic resistance index was 0.47±0.034.

**Introduction**

*Listeria monocytogenes* is an opportunistic foodborne pathogen belonging to the genus *Listeria*, a Gram-positive, non-sporing, facultative anaerobic rod-shaped bacterium [1]. The genus *Listeria* consists of other different recognized species namely *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. marthii*, *L. innocua*, *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae* [2]. Only two of these species are considered pathogenic to humans (*L. monocytogenes*) and animals (*L. monocytogenes* and *L. ivanovii*) [3]. However, sporadic cases of human infections caused by *L. ivanovii*, *L. innocua* and *L. seeligeri* in immunocompromised individuals have been reported [4,5,6] *L. monocytogenes*, the agent of listeriosis has been recognized as an important public health problem due to food safety concerns [2].

Infection with listeriosis is acquired through consumption of contaminated food by susceptible individuals [7] primarily pregnant women, neonates, elderly people, and those with weakened immune systems [8]. In Nigeria, there have been limited reports documenting outbreaks of listeriosis in the country [9]. Recently, South Africa witnessed the world’s largest outbreak of human listeriosis attributed to the consumption of ready-to-eat processed meat products from a food production facility in which were 1060 confirmed laboratory cases with 216 deaths [9,10]. *L. monocytogenes* which is ubiquitous in nature has been isolated from several food and environmental sources including fresh raw and frozen meat and fish products [11,12,13], vegetables [14], several ready-to-eat foods [15,16], milk and milk products [17,18,19], cattle faeces [20], food-associated environment [21,22] and in untreated abattoir wastewater/effluent [23,24]. However, less information is available on the occurrence of *L. monocytogenes* in abattoir by-product from slaught-ered animals.

In the recent past decade, extensive administration of antibiotics has led to misuse of these drugs in humans and animals which has contributed to the widespread antibiotic resistance among foodborne pathogens, *Listeria monocytogenes* inclusive [25]. Presently, antibiotic resistance is a significant public health problem, for instance, in the United States of America thousands of people die from infections caused by antibiotic-resistant bacteria [17,26]. Hence, this study was conducted to get an overview on the occurrence, distribution and antibiogram of *L. monocytogenes* in the liver, spleen, ruminal content of slaughtered cattle and goats and effluent from abat- toir/slaughter slabs in Jos, Nigeria, as a threat to food safety since the organism has the tendency to form biofilms in food processing environment and thereby cross-contaminate food product as seen in many ready-to-eat foods [27,22].

**Materials and methods**

**Study site**

The study was carried out in Jos, Plateau State, Nigeria located in the North-Central geo-political zone of Nigeria (Figure 1). It lies between latitude 9°56’ North and longitude 8°53’ East, having a total land area of 1,821km². Plateau State shares geographic boundaries with four of the thirty-six States of the Federation, Bauchi, Kaduna, Nasarawa and Taraba States at its North-Eastern, North-Western, South-Western and South-Eastern boundaries respectively. It’s comprised of three local governments: Jos East, Jos North and Jos South. Plateau State is the 12th largest State in Nigeria with a population of about three million people. The State capital has only one registered abattoir and many un-registered slaughter slabs [28]; Personal communication) that all serve the populace. The Jos abattoir was established in 1975.

**Figure 1:** Map of Nigeria showing Plateau State and the Study Areas (Source: ArcAGIS).

**Study design**

A cross-sectional study was conducted in which one registered abattoir and three un-registered slaughter slabs were selected for this study i.e. two each from Jos North (Jos Abattoir and Yan-Shanu slaughter slabs) and Jos South (Bukuru and Geyl slaughter slabs) Local Government Areas. Three hundred and sixty (360) samples were collected from the study sites based on convenient sampling method. These are Bukuru (cattle) 72, Geyl (cattle) 72, Jos abattoir (cattle) 72, Jos abattoir (goat) 72 and Yan-Shanu (cattle) 72. Samples collected were 90 Liver, 90 spleen, 90 ruminal content of cattle and goats and 90 effluent samples from abattoir/slaughter slabs were collected, that is, 18 of each of these by-product samples from each of the sampling location.
Sampling

Pooled cattle and goat cut samples of liver, spleen and ruminal content were collected in polythene bags. Two hundred and fifty millilitres (250ml) of effluent samples from the abattoir/slaughter slabs were collected at the discharge point in the slaughter hall. The samples were kept on ice packs at 4°C in Coleman box and transported to the laboratory for analysis. Sampling was performed three times weekly for six weeks.

Isolation of Listeria species

The ISO 11920-1 method for the quantitative isolation and identification of L. monocytogenes was used as described by Indrawattana et al. [29] with modifications. Aseptically, 10g of each sample of spleen, liver and ruminal content were and transferred into sterile stomacher bags and 90ml of 0.1% peptone water was added. They were homogenised using a stom-achor (Stomacher Lab Blender 400) for 4-5 minutes followed by incubation at 37°C for 24 hours. One ml of each sample (effluent and homogenized liver, spleen and ruminal content) was suspended into 9ml of prepared Listeria enrichment broth incorporated with Listeria selective supplement and incubated at 37°C for 24 hours. A loopful of each sample was streaked onto the surface of Listeria selective agar incorporated with Listeria selective supplement and were incubated at 37°C for 24 hours. A loopful of each positive culture identified by the colonial morphology of greyish colonies surrounded by black halos (esculin hydrolysis) with sunken centres were sub-cultured by plating individually onto Tryptone Soya Agar (Oxoid, CMO 131) supplemented by 0.6% Yeast Extract Powder (Oxoid, LP002 1) (TSAYE) and were incubated at 37°C for 24 hours. The suspected colonies were inoculated onto prepared nutrient agar slant and incubated at 37°C for 24 hours and stored at 4°C in the refrigerator for further identification. Phenotypic characterization to species level was performed by subjecting presumptive colonies to Gram staining and various biochemical test such as catalase, oxidase, haemolysis test, esculin hydrolysis, sugar fermentation tests (sucrose, arabinose, mannitol, rhamnose and xylose), methyl red, Voges Proskauer and the use of Microgen™ Listeria-ID by following manufacturer’s instruction.

Antimicrobial susceptibility testing (AST)

The antimicrobial susceptibility test of the Listeria monocytogenes isolates was determined by the Kirby-Bauer disc diffusion method on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). Standard discs were applied using a disc dispenser following the procedures recommended by the Clinical and Laboratory Standards Institute [30]. Three to five well distinct colonies of the confirmed Listeria isolates were transferred into 5ml Brain Heart Infusion Broth (BHIB, Oxoid) and were incubated at 37°C for 24 h. The overnight broth culture was diluted using sterile normal saline to a turbidity equivalent to 0.5 McFarland standard (10^5cfu/ml) and inoculated onto the dried surface of Muller Hinton agar (MHA, Oxoid) plate. Sterile cotton-tipped swab was used to inoculate the plate using the lawn inoculation technique. The inoculated MHA plates were allowed to dry at room temperature and paper discs impregnated sulphanmethoxazole-Trimethoprim (25µg), Kanamycin (30µg), Gentamycin (30µg), Ciprofloxacin (5µg), Nalidixic Acid (30µg), Penicillin G (10 IU), Clindamycin (2µg), Chloramphenicol (30µg), Amoxicillin/Clavulanic Acid 2:1 (30µg), Ampicillin (10µg), Tetracycline (30µg) and Colistin Sulphate (10µg) where placed using antibiotic disc dispenser and incubated at 37°C for 24 hours. After incubation, the diameters (mm) of clear zones of inhibition around each disc was measured and interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines [27] with Staphylococcus aureus ATCC™ 25923 (Pseudomonas aeruginosa ATCC™ 27853 for colistin sulphate) used as control.

Results

Antimicrobial susceptibility testing (AST)

Out of the 360 samples of by-product examined, 152 (42.2%) were positive for Listeria species while 208 (57.8%) samples showed no growth on Listeria agar. Distribution of the 152 Listeria species following biochemical characterization showed that 9 (2.5%) were L. monocytogenes while 16 (4.4%), 121 (33.6%) and 6 (1.7%) were L. grayi, L. ivanovii and L. seeligeri respectively (Table 1).

Distribution of L. monocytogenes isolates by sample type showed that effluent had the highest level of contamination, 6 (6.7%) which was followed by ruminal content, 2 (2.2%) and the least contamination in the liver sample, 1 (1.1%). The result of the chi-square test showed that there was a statistically significant association between the prevalence of Listeria monocytogenes and the sample type in this study (p<0.05) (Table 2).

The level of contamination of the samples with L. monocytogenes showed that Yan-Shanu slaughter slab had the highest occurrence, 5 (6.9%) this was followed by Jos Abattoir cattle with 2 (2.8%) and the least contamination in Gyel slaughter slab and andios abattoir goat with 1 (1.4%) each. There was, however, no statistically significant association between the prevalence of Listeria monocytogenes and sampling locations (p>0.05) (Table 3).

Table 1: Distribution of Listeria species based on total sample collected in Jos North and Jos South LGA of Plateau State using conventional biochemistry testing (n=360).

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. monocytogenes</td>
<td>9</td>
<td>2.5</td>
</tr>
<tr>
<td>L. grayi</td>
<td>16</td>
<td>4.4</td>
</tr>
<tr>
<td>L. ivanovii</td>
<td>121</td>
<td>33.6</td>
</tr>
<tr>
<td>L. seeligeri</td>
<td>6</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>42.2</td>
</tr>
</tbody>
</table>

Table 2: Distribution of Listeria monocytogenes based on sample type in Jos North and Jos South LGA.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>No. Tested</th>
<th>Number Positive (%)</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent</td>
<td>90</td>
<td>6 (6.7)</td>
<td>98.448</td>
<td>0.0001</td>
</tr>
<tr>
<td>Liver</td>
<td>90</td>
<td>1 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruminal content</td>
<td>90</td>
<td>2 (2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>90</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>360</td>
<td>9 (2.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Distribution of Listeria monocytogenes based on sampling location in Jos North and Jos South L.G.A.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. Tested</th>
<th>Number Positive (%)</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bukuru cattle</td>
<td>72</td>
<td>-</td>
<td>21.498</td>
<td>0.1601</td>
</tr>
<tr>
<td>Gyal cattle</td>
<td>72</td>
<td>1 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jos Abattoir cattle</td>
<td>72</td>
<td>2 (2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jos Abattoir goat</td>
<td>72</td>
<td>1 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yan Shanu cattle</td>
<td>72</td>
<td>5 (6.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>360</td>
<td>9 (2.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The antibiotic sensitivity test (Figure 2) showed a high susceptibility to chloramphenicol 88.9% followed by ciprofloxacin 55.6%, amoxycillin and clavulanic acid 55.6% and tetracycline 55.6%. The least susceptibility was obtained for nalidixic acid 11.1% and penicillin G 11.1%. In contrast, a high resistance to ampicillin and colistin sulphate (100%) was observed followed by penicillin G (88.9%). The least resistance was obtained for ciprofloxacin (0%) followed by chloramphenicol (11.1%).

![Figure 2: Distribution of antimicrobial susceptibility testing of Listeria monocytogenes isolates. S: Sensitive; I: Intermediate; R: Resistant.](image)

The antimicrobial resistance profile showed that all the isolates were resistant to at least four antibiotics although the majority (88.9%) of the L. monocytogenes isolates were resistant to more than four antibiotics (Table 4). The mean multiple antibiotic resistance (MAR) index was 0.47±0.034 with the minimum being 0.33 and maximum 0.67 (Table 5).

**Table 4:** Antimicrobial resistance pattern of Listeria monocytogenes isolates from abattoir/slaughter slabs by-products.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Antibiogram</th>
<th>MAR Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCEF07</td>
<td>Effluent</td>
<td>SXT, NA, TE, CT, AMP</td>
<td>0.42</td>
</tr>
<tr>
<td>YSEF28</td>
<td>Effluent</td>
<td>NA, P, CT, AMP</td>
<td>0.33</td>
</tr>
<tr>
<td>JCEF47</td>
<td>Effluent</td>
<td>SXT, CN, P, TE, CT, AMP</td>
<td>0.50</td>
</tr>
<tr>
<td>JCRS54</td>
<td>R. content*</td>
<td>SXT, CN, NA, P, TE, DA, CT, AMP</td>
<td>0.67</td>
</tr>
<tr>
<td>JCLY17</td>
<td>Liver</td>
<td>SXT, NA, P, DA, CT, AMP</td>
<td>0.50</td>
</tr>
<tr>
<td>YSEF78</td>
<td>Effluent</td>
<td>K, P, AMC, C, DA, CT, AMP</td>
<td>0.58</td>
</tr>
<tr>
<td>YSEF85</td>
<td>Effluent</td>
<td>NA, P, AMC, CT, AMP</td>
<td>0.42</td>
</tr>
<tr>
<td>YSEF96</td>
<td>Effluent</td>
<td>NA, P, AMC, CT, AMP</td>
<td>0.42</td>
</tr>
<tr>
<td>GYRC152</td>
<td>R. content*</td>
<td>NA, P, AMC, CT, AMP</td>
<td>0.42</td>
</tr>
</tbody>
</table>

* Ruminal content, SXT: Sulphamethoxazole-Trimetophrom; NA: Nalidixic Acid; TE: Tetracycline; CT: Colistin Sulphate; AMP: Ampicillin; P: Penicillin G; CN: Gentamycin; DA: Clindamycin; K: Kanamycin; C: Chloramphenicol; AMC: Amoxycillin and Clavulanic Acid.

**Table 5:** Multiple drug resistance of the Listeria monocytogenes isolates from abattoir/by-products.

<table>
<thead>
<tr>
<th>Number of antibiotics</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to three antibiotics</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resistance to four antibiotics</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>Resistance to more than four antibiotics</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

This study established an overall prevalence of 42.2% of Listeria species in the abattoir by-product samples in Jos North and Jos South LGA of Plateau State. Similar prevalence of 40% was reported in Iran on chicken carcass by Zeinali et al. [31], 40.8% in Sudan on fresh raw dressed broiler by Alsheikh et al. [32]. A slightly higher prevalence of 58.2% in North-Central Nigeria on raw beef was reported by Chuku et al. [12] and a lower findings of 11.3% in Kano, North-Western Nigeria on some ready to eat foods by Aisha and Kawo [33]. This is probably due the fact that Listeria species are ubiquitous in nature and especially found in temperate regions [34], also, domestic ruminant are known to sustain the micro-organisms in their ruminal environment through a repeated faecal-oral enrichment cycle [35]. Listeria species have been reported in salad vegetables and vegetable salads [14], milk and milk products [36], raw and processed meat [37], abattoir effluent [24] to mention a few.

The prevalence of individual Listeria species was recorded as 2.5%, 4.4%, 33.6% and 1.7% for Listeria monocytogenes, Listeria grayi, Listeria ivanovii and Listeria seeligeri respectively. The results of this study was lower than the report of Omogbai and Esokpunwu [13] in frozen beef and chicken samples sold in Benin city, Nigeria except for Listeria ivanovii which was 30.43% as compared to 33.6% found in this study. Also, Listeria innocua was isolated in their study as while Listeria seeligeri is found in this study.

The overall prevalence of 2.5% obtained for Listeria monocytogenes is higher than the 1.8% observed by Aisha and Kawo [33] from some ready to eat foods in Kano, North-Western Nigeria and also the 1.29% findings of Eruetya et al. [38] from raw cow and goat meat in Port Harcourt, Nigeria. This prevalence is lower than the 36.73% observations of Ebakota et al. [15] from ready to eat foods in Southern Nigeria, also it is lower than the 26.8% reports of Falodun and Amusan [23] from untreated abattoir wastewater in Akinyele, Ibadan, Nigeria as well as lower than the 6.55% findings of Usman et al. [36] from raw milk and milk products in Northern Nigeria. The inconsistencies in the prevalence of Listeria species in both raw and processed food as seen in this and other reports cited here could be attributed to the fact that the pathogen is present in the environment and could be as a result of cross contamination from the meat processing plants and after food processing as seen in ready to eat (RTE) foods [39].

The prevalence of Listeria monocytogenes was highest in effluent (6.7%) as compared to ruminal content (2.2%) and Liver (1.1%) samples. The association was statistically significant (p=0.0001). The relatively higher prevalence in effluent could be attributed to the fact that most Nigerian abattoirs discharge the effluent untreated through drainages unto water bodies that contaminates surface and ground waters with pathogenic micro-organisms that are most time invariably used in washing the carcass and slaughter hall after the slaughtering activities [40,41,42,43]. The relatively high prevalence in the ruminal content may be because of the fact that food animals are a major reservoir of a lot of foodborne pathogens including Listeria monocytogenes [44]. Studies have reported the isolation Listeria species and Listeria monocytogenes in faecal samples of animals that were fed from infected feedlot [21,45]. The prevalence in liver could be attributed to cross-contamination of meat and by-product by pathogenic organisms during processing activities such as washing of carcass with contaminated water sources [46,47], cutting of carcass on bare floor in the
The high resistance of *Listeria monocytogenes* to penicillin G 88.9% and nalidixic acid 77.8% was similar to the work of Şanlıbaba *et al.* [16] where 100% of the isolates were both resistant to penicillin G and nalidixic acid from ready to eat foods in Turkey. Welekidan *et al.* [60] also reported a similar resistance of 76.47% among pregnant women in Tigray region and Vaidya *et al.* [57] observed a resistance of 66.7% from slaughtered goats and pigs in Nagpur, Central India to penicillin G. This finding however does not agree to the report of Osman *et al.* [27] from different ecological niches in Egypt and Aksoy *et al.* [17] in raw milk and dairy products in Turkey where 100% and 93.3% of the isolates were susceptible to penicillin G respectively. Chuku *et al.* [12] also reported a 100% resistance to nalidixic acid while Girma and Abebe [18] a resistance of 30.5%. The 44.4% resistance to sulfamethoxazole-trimethoprim was similar to the findings of Osman *et al.* [27] and Welekidan *et al.* [60] that reported a resistance of 44.4% and 33.3% respectively. The 33.3% resistance to clindamycin observed in this work was lower than the 66.7% and 82.3% findings of Welekidan *et al.* [60] and Owusu-Kwarteng *et al.* [19] respectively while Osman *et al.* [27] and Şanlıbaba *et al.* [16] each reported a much higher resistance of 100% to clindamycin.

Multiple Antibiotic Resistance (MAR) index of the *Listeria monocytogenes* isolates revealed that the mean MAR index was 0.47±0.034. Wong *et al.* [61] stated that a greater than 0.2MAR index points to the fact that the isolates originate from high-risk area where the rate of antibiotic usage is much. This study is one of such where the minimum MAR index was 0.33. The high level of resistance of *L. monocytogenes* to these drugsshould be a great concern to relevant health agencies, regula-tors and the general public. The implication of a multidrug re-sistant pathogen as seen in this study is that, it becomes more patho-genic compared to non-multidrug resistant pathogens[62]. This situation makes treatment regimen unsuccessful in infected pa-tients [63] and there is additional cost of treatment, as well as prolonged hospital stay [64].

Multiple Antibiotic Resistance (MAR) index of the *Listeria monocytogenes* isolates revealed that the mean MAR index was 0.47±0.034. Wong *et al.* [58] stated that a greater than 0.2 MAR index points to the fact that the isolates originate from high-risk area where the rate of antibiotic usage is much. This study is one of such where the minimum MAR index was 0.33. The high level of resistance of *L. monocytogenes* to these drugs should be a great concern to relevant health agencies, regula-tors and the general public. The implication of a multidrug re-sistant pathogen as seen in this study is that, it becomes more patho-genic compared to non-multidrug resistant pathogens[59]. This situation makes treatment regimen unsuccessful in infected patients [60] and there is additional cost of treatment, as well as prolonged hospital stay [61].

**Conclusion**

The results obtained from this study have shown that *Liste-ria monocytogenes* is at 6.7% in effluent, 2.2% in ruminal con-tent and 1.1% in liver abattoir by-products. These are of public health significance based on the fact that the meat processing environment is contaminated with this pathogenic organ-ism and this have been shown to cross contaminate meat by-products (liver). These could result in listeriosis in animals when grazing in the abattoir/slaughter slabs environment which could invariably infect susceptible humans.
The result also showed that 100% of the isolates displayed resistance to more than three antimicrobial agents to which they were tested. The results also showed that ciprofloxacin is the best drug for the treatment of listeriosis since no (0%) isolate exhibited resistance to the drug. This is followed by chloramphenicol and kanamycin where only 11.1% of the isolates each showed resistance to the drugs.

It was discovered from this study that effluent and ruminal content that are discharged untreated into stagnant drainages and piled up in the environment respectively may pose a greater public health threat. This requires that effluent and ruminal content be treated before further use.

References


