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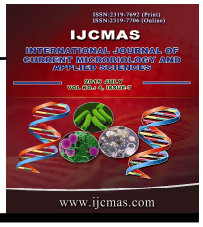

Original Research Article

Contamination and Antibiotic Susceptibility Profile of *Listeria* Species in Frozen and Fresh Chicken Sold in Makurdi, Nigeria

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K e y w o r d s

Listeria species, Contamination, Antibiotic susceptibility, Frozen chicken, Fresh chicken, Makurdi

A B S T R A C T

The main route of *Listeria* infection is by ingestion of food contaminated with the organism particularly those stored at refrigeration temperatures. The aim of this study was to determine the rate of contamination and antibiotic susceptibility profile of *Listeria* species isolated from frozen and fresh chicken sold in major markets within Makurdi metropolis. A total of 120 chicken samples comprising frozen (n = 60) and fresh (n = 60) chickens were purchased from three main markets in Makurdi and bacteriologically examined for the presence of *Listeria* species. Both pathogenic and nonpathogenic *Listeria* species were isolated,and the overall contamination rate was 14.17% (17/120). *Listeria* species identified were *L. grayi* (58.82%), *L. innocua* (17.65%), *L. ivannovii* (11.76%), and *L. welshimeri* (11.76%). Contamination of frozen chicken was significantly associated with selling sites (P< 0.05) with High-Level market having the highest contamination rate (50.0%). For fresh chicken, however, point of sales was not significantly associated with rate of listerial contamination (P>0.05). Antibiotic sensitivity profile showed that the contaminants were resistant to gentamycin 82.35% (14/17), amoxicillin 70.59% (12/17), and erythromycin 58.82% (10/17), but highly susceptible to streptomycin (100%), recophin (94.12%), ciproflaxacin (88.24%), zinacef (88.24%) and septrin (72.2%). The rate at which commercially frozen and fresh chicken sold in Makurdi main markets were contaminated with *Listeria* species is an indication that these poultry products could serve as a source of listerial infections for humans.

Introduction

The obviously increased bacterial contamination in the food industry bacteria has raised great concerns by consumers. Many of these pathogenic bacteria which are contaminants of meat (fresh or frozen) may get in during production and processing through sources such as the air, contaminated water, soil, processing surfaces, processing equipment, and during distribution (Ikeh *et al.*, 2010). Following slaughter and dressing, the carcasses of animals and birds contain different types of

microorganisms predominantly bacteria which originate from the skin, hair, feathers, gastrointestinal tract, the environment of the feedlot and pasture including the slaughtering facilities and the environment (Ray, 2004).

The contamination of meat in the open market is due mainly to undue exposure which makes it amenable to spoilage (Ray, 2004; Okonkwo *et al.,* 2014). Exceptional attention should be paid to poultry meat production because live animals are hosts to a large number of different microorganisms residing on their skin, feathers or in their alimentary tract.

Contamination of meat and meat products has resulted in an increased number of meat borne diseases such as Salmonellosis, Campylobacteriosis, Listeriosis, *E. coli* enteritis and food poisoning by *Clostridium* and *Staphylococcus* spp.

Listeria monocytogenes the causative agent of human and veterinary listeriosis has gradually become an important food-associated pathogen with high fatality rate. The pathogen is primarily transmitted through foods like: milk, milk products, meat and meat products, fish, eggs and egg products, fruits and vegetables (Gordana *et al.,* 2010). However, sporadic listeriosis remains the most frequent manifestation of the illness (Gilot *et al.,* 1996) although few epidemic outbreaks have been recorded in United States and other parts of the European Union (Churchill *et al.,* 2006).

In Nigeria, few sporadic cases of listeriosis have been reported (Chukwu *et al.,* 2006) but today there is little or no information there is no data on outbreak of human listeriosis. Furthermore, the sources of contamination are most times unknown. In Nigeria various surveys on food have reported occurrence of *L. monocytogenes* in different food products like raw milk, smoked fish (Salihu *et al.,* 2008), beef, pork, goat meat, poultry, fish and vegetable products (Ikeh *et al.,* 2010), fresh soft cheese 'wara' (Adetunji and Adegoke, 2008) and ready-to-eat foods such as 'Deli' salad, 'Kilishi' and 'Suya' meat (Hemen *et al.,* 2013). In spite of all these studies, there is no data on prevalence or contamination rate of *Listeria* species in poultry products sold in Makurdi. Hence the need to investigate the contamination rate of *Listeria* species in poultry products sold in Makurdi metropolis.

Materials and Methods

A total of 120 chicken samples were collected from three markets in Makurdi namely: High-Level, Railway, and Wurukum markets respectively between August and December, 2014.

The chicken samples consisted of frozen chicken meat (n = 60) and fresh chicken meat (n = 60). Frozen and fresh chicken meat samples were collected aseptically in clean sterile polyethylene bags, labeled, and transported in insulated coolers containing ice packs to the Veterinary Pathology and Microbiology Laboratory, University of Agriculture Makurdi for bacteriological examination.

Cultural techniques

The cultural techniques for pre-enrichment, enrichment and plating described by OIE Terrestrial Manual (2014) and Ohue *et al.* (2014) were used with slight modifications. Twenty-five grams (25 g) of chicken meat was pounded in a sterile plastic mortar and homogenized in 225 ml of primary enrichment culture of Listeria enrichment broth without supplement (Oxoid) in sterile Erlenmeyer's flasks and incubated at 30°C for 24 hours. One milliliter (1 ml) of the primary enrichment culture was then added

to 9 ml of enrichment broth with supplement and incubated at 37°C for 24 hours. A loop full of the enrichment broth was streaked onto Listeria selective agar (Oxoid) and the culture plates were incubated at 37°C for 24 to 48 hours.

Identification of isolates

The 48 hour culture plates were examined for black colonies with black to brown background zone produced by aesculin hydrolysis, typical of *Listeria* species. Presumptive colonies from the culture medium were further identified using Gram staining and catalase test. The isolates were further tested for haemolysis on blood agar and carbohydrate fermentation using mannitol, rhamnose and xylose as described by Hitchins and Jinnemans (2011).

Antimicrobial susceptibility test

Antibiotic susceptibility of isolates was performed using the disk diffusion method as described by Ohue *et al.* (2014) using Mueller-Hinton agar. The diameter of zones of inhibition were measured using a measuring rule and interpreted as susceptible (S), resistant (R) and intermediate (I) according to Clinical Laboratory Standards Institute (CLSI, 2006) guidelines. Inoculum load was prepared using 0.5 MacFarlands turbidity standard (approximately 10^8 cfu/ml).

Media preparation

All media were prepared according to manufacturers' instructions. The suspensions were autoclaved at121°C for 15 minutes, cooled to 45°C and aseptically poured into Petri dishes and allowed to gel. Plates were dried in an oven (Hospibrand USA GZX-Cf.400) before inoculation.

Results and Discussion

Poultry products comprising of fresh and frozen chicken were screened for presence of *Listeria* species. Results showed that frozen chicken had the highest contamination rate of 21.67% (n=13) while fresh chicken was least contaminated with 6.67% (n=4). The different species of *Listeria* identified were *L. grayi* (58.3%); *L. innocua* (22.2%); *L. ivannovi* (11.1%); and *L. welshimeri* (5.6%) (Table 1 & 2).

Contamination rates of poultry products purchased from three main markets in Makurdi were statistically compared. Contamination of frozen chicken meat by listerial species was highest in High-Level market (50.00%) and was least in Wurukum market (1.0%). Contamination rate among markets was statistically significant ($P < 0.05$) (Table 3). Contamination rate of fresh chicken meat in the three markets studied did not differ significantly from each other ($P > 0.05$) (Table 4).

Results from the listerial susceptibility to antibiotics showed that *L. grayi*; *L. innocua*; and *L. ivannovi* isolates were resistance to amoxicillin (75.0%), erythromycin (11.1%), and gentamycin (11.1%). The *L. grayi*; *L. innocua*; and *L. ivannovi* isolates were however susceptible to streptomycin (100%), ciprofloxacin (88%), pefloxacin (75.0%), and ampiclox (75.0%) (Fig. 1). In this study,it has been shown that fresh and frozen chicken products were contaminated with *L. innocua, L. ivannovi, L. welshimeri,* and *L. grayi* were isolated which could be an indication of poor hygienic handling by the butchers. There is also a possibility of cross contamination during processing. These findings agree with those of Katarzyna *et al.* (2006) that *L. innocua,L. welshimeri, L. grayi,* and *L. seeligeri* were isolated fromraw chicken parts.

Table.1 Contamination rate of chicken sold in Makurdi Markets

Poultry product	No. of Samples Examined (%)	No. contaminated by *Listeria* (%)
Frozen Chicken	60 (100.0)	13 (21.67)
Fresh Chicken	60 (100.0)	4 (6.67)
TOTAL	**120 (100.0)**	**17 (14.17)**

Table.2 Distribution of *Listeria* spp. in chicken sold in Makurdi Markets

Poultry Product	Distribution of *Listeria* species (%)			
	L. grayi	*L. innocua*	*L. ivannovii*	*L. welshimeri*
Frozen Chicken	8 (61.54)	2 (15.38)	1 (7.69)	2 (15.38)
Fresh Chicken	2 (50.0)	1 (25.00)	1 (25.00)	0 (0.00)
TOTAL	**10 (58.82)**	**3 (17.65)**	**2 (11.76)**	**2 (11.76)**

Table.3 Listerial Contamination rates of frozen chicken sold in selected Makurdi Markets

Sample Source	No. of Samples Examined (%)	No. Contaminated (%)
High-Level Market	20 (100.00)	10 (50.00)
Railway Market	20 (100.00)	2 (10.00)
Wurukum Market	20 (100.00)	1 (5.00)
TOTAL	**60 (100.00)**	**13 (21.70)**

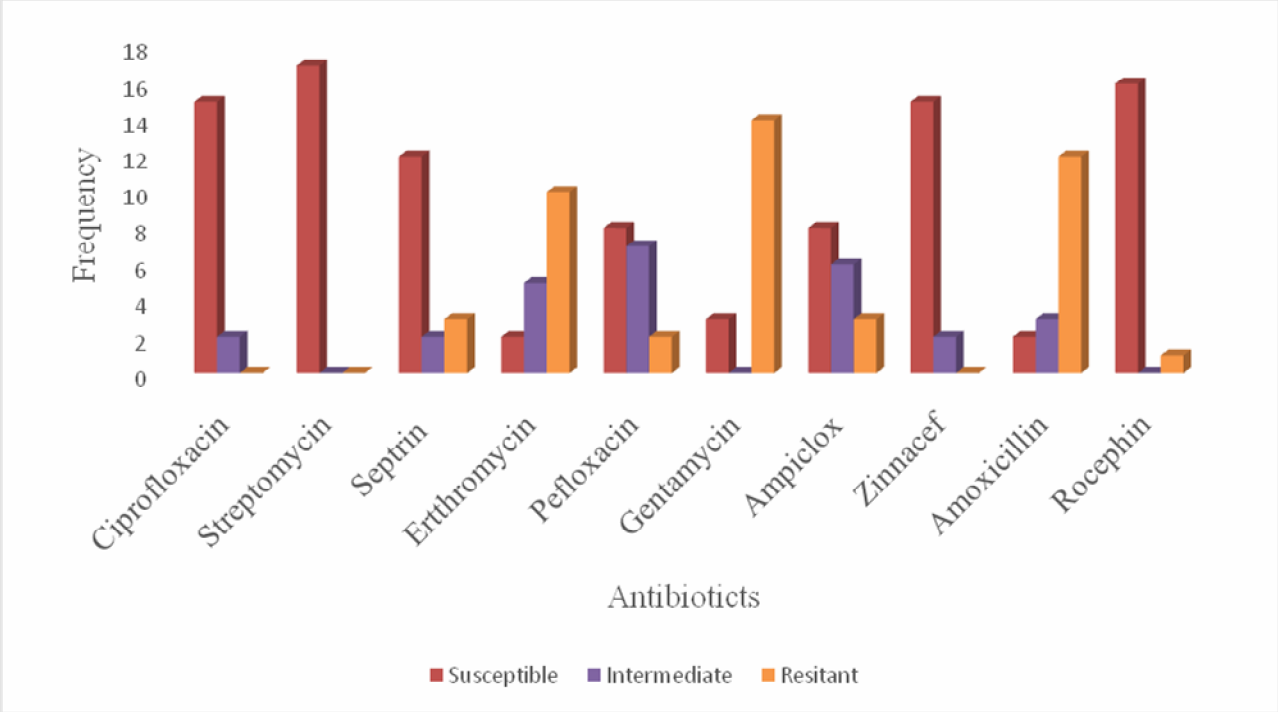
χ^2 =14.34, df = 2, P = 0.0008. (P □ 0.05)

Table.4 Listerial contamination rates of fresh chicken sold in selected Makurdi Markets

Sample Source	No. of Samples Examined (%)	No. contaminated (%)
High-Level Market	20 (100.00)	1 (5.00)
Railway Market	20 (100.00)	1 (5.00)
Wurukum Market	20 (100.00)	2 (10.00)
TOTAL	**60 (100)**	**4 (6.67)**

χ^2 =0.5357, df = 2, P = 0.765 (P > 0.05)

Fig.1 Antimicrobial profile of *Listeria* spp. from frozen and fresh chicken



Similarly, Ashraf *et al.* (2010) recovered *Listeria* spp. from chicken meat samples in Egypt and Ikeh *et al.* (2010) isolated *L. monocytogenes, L. murrayi grayi* and *L. seeligeri, L. ivanovii, L. innocula, and L. welshimeri.*

Ghasemian-Safaei *et al.* (2011) had reported the presence of bacterial contamination in poultry product and Kozacinski *et al.* (2006) presence of *Enterobacteria* in chicken breasts samples.

This finding also agrees with those of Kozacinski *et al.* (2006) and Bhandari *et al.* (2013) who reported contamination at any stage of the production process in meat and meat products.

The results of this study suggest that the *Listeria* spp. were highly resistant to amoxicilin. Resistance to gentamycin and erythromycin was less. The *L. grayi, L. innocua,* and *L. ivannovi*isolates were however highly susceptible to ciprofloxacin, streptomycin, zinnacef. This finding collaborates the reports of Ennaji *et al.* (2008) who observed high susceptibility of listerial isolates to antibiotics. Ennaji *et al.* (2008) had observed that listerial isolates were resistant to septrin (sulphamethaxozole), gentamycin and erythromycin in contrast to the findings of this study. The discrepancy in these findings may be attributed to excess concentration of the antibiotics in the commercially prepared antibiotic discs which did not use the minimum standard concentrations for the antibiotics as documented in the CLSI standard (2006). The CLSI standard (2006) is not always adhered to according to Schroeder *et al.* (2002). Indiscriminate use of antibiotics in animals may be another contributing factor. Safdara and Armstrong (2003) and Chukwu *et al.* (2006) had observed a continuing pattern of emergence of strains of *Listeria* spp. isolated from food and clinical cases of listeriosis which are

resistant to one or more antibiotics. Although the incidence of antibiotic resistance is currently low, the range of antibiotics to which resistance has been acquired is wide. It is of concern that this expanding range now includes a number of first line antibiotics such as penicillin, ampicillin, tetracycline and gentamycin used in the treatment of listeriosis.

This study has confirmed a high prevalence of *Listeria* species in frozen and fresh chicken sold in Makurdi, Nigeria. This study has also demonstrated high antibiotic susceptibility of test organisms to ciprofloxacin, streptomycin, zinnacef and resistance to amoxicilin, septrin, gentamycin and erythromycin. This study thus affirms that the isolation of *Listeria* species in poultry products could serve as a source of contamination of chicken meat meant for human consumption and thus pose a health risk for high-risk individuals. Consequently consumers of chicken should ensure that meat and other poultry products are boiled properly before consumption. In addition market administrators and health inspectors should advocate improvement in hygienic conditions of slaughter houses and other meat processing sites by meat sellers to avoid cross contamination which is a major factor in the transmission of *Listeria*.

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