ORIGINAL ARTICLE

Prevalence and antibiotic susceptibility profiles of *Listeria monocytogenes* contamination of chicken flocks and meat in Oyo State, south-western Nigeria: Public health implications

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Keywords

Listeria monocytogenes • Meat contamination • Public health

Summary

Introduction. *Food contamination with Listeria monocytogenes is on the increase posing threats to public health with growing trends in food products recalls due to suspected Listeria contamination.*
Methods. *We conducted a cross-sectional study to determine the prevalence and antibiotic susceptibility profiles of Listeria monocytogenes (Lm) among 71 randomly selected poultry farms in Oyo State, Nigeria. A total of 450 samples comprising cloacal swabs (426) and randomly selected dressed chicken meat (24) were cultured for Lm isolation using BrillianceTM Selective Listeria Agar with antibiotics and microbial load count with Nutrient Agar. Further identification was done using microscopic, biochemical characterization and antibiotic sensitivity tests. Data were analysed using bivariate analysis and student t-test.*
Results. *An overall prevalence of 91.8% Lm contamination was obtained comprising 91.5% (390/426) in cloacal swabs and 95.8% (23/24) in meat. The prevalence of Lm in cloacal samples was significantly associated with poultry type (p = 0.008) and breed (p = 0.000. In addition, all the flocks had at least one positive sample yielding 100% flock prevalence. Antibiotic sensitivity test revealed that most of the isolates were resistant to common antibiotics like Ampicillin-cloxacillin and cefuroxime.*
Conclusions. *The results revealed a high level of contamination with Lm in the poultry flock and meat and the observed resistance to most common antibiotics has implications for future disease control as well as public health. There is need to step up routine screening of food animal products for Listeria contamination as well as measures towards reducing such contaminations.*

Introduction

Listeria monocytogenes (Lm) is a facultative anaerobic bacterium which can grow and reproduce inside the host's cells, making it one of the most virulent food-borne pathogens. It belongs to the genus *Listeria*. *Listeria* spp. is widely distributed in environment. The genus consists of six species i.e., *Listeria monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri* and *L. grayi,* of which only *L. monocytogenes* is the primary human pathogen although there have been rare reports of illnesses caused by *L. seeligeri* and *L. ivanovii* [1-3]. *Listeria monocytogenes*, commonly referred to as *Listeria*, is a pathogen that causes listeriosis, a severe human illness [4, 5]. It is unlike most other food-borne pathogens because it can grow and multiply at proper refrigeration temperatures [6].
In addition, *Listeria* is widely distributed in nature [7], and has been recovered from farm fields, vegetables, animals and other environments such as surfaces of food processing facilities, retail stores and home kitchens and ready-to-eat foods [8-10]. *Listeria monocytogenes* represents a constant challenge for the food industry, health regulatory officials and consumers [11] since it remains one of the most virulent foodborne pathogens for immunodeficient individuals, It has been extensively studied over the past few decades due to its high case/fatality rate (20-30%), its high burden of healthcare costs during chronic episodes of infection and its ability to survive for longer periods under adverse environmental conditions than many other non-spore-forming bacteria [12]. In man, outbreaks usually occur following consumption of unpasteurized milk, contaminated cheeses and other dairy products. Reports of outbreaks have also followed ingestion of undercooked meat, poultry [13] as well as coleslaw where it was first recognized as a food-borne zoonosis [14]. It is frequently present in the gut of cattle, poultry and pigs and can be transmitted to ready-to-eat (RTE) foods as well as raw meat products [7]. *Listeria* species are isolated from a diversity of environmental sources, including decaying vegetation, soil, water, effluents, a large variety of foods, and the faeces of humans and animals [15]. Most reported isolations of this species were from abortions, stillbirths, and neonatal septicemias in sheep and cattle [16, 17].
Listeria monocytogenes is a major contaminant of RTE food and food products. Packaged raw foods can represent a potential source of contamination when opened at home, and listeriosis is associated with the consumption

of such undercooked raw foods [5]. Human to human transmission is rare, except in cases of pregnancy where infected mothers transmit the infection via the placenta to the unborn child. This results in abortion, still birth or death of newly-born infant [18]. Transmission in domestic animals can occur by ingestion of contaminated feed and poor quality silage with pH greater than 5.5, hence the name "silage disease" [19]. Outbreaks usually occur as septicaemia, meningoencephalitis (circling disease), and abortion.

There has been a dearth of information on the epidemiology of listeriosis in most African countries, including Nigeria [20] with only few reports, when compared to Europe and USA [21]. This is because the organism seems not to have been given attention as required. While antibiotic resistance has been reported severally in literature with clinical isolates from human beings, recent evidences however, show that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them [22]. 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 [23]. 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 [23, 24]. Despite these and the increase in the consumption of poultry products coupled with enormous untrained hands in the poultry industry in Nigeria and the associated public health implications, there is paucity of information on the prevalence and antibiotic susceptibility profiles of *L. monocytogenes* among commercial chickens as well as raw processed chicken meat; hence, this study.

Methods

Study site, design, population and sampling

The study was carried out in 13 Local Government Areas (LGAs) known for the presence of high number poultry industries through a pilot survey across three Senatorial Districts of Oyo State, south-western Nigeria. The state was chosen as it possesses the majority of poultry industries in the region aside the backyard small scale poultry farming being practised by many. In addition, consumption of chicken and other poultry products is increasingly high in the state. This cross-sectional study involved a total of 71farms randomly selected from 100 available farms with different poultry types (layers, broilers), breeds, management (deep litter, battery cage) and biosecurity levels (high, average, low) located in the 13 LGAs of the state. The purpose of the study as well as the potential benefits was explained to the farm owners and they were told that participation was voluntary. It was also emphasized that declining participation did not have any attached penalty and that participation would not have any negative effects on their farms. The total number of poultry farms sampled was based on random selection of three of every four poultry farms through a transect walk guided by an initial pilot survey conducted. However, four of the selected farms declined participation. At each of the participating farms, cloacal swabs were collected using sterile swabs to scoop about one gram from each randomly selected chicken. 1ml of peptone water was then dispensed into each of the swab containers to moisten the samples in order to prevent the samples from drying up. Meat samples were also collected from points of retail into sterile sample bags. These were then placed in coolers containing ice packs for transportation to the Meat Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Nigeria for processing.

Microbiological analysis for *Listeria monocytogenes*

isolation was done using a slight modification of the methods described by Gibbons et al. [25] and Indrawattana et al. [26]. Peptone water was prepared by dissolving 15g of the powder in 1000mls distilled water and autoclaved at 121°C for 15min. Nutrient Agar was prepared by dissolving 28g of the powder in 1000mls of distilled water and autoclaved at 121°C for 15min. Listeria Selective Agar (LSA) (Brilliance™) was prepared by dissolving 33.6g of the powder base in 1000ml of distilled water, autoclaved for 15min at 121°C, cooled to 40°C and LSA antibiotics supplements was added. One gram of each sample was homogenized and transferred into a test tube containing sterile and freshly prepared peptone water. This was incubated at 37°C for 18 hours to 24 hours to revive viable but non-culturable cells. Thereafter, 100ul (0.1ml) each of the peptone water culture was transferred to a freshly prepared LSA and spread plated. Incubation was done at 37°C for 36-48 hours. Following incubation, discrete bacterial colonies were then counted from the incubated LSA for *Listeria monocytogenes* using the colony counter. Counts were transformed to colony forming unit (CFU) [27, 28]. *Listeria monocytogenes (Lm)* colonies appeared as green colonies with opaque white halos. Discrete *Lm* colonies from the LSA plates were then streaked onto freshly prepared LSA plates to obtain pure listeria isolates and the streaked plates were incubated at 37°C for 36-48 hours. Pure *Listeria monocytogenes* isolates were gram stained, then subjected to various morphological and biochemical tests which included catalase, oxidase and sugar fermentation using Glucose, Mannitol, Sucrose, Maltose, Fructose and Lactose. Phenolphtalein was used as indicator.

Assessment of the microbial load on samples screened

Serial dilution of each sample was also done up to the 6-fold dilutions, using freshly-prepared peptone water. 100ul (0.1ml) each of the 4th and 6th dilutions were then spread plated on nutrient agar plates and incubated at 37°C for 18-24 hours for counting. Following incubation, discrete bacterial colonies were then counted from the incubated nutrient agar plates using the colony coun-

ter. Counts were transformed to colony forming unit (CFU) and Log CFU.

Antibiotic susceptibility testing

This was performed using the Kirby-Bauer method (Disc diffusion Technique) [29]. The sensitivity discs were specifically designed and contained appropriate concentrations of different Gram positive antibiotics which include: ciprofoxacin (10µg/disc), norflaxacin (10µg/disc), gentamycin (10µg/disc), streptomycin (30µg/disc). Pure isolates were closely streaked onto the surface of Nutrient agar plates. The plates were then incubated at 37°C for 18-24 hours. Following incubation, they were observed for zones of inhibition surrounding each disc.

Data analysis

Data were analyzed using SPSS version 15. Chi-square test was used to test for association between the variables and prevalence of *Listeria monocytogenes*. Mean differences were analyzed using student's t-test (paired). Colonies counted were converted to colony forming units (CFU/ml). This was then transformed to base 10 Logarithms (CFU/ml). Mean standard deviation of CFU and Log10 CFU were calculated per sample type. Bacteria counts at the two different dilutions were compared among the sample types using paired t-test. The prevalence of *Listeria monocytogenes* contamination was calculated by dividing number of contaminated samples with the total number of samples collected. The epidemiological unit was the flock. A flock was considered contaminated by *Listeria monocytogenes* if at least one sample taken from the poultry house tested positive. The outcome variable "*Listeria monocytogenes* status" was dichotomous (contaminated (positive) versus non-contaminated (negative) flock). Prevalence was calculated based on the 100cfu/unit limit set by the European Commission Regulation (EC) No.2073/2005 on microbiological criteria for foodstuffs [30].

Results

Prevalence of *Listeria monocytogenes*

Of the 450 samples screened in this study, an overall prevalence of *Lm* contamination was found to be 91.8% comprising 95.8% (23/24) in meat and 91.5% (390/426) in cloacal swabs. All the flocks sampled had at least one positive sample yielding a flock prevalence of 100.0%. Cloacal samples from broilers had significantly higher prevalence (98.8%) than 89.8% from the layers (Tab. I). *Listeria monocytogenes* prevalence was highest among the Leghorn White (98.5%) and least among the Isa Brown breed (85.6%). Samples from poultry raised on deep litter (92.6%) and those from farms with low biosecurity level (93.2%) also recorded higher *Lm* prevalence. Overall, poultry type (X^2= 7.13; p =0.008); breed (X^2 = 15.25; p = 0.000), but not management (X^2 = 1.09; p = 0.297) as well as biosecurity level (X^2 = 0.173; p = 0.917) were significantly associated with the prevalence of *Lm* among the cloacal samples obtained (Tab. I).

Total bacteria count and antibiotic sensitivity test

Table II shows the comparison of bacteria counts (log CFU/ml) obtained at two different dilutions based on sample types. Mean bacteria counts obtained at 10^{-6} dilution were significantly higher (p = 0.0001) than those obtained at 10^{-4} dilution when compared across the sample type. The variations in mean logCFU/ml differences were significant across sample types (p = 0.0001).
A 100% resistance to both ampicillin-cloxacillin (30 ug) and cefuroxime (20 ug) antibiotics was demonstrated by the *Lm* isolates tested while the highest sensitivity

Tab. I. Occurrence of Listeria ***monocytogenes*** contamination based on poultry types, breed, management and biosecurity levels.

Variables	Category	Positive (%)	Negative (%)	Total	X^2; P value
Poultry type	Broilers	83 (98.8)	1 (1.2)	84	7.13; 0.008
	Layers	307 (89.8)	35 (10.2)	342	
Breed	Isa Brown	154 (85.6)	26 (14.4)	180	
	Nera Black	137 (95.1)	7 (4.9)	144	15.25; 0.000
	Leghorn White	65 (98.5)	1 (1.5)	66	
	Others*	34 (94.4)	2 (5.6)	36	
Management	Deep litter	261 (92.6)	21 (7.4)	282	1.09; 0.297
	Battery cage	129 (89.6)	15 (10.4)	144	
Biosecurity level	High	287 (91.4)	27 (8.6)	314	
	Average	62 (91.2)	6 (8.8)	68	0.173; 0.917
	Low	41 (93.2)	3 (6.8)	44	

*Harco Black, Anak White, Cobb USA

Tab. II. Total bacterial counts among the different samples taken (log CFU/ml).

Sample	1st Dilution(10^{-4})			2nd Dilution(10^{-6})			Paired t-test		
	Min	Max	Mean±SD	Min	Max	Mean ±SD	t	d_f	p-value
Cloaca	5.00	7.16	6.69± 0.25	7.00	9.00	8.43 ± 0.31	179.70	425	0.0001
Meat	6.41	7.15	6.71± 0.19	7.85	8.78	8.43 ± 0.25	44.67	23	0.0001

Tab. III. Antibiotic susceptibility of the *Listeria monocytogenes* isolates.

Antibiotics	Number of isolates tested	Amount sensitive	% sensitivity
Amocillin clavulanate(30ug)	72	62	86.1
Ciprofloxacin(10ug)	80	35	43.8
Cloxacillin(5ug)	72	26	36.1
Ceftriaxone(25ug)	80	26	32.5
Gentamicin sulphate(10ug)	72	20	27.8
Streptomycin sulphate (30ug)	80	20	25.0
Pefloxacin(10ug)	80	14	17.5
Erythromycin(5ug)	72	12	16.7
Co-trimoxazole(30ug)	88	11	12.5
Erythromycin(10ug)	72	9	12.5
Amoxacillin(30ug)	80	5	6.3
Ampicillin-cloxacillin(30ug)	80	0	0
Cefuroxime(20ug)	80	0	0

(86.1%) was obtained with amocillin clavulanate (30ug) (Tab. III).

Discussion

The overall high prevalence of 91.8% obtained in this study shows that *Listeria monocytogenes* is a common and constant contaminant of chicken flocks and chicken meat in the study area. This is similar to the findings of Gaffa & Ayo [31] and Chukwu et al. [32] in ready-to-eat (RTE) dairy products; and Nwachukwu et al., [33] in *Kunu*. Our findings further corroborate previous reports that *Listeria monocytogenes* is an important food-borne pathogen and is widely distributed in food, environmental and clinical samples [2, 34, 35]. As observed from our findings, the meat samples had higher incidence of *L. monocytogenes* (95.8%) when compared to cloacal samples (91.5%). These higher counts in meat could have resulted from the unhygienic handling practices of meat handlers and processors. As reported, contamination usually arises from unwholesome contacts of meat with excretions from skin, mouth and nose of the meat processors [36, 37]. It also suggests likely cross-contamination of raw processed chicken by improperly cleaned and disinfected processing environment and to a lesser degree from the live chicken. This finding concurs with similar findings by Cox et al. [38] and Kanarat et al. [39] which put processing as a major hazard of cross-contamination. The very high prevalence in raw processed chicken meat samples in this study is similar to the report by Gibbons et al. [25] which indicated 90.9% prevalence in raw meat. These findings coupled with poor food handling practices in the study area therefore portends serious health hazards to the public considering possible contamination with other raw food items during food preparation.

Comparatively, most Listeria cases are reported in high-income countries, while cases are much more likely to go unreported in developing countries. Most cases of listeriosis are sporadic and have been reported in high-income countries, where incidence is quite low but fatality rate is high [40]. Recently, Effimia [41] reported a 14.4% prevalence of *L. monocytogenes* in ready-to-eat food products in Greece while Wu et al. [42] observed a 20% prevalence in retail foods in China. Important outbreaks have also occurred-for example, an outbreak of listeriosis from cantaloupes in Colorado, USA, in 2011 resulted in infection of 147 people and 33 deaths, making it the deadliest recorded US foodborne outbreak since the US Centers for Disease Control and Prevention (CDC) began tracking outbreaks in the 1970s [43-44]. Listeriosis often results in admission to intensive-care units, which makes *L. monocytogenes* the third most costly foodborne pathogen in the USA per case in 2010, after *Clostridium botulinum* and *Vibrio vulnificus* [45]. Ivanek and colleagues [46] estimated that the annual cost of *L. monocytogenes* in the USA was US$2·3 billion to 22 billion, and the annual benefit of listeria food safety measures was $0·01 billion to 2·4 billion.

Our findings also observed a higher *Lm* prevalence among poultry flocks on deep litter than those in battery cage system. A previous report indicated that *Lm* can survive and multiply in wet litter [47] and thus serves as a source of contamination to the poultry flock. This may also explain the higher *Lm* prevalence recorded among broilers than layers in this study since broilers were in most cases raised on deep litter system. Litter should therefore be regularly changed and be protected from moisture. Also, it should always be stored in an enclosed location in order to protect it from pests such as wild bird so as to avoid contamination by wild life.

Similarly, the results of this study also suggest a significant association between the breeds of poultry flocks and *Lm* prevalence, with the Isa Brown breed showing the least prevalence. This could be as a result of possible varying resistance associated with different breed types. A further research into the genetic variations of breeds of poultry with reference to resistance/susceptibility to disease organism is required. On the other hand, while there was no statistically significant association between *Lm* prevalence and biosecurity levels of the different

farms, *Lm* prevalence was highest in farms with low biosecurity level. Previous studies have also showed that farms with low biosecurity level have increased risk of *Lm* contamination [47, 48].

In addition, most of the *Listeria monocytogenes* isolates obtained in this study showed profound resistance to the majority of the common antibiotics with 100% resistance to ampicillin cloxacillin and cefuroxime. This observation suggests a gross antibiotic abuse among poultry farmers in the study area. A similar report was previously made by Adetunji and Ishola [49] and Nwachukwu et al. [33] who revealed a profound resistance to Ampicillin , which is the drug of choice for treating listeriosis. It was, however in contrast to the report by David and Odeyemi [50] who found that broad-spectrum drugs like chloramphenicol and fluoroquinolones were significantly effective against this organism. Again, the susceptibility of most of the *Listeria monocytogenes* to gentamicin sulphate in this study is similar to previous reports [51, 52] which indicated susceptibility of all the *L. monocytogenes* obtained to this antimicrobial agent. The susceptibility of most of the *L. monocytogenes* in this study and previous studies to gentamicin sulphate plausibly suggests that this antimicrobial remains an alternative regimen against the organism. Given the multiple resistance shown by the *L. monocytogenes* to antimicrobial agents, the implication could be that the cost of treatment will be very high when humans are infected with these zoonotic pathogens; assertions which are in agreement with other reports [52, 53].

Similarly, the high incidence of *Listeria monocytogenes* in cloacal samples (64.8%) may be attributed to the constant ingestion of listeria-contaminated feed and water. This is similar to findings by Schlech et al. [14] and Gravani [34] which stated that listeria are mainly found in soil, silage and water. Though, the gut of birds is a usual habitat for *Listeria monocytogenes* [54]; Skovgaard [55] and Larpent [56] reported a common occurrence of *Liseria monocytogenes* in animal feaces.

Despite the high prevalence of *Listeria monocytogenes* in this study, most of the chickens showed no sign of infection. This further reiterates the claim by Cox et al. [38], that chickens are faecal carriers of the organism and may contaminate the litter and environment of the poultry house. Also, there seemed to be no significant increase in *Lm* counts with total microbial load, as samples with highest *Lm* counts did not necessarily have the highest microbial load, and vice-versa. This could be explained by the fact that *Lm* is very hardy and persists in the environment, resisting most cleaning and disinfectant techniques, unlike many other bacteria which are eliminated by cleaning and disinfecting [49, 57].

Conclusions

This study showed a high overall incidence (91.8%) of *Listeria monocytogenes* in poultry flocks and poultry meat in Oyo state, Nigeria. The higher incidence in meat suggests post-slaughter contamination and portends health hazards to the public through contact between these raw meat and other processed foods. It also shows that poultry flock types and breeds were significant factors associated with *Lm* contamination. In addition, the resistance of *Listeria monocytogenes* isolates to most of the antibiotics in this study is a matter of concern both to the future management of poultry diseases as well as public health. We therefore recommend that farm –to-fork principles of hygiene should be stepped up particularly among poultry and other food handlers in order to limit contamination with food pathogens. Standard Operating Procedures (SOP) and Hazard analysis and critical control points (HACCP) should be developed and implemented by poultry regulatory agencies such as Poultry Association of Nigeria (PAN) and Poultry Farmers of Nigeria (PFN). Government should enforce prompt registration and periodic monitoring of all poultry farms and abattoirs in order to institute measures to check the sanitary levels of farms and abattoirs and enforce strict adherence to hygiene standards on a continual basis. Farmers should be enlightened on appropriate antibiotic usage and withdrawal period.

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Authors' contributions

OOI developed the concept of the study and wrote the manuscript; JIM did the sample collection and analysis and was involved in the writing of the manuscript; HKA did the statistical analysis of the data and was involved in the writing of the manuscript.

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