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# PREVALENCE OF LISTERIA SPECIES IN READY TO TAKE MILK AND MEAT PRODUCTS IN NAIROBI AND ITS ENVIRONS, KENYA

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## ABSTRACT

Listeriosis is one of the leading causes of death in food-borne infections globally. The disease has mortality rates between 30-50%, especially in high-risk groups. The disease is caused by *Listeria monocytogenes*, a pathogenic bacterium in the genus *Listeria*. Currently, in Kenya, there's limited information on the existence of *Listeria spp.* in milk and meat products. Therefore, the present study aimed to ascertain the incidence of *Listeria spp*. in ready-to-eat meat and milk products in the city of Nairobi and its surroundings. We collected 570 meat and milk products from selected retail markets. Isolation of *Listeria spp.* was carried out per the bacteriological analytical manual protocol of the food and drug administration. Identification of suspected colonies was done through colonial morphology and biochemical tests. Confirmation of the genus and species of the isolates was done through multiplex PCR. Out of the total samples, 8.59% were confirmed for *Listeria spp*. Out of these isolates, 21(42.8%) were found in milk products such as milk powder 1/17(5.8%), short life pasteurized milk 1/66(1.5%), long life pasteurized milk 3/62(4.83%) and pasteurized milk from dispensing machines 16/20(80%). The rest, 28/49(57.2%) were obtained from meat products namely, ham 2/37(5.4%), brawn 13/73(17.8%), polony 8/27(29.6%), salami 1/6(16.7%) and ready to eat meat bites 4/77(5.19%). *Listeria monocytogenes* were detected in 22(3.86%) samples, with the highest prevalence being from milk from dispensing machines (68.18%). Of the other *Listeria* isolates, 27/49(55%) and 2(7.4%) were identified as *Listeria welshimeri*, while 3(11.11%) were identified as *Listeria innocua*. The remaining isolates were unidentified *Listeria*. The study concluded that *Listeria* spp. and *Listeria monocytogenes* in particular is present in milk and meat products sold in retail markets in Nairobi and its environs.

***Keywords*:** *Listeria*, Meat, Milk, PCR, Zoonosis

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## 1. INTRODUCTION

*Listeria spp.* are rod shaped bacteria of 0.4-0.5µm by 1-1.5µm in size, gram positive, motile at 10°C to 25°C, non-spore forming, facultative anaerobic and found in a wide range of environments including water, soil, effluent and a variety of foods (Vazquez-Boland et al. 2001; Liu 2006; Liu 2013; Schlech 2019; Iwu and Okoh 2020). Recent studies have identified up to 18 species namely; *L. monocytogenes, L. innocua, L. ivanovii, L. grayi, L. welshimeri, L. marthii, L. seeligeri, L. fleischmannii, L. rocourtiae, L. weihenstephanensis, L. aquatica, L. cornellensis, L. newyorkensis, L. floridensis, L. grandensis, L. riparia, L. costaricensis* and *L. booriae* (Orsi and Wiedmann 2016; Schardt et al. 2017). Of the 18 species, only *L. ivanovii* and *L. monocytogenes* are found to be pathogenic in animals as well as man (Robinson et al. 2000; Liu 2013; Orsi and Wiedmann 2016; Ahmed 2019; Feng et al. 2020; Liu et al. 2020).

*Listeria monocytogenes* is the causative agent of Listeriosis, a foodborne zoonotic disease, that is said to be the leading cause of death in reported cases of food poisoning, often having a mortality rate of between 30-50% in some cases (Lindback et al. 2011). The organism is widespread in nature and found in a wide variety of natural environments such as soil, plants, silage, sewage and water (Tchatchouang et al. 2020). The reservoirs for infection are the soil and the intestinal tracts of infected asymptomatic animals such as domestic animals, wild animals, fish and birds (OIE 2014). These animals can then shed the organisms through faeces, milk, uterine discharges, nasal discharges and urine (MVM 2016). The pathogen is a common contaminant of a wide range of food products, including raw vegetables, raw milk, raw meat, soft cheese, fish, poultry and minimally processed foods that do not require any significant heat processing before consumption. Processed foods may get contaminated during the production process from the raw product to the final consumer (Thakur et al. 2018).

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Infection in humans is through ingestion of a wide range of contaminated foods such as meat products, dairy products especially soft cheese, salad vegetables, fish and sea food products, delicatessen products and industrially produced refrigerated ready to eat foods that don't require further cooking or reheating (Vazquez-Boland et al. 2001; Ponniah et al. 2010; CDC 2011; OIE 2014). The infective dose of Listeriosis has been estimated to be the consumption of food containing between $10^6$-$10^8$ cells of *Listeria monocytogenes*. However, it is largely dependent on the immunological status of the host (Arun 2008). The groups with the highest risk of Listeriosis are pregnant women, neonates, the elderly and the immunocompromised (Montero et al. 2015; Craig et al. 2019; Jeffs et al. 2020) where it manifests itself through septicemia, meningitis, encephalitis, gastroenteritis and spontaneous abortions or still births in pregnant women (Liu 2006; OIE 2014; Girma et al. 2021; Heidarzadeh et al. 2021).

A lot of studies have been conducted to determine the occurrence of *Listeria spp*. in foods worldwide. In Kenya, however, there's limited information on this and therefore this study was conducted to determine the prevalence of *Listeria spp*. in ready to eat meat and milk products in the county of Nairobi and its environs.

## 2. MATERIALS AND METHODS

### 2.1. Study Design and Sampling

A total of 570 samples were collected by simple random sampling from selected supermarkets in 45 suburbs and urban centers within 4 counties that make up the Nairobi metropolitan region (Nairobi, Kiambu, Machakos and Kajiado) between March 2017 and October 2018. Samples collected were milk from vending machines, packaged milk (short life and long life), *mala* (sour milk), ice cream, milk powder, yoghurt, brawn, ham, ready to eat meat bites, polony and salami. The samples were checked for expiry dates, properly labelled and placed in a cool box containing ice packs and transported to the research laboratory at the Department of Public Health, Pharmacology and Toxicology at the University of Nairobi for analysis. Aseptic techniques were observed to avoid contamination of the sample from the collection site to the laboratory.

### 2.2. Isolation and Identification

Isolation and identification were carried out as per the United States Food and Drug Administration (FDA)/ Bacteriological Analytical Method 2011 (BAM 2011) with slight modifications. Briefly 25g or 25ml of the products were placed in sterile stomacher bags after which 225 ml of Listeria Enrichment Broth (CM0862B Oxoid® UK) with supplements (SR0141E Oxoid® UK) prepared as per the manufacturer's instructions was added. Homogenization was done using a stomacher machine (Stomacher 400 Lab Blender) for 1 minute in normal speed after which the samples were incubated for 48 hours at 30°C (Sekonic pocketcorder incubator, Japan). A loopful of the enriched sample was then subcultured for 24 hours at 37°C in Listeria Selective Agar (CM0856B Oxoid® UK) containing selective supplements (SR0140E Oxoid® UK).

Grey colonies with a black surrounding were identified as possible *Listeria* colonies. Gram staining was conducted using the recommended protocol and gram-positive short rods were tentatively identified as belonging to the genus *Listeria*. The oxidase test was performed by touching and spreading an isolated *Listeria* colony on an oxidase disc (Himedia® India) and the reaction observed within 10 seconds while the catalase test was performed by picking an isolated *Listeria* colony and exposing it to a drop of 3% hydrogen peroxide solution on a sterile Petri dish. Four distinct colonies from each plate were stored in cryotubes containing 10% skimmed milk (LP0031B Oxoid® UK) at a temperature of -20°C until required for molecular identification of genus and species.

### 2.3. Reviving of Stored Colonies

The colonies stored in skimmed milk were thawed and revived by streaking a loopful on Tryptone Soy Agar (TSA) base (CM0131B Oxoid® UK) and incubating for 24 hours at 37°C. DNA was extracted from distinct white colonies which were indicative of *Listeria spp*.

### 2.4. DNA Extraction

This was performed by placing a colony of *Listeria spp.* in Eppendorf tube containing 200µL distilled water and heating at 100°C for 10 minutes in a water bath (Monday et al. 2007). The boiled suspension was then let to cool before being centrifuged (Eppendorf centrifuge 5424R, Hamburg, Germany) at 15,000 rpm for four minutes. The supernatant was transferred in a new DNAase/ RNAase free Eppendorf tube and stored at -20°C until required for PCR analysis.

### 2.5. Listeria Genus and Species Identification by Multiplex Polymerase Chain Reaction (mPCR)

A multiplex PCR (mPCR) was done to identify the genus and six species of Listeria (*L. monocytogenes, L. innocua, L. ivanovii, L. grayi, L. seeligeri* and *L. welshimeri*) among the isolates. The PCR protocol described by Mazza et al. (2015) for genus and species identification was followed. The forward and reverse primer sequences,

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amplicon sizes and target genes are as indicated in Table 1. The reaction was performed in a final reaction volume of 25µL containing 2.5µL of 10X PCR buffer, 1U of Taq DNA Polymerase (New England BioLabs® Inc.), 0.25µL of 100 mM $MgCl_2$. 2.0µL of 2.5mM of each dNTPs, 5µL of DNA lysate template and the primers for genus and each species-specific gene at the given concentration. M/S New England BioLabs (NEB, USA) supplied all PCR reagents except the primers which were procured from Inqaba Biotec ™ (South Africa). *Listeria monocytogenes* ATCC 19115 was used as the positive control while DNase/RNase free distilled water was used as negative control.

**Table 1:** Target genes, primer pairs sequences and amplicon sizes for the six *Listeria* species

| Species | Target Gene | Primer pair sequences | Amplicon size (bp) | References |
|---|---|---|---|---|
| *Listeria species* | *prs* | F: 5′-GCTGAAGAGATTGCGAAAGAAG-3′<br>R: 5′-CAAAGAAACCTTGGATTTGCGG-3′ | 370 | Doumith et al. (2004) |
| *L. monocytogenes* | *Lmo1030* | F:5′-GCTTGTATTCACTTGGATTTGTCTGG-3′<br>R: 5′-ACCATCCGCATATCTCAGCCAACT-3′ | 509 | Mazza et al. (2015) |
| *L. ivanovii* | *namA* | F: 5′-CGAATTCCTTATTCACTTGAGC-3′<br>R: 5′-GGTGCTGCGAACTTAACTCA-3′ | 463 | |
| *L. innocua* | *Lin0464* | F: 5′-CGCATTTATCGCCAAAACTC-3′<br>R: 5′-TCGTGACATAGACGCGATTG-3′ | 749 | |
| *L. grayi* | *Oxidoreductasi* | F:5′-GCGGATAAAGGTGTTCGGGTCAA-3′<br>R: 5′-ATTTGCTATCGTCCGAGGCTAGG-3′ | 201 | |
| *L. seelingeri* | *Lmo0333* | F: 5′-GTACCTGCTGGGAGTACATA-3′<br>R: 5′-CTGTCTCCATATCCGTACAG-3′ | 673 | |
| *L. welshimeri* | *scrA* | F: 5′-CGTGGCACAATAGCAATCTG-3′<br>R: 5′-GACATGCCTGCTGAACTAGA-3′ | 281 | |

The amplification was carried out in a PCR thermal cycler (Applied biosystems ™ Veriti 96 well thermal cycler) with an initial denaturation step at 94°C for 5 minutes; annealing at 58°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. Agarose gel (1.5%; w/v) in TAE (Tris–acetate–ethylenediamine tetra acetic acid) buffer was used to electrophorese PCR product. Then were stained with ethidium bromide (0.05mg/µL) and envisioned under UV light and the images acquired by the UVP Gelmax® imager.

## 3. RESULTS

From the 570 milk and meat samples collected and cultured for bacteriological identification, 49 (8.59%) samples had isolates that showed growth characteristics similar to those of *Listeria spp*. namely small grey colonies with a black surrounding (Fig. 1). Twenty-one (42.86%) of the isolates were from milk and milk products while the rest (57.14%) were from meat products. On gram staining of these colonies, gram positive short rods indicative of *Listeria spp*. was observed. All the 49 Listeria isolates were positive for the catalase test and negative for the oxidase test indicative of *Listeria spp*.

**Fig. 1:** Small grey colonies and a black surrounding indicative of the growth of *Listeria spp* in Listeria Selective Agar (CM0856B Oxoid® UK) containing selective supplements (SR0140E Oxoid® UK).

Results of the MPCR confirmed that all the 49 biochemically identified isolates belonged to *Listeria spp*. after amplification of a 370 bp region of the *prs* gene. Of the twenty-one isolates from milk and milk products, 1 isolate (4.76%) was from milk powder, 1 (4.76%) from short life pasteurized milk, 3 (14.29%) from long life milk and 16 (76.19%) from pasteurized milk obtained from milk dispensing machines. Of the twenty-eight confirmed isolates from meat and meat products 2 isolates (7.14%) were from ham, 13 (46.43%) from brawn, 8 (28.57%) from polony, 1 (3.57%) from salami and 4 (14.28%) from ready to eat meat bites. There were no isolates from samples of *mala* (fermented milk), yoghurt, ice cream, cheese and milk cream. The overall prevalence of *Listeria spp*. in the sampled milk and meat products is as shown in Table 2.

Speciation of the *Listeria* isolates showed that of the 49 isolates, 22(44.9%) were *Listeria monocytogenes* as identified by the amplification of a 509bp region of the gene *"Lmo1030"*. Of these isolates, 17/22(77.27%) were from milk and milk

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**Table 2:** Overall prevalence of *Listeria spp.* in meat and milk products

| Product Type | # Samples collected | Listeria positive samples by PCR |
|---|---|---|
| Milk and milk products | | |
| Milk powder | 17 | 1 (5.8) |
| Short life milk | 67 | 1 (1.5) |
| Long life milk | 65 | 3 (4.6) |
| Dispenser milk | 36 | 16 (44.4) |
| *Mala* | 27 | 0 (0) |
| Ice cream | 24 | 0 (0) |
| Yoghurt | 109 | 0 (0) |
| Cheese and milk cream | 5 | 0 (0) |
| Meat and meat products | | |
| Polony | 27 | 8 (29.6) |
| Brawn | 73 | 13 (17.8) |
| Ham | 37 | 2 (5.4) |
| Salami | 6 | 1 (16) |
| Ready to eat meat bites | 77 | 4 (5.1) |
| Total | 570 | 49 (8.60) |

Values in parenthesis indicate percentage.

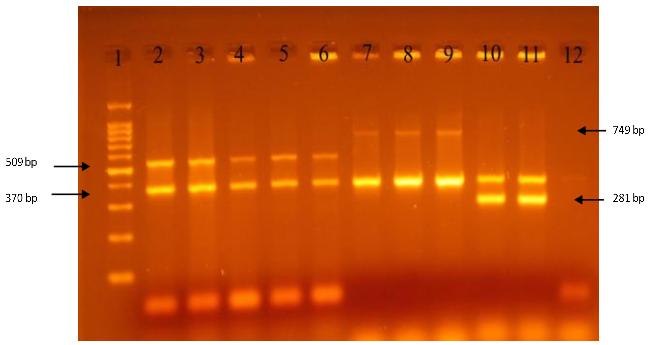


**Fig. 2:** Ethidium bromide stained 1.5% agarose gel electrophoresis of Listeria spp. isolates in mPCR using prs (370 bp), Lmo1030 (509bp), scrA (281bp) and Lin0464 (749bp) genes. Lane 1, 100 bp DNA ladder, Lane 2 *L. monocytogenes* positive control (ATCC19115), Lanes 3, 4, 5, 6 products amplified from DNA of *L. monocytogenes* isolates, Lanes 7, 8, 9 products amplified from DNA of *L. innocua* isolates, Lane 10 and 11 products amplified from DNA of *L. welshimeri* isolates, Lane 12 negative control.

products while 5/22(22.72%) were from ready to eat meat products. The maximum incidence, 15/22(68.18%) was from dispensed milk followed by 9.09% each from polony and brawn while the lowest, 4.54% was from short and long-life milk and ham. Among the non-*L. monocytogenes* 55.1%(27), 7.4%(2/27) were identified as *Listeria welshimeri* by amplification of a 281 bp region of the *scrA* gene through PCR while 11.1%(3/27) were identified as *Listeria innocua* by amplification of a 749 bp region of the *Lin0464* gene (Fig. 2). The remaining isolates, 81.5%(22/27) were not found to belong to any of the six tested *Listeria spp*. (Table 3).

**Table 3:** Distribution of isolated Listeria species in milk and meat products

| Product Type | No. of samples collected | *L. monocytogenes* | *L. innocua* | *L. welshimeri* | Unidentified *Listeria spp* |
|---|---|---|---|---|---|
| Milk and milk products | | | | | |
| Milk Powder | 17 | 0 (0) | 0 (0) | 0 (0) | 1 (5.8) |
| Short life milk | 67 | 1 (1.5) | 0 (0) | 0 (0) | 0 (0) |
| Long life milk | 65 | 1 (1.5) | 0 (0) | 0 (0) | 2 (3.1) |
| Dispenser milk | 36 | 15 (42) | 0 (0) | 0 (0) | 1 (2.8) |
| Mala | 27 | - | - | - | - |
| Ice cream | 24 | - | - | - | - |
| Yoghurt | 109 | - | - | - | - |
| Cheese and Milk cream | 5 | - | - | - | - |
| Total | 350 | 17 (4.86) | 0 (0) | 0 (0) | 4 (1.14) |
| Meat and meat products | | | | | |
| Polony | 27 | 2 (7.4) | 0 (0) | 1 (3.7) | 5 (18.5) |
| Brawn | 73 | 2 (2.7) | 3 (4.1) | 0 (0) | 8 (10.9) |
| Ham | 37 | 1 (2.7) | 0 (0) | 1 (2.7) | 0 (0) |
| Salami | 6 | 0 (0) | 0 (0) | 0 (0) | 1 (16.6) |
| Ready to eat meat bites | 77 | 0 (0) | 0 (0) | 0 (0) | 4 (5.2) |
| Total | 220 | 5 (2.27) | 3 (1.36) | 2 (0.91) | 18 (8.18) |
| Grand Total | 570 (100) | 22 (3.86) | 3 (0.53) | 2 (0.35) | 22 (3.86) |

Values in parenthesis indicate percentage.

## 4. DISCUSSION

In the present study, *Listeria spp*. were detected in ready-to-take milk and meat products with an overall prevalence of 8.59%. This was lower than what was reported in ready to eat foods of animal origin in Ethiopia which was between 25-28.4% (Derra et al. 2013; Garedew et al. 2015; Seyoum et al. 2015), in Turkey 20.4%, (Şanlibaba et al. 2018), and in Thailand, 16.5%, (Vongkamjan et al. 2016). However, it was consistent with the 9.3% that was reported in Algiers, Algeria by Bouayad and Hamdi (2012) and 9% in India by Nayak et al. (2015). The overall prevalence of *Listeria spp*. was higher in meat products than in milk products in the current study. This finding could have been as a result of the processing methods in meat products which may introduce a higher level of contamination especially during slaughter and evisceration (Bouayad et al. 2015; Kurpas et al. 2018).

This study reports an overall prevalence of *Listeria monocytogenes* of 3.86% which is consistent with the 4.1% reported in Addis Ababa, Ethiopia by Derra et al. (2013), 4.3% reported by Morobe et al. (2009) in Gaborone, Botswana 2.6% reported by Bouayad and Hamdi (2012) in Algiers, Algeria and 2.5% reported by Gelbicova and Karpiskova (2009) in the Czech Republic.

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This prevalence of *Listeria spp.* is higher than what was reported in Egypt by Osman et al. (2020) in retail food samples and lower than what was reported by Garedew et al. (2015) and Seyoum et al. (2015) in Ethiopia. The differences in findings may be attributed to differences in food item composition or the hygienic standards of the processing plants (Garedew et al. 2015).

The prevalence of *Listeria monocytogenes* in the current study was higher in milk and milk products than in ready-to-eat meat products with the highest prevalence being recorded in pasteurized milk from vending machines. The organism was also isolated from long life and short life pasteurized milk. Milk is one of the most important foods consumed by humans in addition to being a good culture medium for microorganisms including *Listeria monocytogenes* (Lee et al. 2019; Possas et al. 2022). Apparently healthy milk producing animals may shed *Listeria spp*. in milk throughout the lactation period and contribute to an increased risk of milk product contamination (Farber and Peterkin 1991). In studies conducted in Egypt on the prevalence of *Listeria spp*. in goat, sheep, cow, buffalo and camel udder milk, varying levels of *Listeria spp*. were reported with camel milk having the lowest prevalence of *Listeria monocytogenes* (Osman et al. 2014; Osman et al. 2014; Osman et al. 2016).

The process of pasteurization is meant to eliminate pathogens in milk and therefore the presence of *Listeria monocytogenes* in pasteurized milk may be due to contamination after pasteurization or faults in technology during pasteurization either due to inadequate temperature or a decreased pasteurization time (Navratilova et al. 2004; Lee et al. 2019). The findings from the present study are consistent with findings by Sreeja et al. (2016) and Sheela and Shrinithivihahshini (2017) in India and Navratilova et al. (2004) in Czech Republic who reported presence of *Listeria monocytogenes* in pasteurized packaged milk. They are also consistent with what has been previously reported in Kenya on the inadequacy of pasteurization in packaged milk (Mwangi et al. 2000; Nato et al. 2016). It was noted that milk sold through vending machines was brought in using metallic cans from processors and dispensed to consumers. Contamination could have occurred through improper handling of this milk during transfer to the vending machines, inadequate cleaning of the vending machines and the subsequent formation of biofilms that are able to embed themselves on food processing surfaces and niches. These biofilms are able to resist biocides and stress conditions including cleaning and disinfection (Colagiorgi et al. 2017). The isolation of *Listeria monocytogenes* in ready to eat meat products could have been due to cross contamination during processing, inadequate heat treatment, inadequate physical separation between raw and cooked product and poor sanitation (Şanlibaba et al. 2018).

There was no isolation of *Listeria spp*. from yoghurt, *mala* and ice cream. This finding is consistent with studies by Abrahão et al. (2008), Akman et al. (2004) and Mugampoza et al. (2011) who reported no isolation from ice cream and fermented milk products in Brazil, Turkey and Uganda respectively. The absence of *L. monocytogenes* in these products could have been due to use of Ultra High Temperature (UHT) processed milk in their production or low PH and effect of bacteriocins produced by lactic acid bacteria in the fermented products (Abrahão et al. 2008; Mugampoza et al. 2011).

This study also reports the isolation of *Listeria innocua* and *Listeria Welshimeri* from milk, milk products and ready to eat meat products although in a lower overall prevalence that what was reported in Ethiopia by Garedew et al. (2015) and in India by Nayak et al. (2015). Due to the frequent occurrence of *L. innocua* in foods, it can be considered an indicator bacterium for the presence of *L. monocytogenes* (Bubert et al. 1999).

There was also a significant portion of unidentified *Listeria spp*. which were mainly present in meat brawn, polony and ready to eat meat bites. These other *Listeria* could belong to any of the 12 species that were not tested for since the study was focusing on the six most common species of *Listeria* which are related with animal hosts. These other 12 *Listeria spp*. are mainly found in the environment such as water, soil and decaying plant matter (Orsi and Wiedmann 2016). Their presence in food therefore could indicate contamination of processing equipment.

## 5. Conclusion

From this study we conclude that *Listeria spp.* and *Listeria monocytogenes* in particular was present in milk and meat products sold in supermarkets in Nairobi and its environs. We recommend strict regulation of food processing especially pasteurization and post pasteurization handling of food products to ensure that food safety standards are achieved and that ready-to-eat products reach the consumers within the acceptable safety limits.

## Author's Contribution

Kabui KK, Gicheru MM, Gathura PB and Mainga AO conceived the idea, prepared the study design. Kabui KK, Mainga AO and Nduhiu JG collected the data. All authors analyzed the data, interpreted the data, and drafted and revised the article.

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