



# PREVALENCE AND ANTIBIOTICS SUSCEPTIBILITY PROFILE OF *Listeria monocytogenes* ISOLATED FROM PROCESSED AND UNPROCESSED MEAT PRODUCTS

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## ABSTRACT

The antibiotics susceptibility pattern of *Listeria monocytogenes* isolated from processed and unprocessed meats were investigated. Isolation of *Listeria monocytogenes* was done using the FDA BAM method. A total of fifty (50) retail meat samples consisting of twenty (20) raw meats, 15 fresh processed meat samples and 15 processed ready-to-eat samples were analyzed on *Listeria Selective Agar* and *Listeria Chromogenic Agar* following pre-enrichment. Isolates were screened for their susceptibility to ten selected antibiotics by the standard disk diffusion method. *Listeria* spp. were isolated from 29 (58%) of the samples, out of which 14 (28%) were *Listeria monocytogenes*. *Listeria monocytogenes* was significantly higher in unprocessed meat 9 (45%) than in processed ready-to-eat meat products which were 5 (33%). No *Listeria* isolates were found in fresh processed meat products comprising of smoked bacon, sausages, beef mortadella, minced meat and beef salami. High susceptibility was displayed to ciprofloxacin (100%), penicillin (100%), gentamicin (100%), streptomycin (92.9%), erythromycin (92.9%), sulphamethoxazole – trimethoprim (71%) and amoxicillin (64.9%). Highest resistance was displayed to cloxacillin (93%) and ceftriaxone (78%). Results in this study showed *Listeria monocytogenes* from the analyzed meat samples to be highly susceptible to the antibiotics commonly used in the treatment of human and veterinary listeriosis.

**Keywords:** *Antibiotics Resistance, Listeria monocytogenes, Listeriosis, Processed meats.*

## INTRODUCTION

*Listeria monocytogenes* is a foodborne pathogen that is widely distributed in the environment and can also be found in the gastrointestinal tract of individuals who remain as asymptomatic carriers (Mead *et al.*, 1999). It can cause sporadic and epidemic outbreaks of listeriosis worldwide as a result of consumption of contaminated foods (Bula *et al.*, 1995). Among foods that are easily contaminated with *Listeria monocytogenes* are ready-to-eat (RTE) food such as sausage, burger; unpasteurized dairy foods (cheese and milk); cured and raw meats (Schlech and Acheson, 2000). *Listeria monocytogenes* is of particular concern in raw, undercooked or ready-to-eat foodstuffs because the organism is ubiquitous, and processed foods are easily contaminated with raw foods in food-processing industries and at homes. In the United States, it is estimated that there are 76 million cases of foodborne illness each year. According to Mead *et al.*, (1999), the incidence of listeriosis is considered low with an average of 2500 infections yearly. However, a mortality rate is considerably higher than the common infections from other foodborne pathogens such as *Escherichia coli* 0157: H7, *Campylobacter* spp. and *Salmonella* spp. where it can be as high as 20-30% regardless of antimicrobial treatment. Thus, it indicates that the prevalence of *Listeria monocytogenes* in foods poses a significant danger.

The detection of *Listeria monocytogenes* in meat of particular concern in terms of consumer safety, as this organism is capable of growing on both raw and cooked meat at refrigeration temperatures (Walker, 2000). On the other hand, during further processing of raw meat into meat products *Listeria monocytogenes* can be introduced, where the amount depends on the extent of cross contamination, personal and general hygienic measures and the process parameters (Glass and Doyle, 1999). In addition, assessing the prevalence of *Listeria monocytogenes* in processed meats is particularly important, since these products are often consumed after a brief heat treatment, this may not be sufficient to kill viable cells.

*Listeria monocytogenes* has fair stability over antibiotic susceptibility, but in relatively recent time, reports of emergence of antibiotic resistant *Listeria monocytogenes* recovered from food, environment and from sporadic cases of human listeriosis have remained of significant public health concern. Currently, the treatment of choice for listeriosis is a  $\beta$ -lactam antibiotic (e.g. penicillin or ampicillin), alone or in combination with an aminoglycoside (e.g. gentamicin) in case of immune-compromised

patients (Hof, 2003). The second choice is the combination of trimethoprim and a sulfonamide (e.g. sulphamethoxazole), especially for patients allergic to  $\beta$ -lactams. But multidrug resistance to erythromycin, tetracycline, dicloxacillin, and trimethoprim-sulfamethoxazole has been reported (Rodax 2004; Brooks *et al.*, 2004).

In Northern Nigeria and North Africa, it was reported that most strains of *Listeria monocytogenes* were sensitive/susceptible to ampicillin, erythromycin and other common antibiotics. Surprisingly, the same research reported that the organism was found resistant to cephalosporin, nitrofurantoin, tetracycline, and chloramphenicol at in vitro levels (Onyemelukwe *et al.*, 1983, Cherubin *et al.*, 1991, Adetunji and Adegoke, 2008). In Western Nigeria, a multi-antibiotic resistance of *Listeria monocytogenes* has been reported (David and Odeyemi, 2007).

*Listeria monocytogenes* is a significant problem for the food industries due to its ability to survive and grow under adverse conditions (e.g. low temperature, pH, water activity, e.t.c.) that are not tolerated by other non-spore forming foodborne pathogens (Montville and Mathews, 2005).

Due to high risk of listeriosis and low infective dose, there is "Zero tolerance" policy for *Listeria monocytogenes* in ready-to-eat (RTE) foods in the U.S. Since the organism can grow at temperatures as low as 1°C, it poses a serious food safety hazard in refrigerated RTE products. Among RTE foods, meat and poultry products are the leading vehicles of listeriosis (Jay *et al.*, 2005). RTE meat products, such as frankfurter that have received heat treatments followed by cooling in brine before packaging, may provide a more favourable environment for growth of *Listeria monocytogenes* because of the decreased competitive microflora and high salt tolerance of his organism (Doyle *et al.*, 2001).

Despite efficient antibiotic therapy, listeriosis represents a public health problem since it is fatal in up to 30% of the cases. This threatening nature of listeriosis also prompted the World Health Organization (WHO) to suggest that various food products must be frequently investigated for the presence of *Listeria monocytogenes* on a worldwide basis (WHO, 2000). This study investigated the prevalence and the antibiotic susceptibility of *Listeria monocytogenes* in processed and unprocessed meat products.

## MATERIALS AND METHODS

A total of fifty (50) retail meat samples consisting of (20) raw meat samples were collected from five open-

air markets in Lagos metropolis; (15) fresh processed meat samples which included (beef salami (3), sausage (3), smoked flavored bacon (3), minced meat (3) and beef mortadella (3), were collected from retail stores and supermarkets in Lagos; and (15) processed ready-to-eat meat samples consisting of packaged Kilishi (4), unpackaged Kilishi (4), suya (4) and stick-peppered suya (3) were collected from open markets in Ketu; Agege and Akoka area of Lagos metropolis and also from supermarket.

Samples were collected and transported to the laboratory in sterile polyethylene bags, packed in ice pack containers. Samples were stored in 4°C refrigerator and were analyzed immediately.

Isolation of *Listeria monocytogenes* from processed and unprocessed meat products was done using the FDA bacteriological and analytical method (BAM) (FDA BAM method) procedure. One ml of the meat filtrate of all the 50 samples were added to 9 ml of Buffered *Listeria* enrichment both (Oxoid, UK) supplemented with *Listeria* selective enrichment supplement (Oxoid, UK), and incubated for 24 hours at 30°C. This was done to resuscitate stressed cells. *Listeria* Selective agar (Oxford formulation) (Oxoid, UK) supplemented with *Listeria* Selective supplement (Oxford formulation) (Oxoid, UK) agar plates were inoculated by spreading a loopful taken from the enrichment broth. These selective agar plates were incubated for 24 hours at 37°C. Suspected colonies appeared grayish colonies surrounded by black halos and sunken centers with possible green sheen. The resulting colonies were further streaked on Chromogenic *Listeria* Agar plates supplemented with Brilliance *Listeria* differential supplement. Incubation was done aerobically at 35-37°C for 18-24 hrs.

Colonies that conform to the morphology of *Listeria monocytogenes* were purified and kept for identification tests. They were subjected to recommended biochemical tests. The biochemical tests included Christie, Atkins, and Munch-Peterson (CAMP) test, xylose sugar fermentation, catalase, oxidase, and haemolysis tests. The commercially available Oxoid biochemical identification system (O.B.I.S) mono kit was further used.

The antibiotic susceptibility of the isolated *Listeria monocytogenes* strains was determined by the disk diffusion method as described in Madigan *et al.*,

(2006) on Mueller Hinton agar (Lab M, LAB039). The Gram positive antibiotic disc used included ceftriaxone (25µg), ciprofloxacin (10 µg), streptomycin (30 µg), sulphamethoxazole-trimethoprim (30 µg), erythromycin (10 µg), penicillin (10µg), gentamicin (10µg), cloxacillin (30 µg), cefuroxime (20µg), and amoxicillin (30 µg).

Four colonies each of all identified *Listeria monocytogenes* were transferred into 10mls of Nutrient Broth in test tubes. The test tubes were incubated at 37 °C for 24 hours till very turbid. This was then adjusted to 0.5 McFarland standard. Sterile cotton swab was dipped into the suspension and pressed firmly on the surface of the Mueller Hinton Agar plate was inoculated by streaking the entire surface. The seeded plates were then allowed to dry for no more than 15min. the antibiotic discs were placed on the agar using sterile force making sure it made an immediate and complete contact with the agar surface. The plates were incubated at 37°C for 24 hours.

The diameter of the zone of clearance (including the diameter of the disk) was observed and measured with a ruler to the nearest whole millimeter. Zones of inhibition > 17mm were recorded as susceptible, intermediate and resistant based on the interpretive guidelines of Clinical Laboratory Standard Institute (CLSI) (2000). The results were analysed statistically using the statistical software SPSS version 12.0 to determine the significance of the results.

## RESULTS AND DISCUSSION

A total of fifty (50) samples of meat and meat products were examined, 29 (58%) were positive for *Listeria* spp. out of which 14 (28%) were positive for *Listeria monocytogenes*. Prevalence of *Listeria monocytogenes* was higher in unprocessed meat 9 (45%) than in processed ready-to-eat meat products which were 5 (33%). No *Listeria monocytogenes* were found in fresh processed product. Out of the 14 isolated *Listeria monocytogenes*, 9 (64.3%) were isolated from the unprocessed meat while 5 (35.7%) were isolated from the processed meat. The distribution of the isolates into respective product type is given in Table 1.

Table 1: Prevalence of *Listeria monocytogenes* Isolated from Processed and Unprocessed Meat Products

No of positive Sample (%)				
Product Category	Sample Code	Product type	Listeria spp.	L. monocytogenes
Unprocessed	A, B, C, D, E,	Raw meat	16(80)	9(45)
	F, G, H, I, J, K,			
	O, P, L, M, N,			
	Q, R, S and T			
Processed	1, 2, 3, 4, 5, 6,	Fresh Processed meat	0	0
	7, 8, 9, 10, 11,			
	12, 13, 14 and 15			
	PR1, PR2, PR3	Processed Ready-to-eat meat products	13 (86,7)	5 (33.3)
	PR4, PR5, PR6			
	PR7, PR8, PR9			
	PR10, PR11,			
	PR12, PR13,			
	PR14 and PR15			
<b>Total</b>			<b>29 (58)</b>	<b>14 (28)</b>

The high occurrence of *Listeria monocytogenes* in raw meat is expected, because *Listeria monocytogenes* is ubiquitous in the environment (Vitals *et al.*, 2004). Furthermore, the method of slaughter and evisceration allows ample opportunity for contamination to occur. People handling meat at different levels can also be sources of contamination. This finding similar to the reports of other studies which reported a 30 to 70% prevalence of *Listeria monocytogenes* in raw meat (Dhanashee *et al.*, 2003; Vitals *et al.*, 2004). It is also in accordance with the incidence of 38-50% reported by McGowan *et al.*, 2004. However, other studies have shown a lower incidence of the pathogen in raw meats (5% and 17%) (Rorvik *et al.*, 2001; De Simon *et al.*, 2002). Raw meats are cooked before consumption and, although *Listeria monocytogenes* is relatively heat resistant, if the cooking of raw meats is up to a temperature of 70°C for 2 minutes; one can assume that this will be sufficient to kill *Listeria monocytogenes* (Boyle *et al.*, 1999). It is necessary to improve hygiene and provide adequate storage conditions from slaughter houses through meat sellers to avoid growth of the pathogen to high levels, because across contamination represents the major factor in the introduction of *Listeria monocytogenes* to meats (Tompkin *et al.*, 1992).

An analysis of the fresh processed meat samples such as sausages, beef mortadella, beef salami minced meat and smoked bacon showed no contamination with *Listeria* spp. and *Listeria monocytogenes*. The absence of *Listeria monocytogenes* in these products might be attributed to factors such as high salt concentration and low water activity making them unsuitable for the growth of the pathogen. The drying

stage of cured raw sausage causes decrease in the water holding capacity to less than 90 aw and also does the pH, which approaches the isoelectric point (Tyopponen, *et al.*, 2003). Also, the addition of salt to the sausage mix limits water activity, thereby inhibiting the growth of many spoilage and pathogenic bacteria including *Listeria monocytogenes*.

The processed ready-to-eat meat product (suya and Kilishi), 33% were positive for *Listeria monocytogenes*. The products underwent procedures of processing like smoking and drying that should make them safe for direct consumption. However, because of the substrate properties and storage conditions, these products are adequate for the development of the pathogen. Slicing of cooked meat products in the case of suya and Kilishi has been shown to be a critical point in contamination and transfer of *Listeria monocytogenes* from the hands to the meat products (Tompkin, 2002). Jones *et al.* 2001 reported the water activity of Kilishi to be 0.59 aw indicating it to be a very dry meat product. The presence of the pathogen in Kilishi is somewhat surprising, because low water activity has been shown to profoundly limit the growth and multiplication of the pathogen in meats (Vermont *et al.*, 2007). In the present study, 33% of RTE meat samples analyzed contained *Listeria monocytogenes*, which reflected the need for better control of the post-processing environment as well as the storage conditions. The occurrence of *Listeria monocytogenes* in suya and Kilishi is of public health significance because these products are eaten without further processing.

High susceptibility was displayed to ciprofloxacin (100%), penicillin (100%), gentamicin

(100%), streptomycin (92.9%), erythromycin (92.9%), sulphamethoxazole – trimethoprim (71%) and amoxicillin (64.9%). All *Listeria monocytogenes* strains except one were found to be resistant to one or more of the ten antibiotics used. The isolates showed varied degree of resistance to antibiotics. Generally, the resistance of *Listeria monocytogenes* in this study was displayed to five out of the ten antibiotics (ceftriaxone, ampicillin/cloxacillin, sulphamethoxazole-trimethoprim, streptomycin, and erythromycin). Highest resistance was displayed to cloxacillin (93%) and ceftriaxone (78%) (Figure 1).

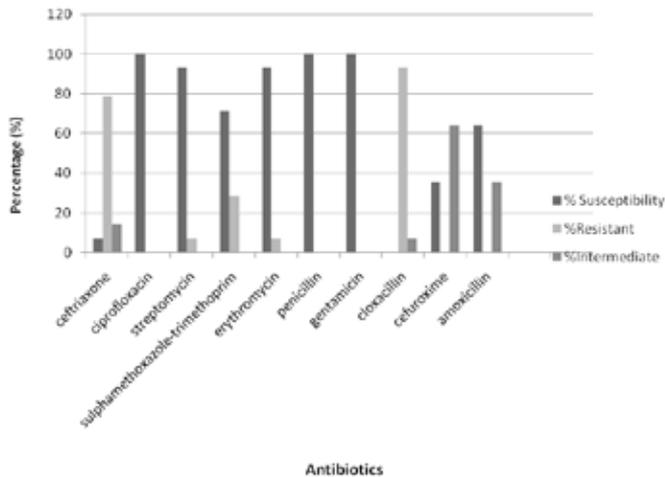


Figure 1: Antibiotics susceptibility pattern of *Listeria monocytogenes* isolate from processed and unprocessed meats products.

The result of this study suggested that the overall incidence of antibiotic resistance in *Listeria monocytogenes* is still relatively low. There has been a continuing pattern of the emergence of strains of *Listeria* spp. isolated from food and clinical cases of listeriosis which are resistant to one or more antibiotics (Chukwu *et al.*, 2006; Safdara and Armstrong, 2003). 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 antibiotics used to treat listeriosis, e.g. penicillin, ampicillin, tetracycline and gentamicin. Moreover, although the isolation of resistant strains from various sources has been reported (Rodax, 2004).

Ampicillin or penicillin plus gentamicin remain the treatment of choice for most manifestations of listeriosis. Sulphamethoxazole-trimethoprim is considered to be a second choice therapy; vancocycin and erythromycin are also used respectively to treat bacteremia and pregnant women diagnosed with listeriosis (Charpentier and Courvalin, 1999). The results from this study showed that *Listeria monocytogenes* stains are susceptible to the first choice antibiotics used in treatment of listeriosis which are ampicillin (65%) and gentamicin (100%) and also

susceptible to sulphamethoxazole – trimethoprim (71%) used as second choice antibiotics in treatment of listeriosis especially in patients allergic to penicillin. The major of the strains isolated in this study are susceptible to the antibiotics commonly used in veterinary and human listeriosis, even if more than one strain was resistant to amoxicillin, erythromycin, and sulphamethoxazole-trimethoprim.

A similar pattern of resistance has also been found by other authors (Aurelli *et al.*, 2003) suggesting the worldwide increase in antibiotic resistance.

*Listeria monocytogenes* strains isolated in this study were highly resistant to ampicillin/cloxacillin (93%). Its widespread use under the brand name ampiclox in human and veterinary therapy, alongside the length of time over which it has been available in Nigeria and other countries of the world could account for this trend. It is known that *L. monocytogenes* can either acquire, or transfer antibiotic resistances' genes from plasmid and transposons of other bacterial species (including *Enterococcus* spp. either in vivo or in vitro in the intestinal tract (Pourshaban *et al.*, 2002). This is very important because bacteria that acquire new resistance are not disrupted by antibiotics during a therapy.

## CONCLUSION

The presence of *Listeria monocytogenes* in a variety of raw and processed ready-to-eat meat products and absence in fresh processed meat products has been demonstrated in this study. Overall, the majority of the strains isolated in this study are susceptible to the antibiotics commonly used in veterinary and listeriosis treatment. The study also suggested the need for improved food safety through the implementation of hygienic measures at all levels from product to consumption with particular emphasis on ready-to-eat food items which require no further heat treatment. A continued surveillance on *Listeria monocytogenes* prevalence and on emerging antibiotics resistance is important. This will identify foods that can represent a risk for the population and ensure effective treatment of listeriosis. The results obtained from this study provide an important baseline for the contamination status of meat products with *L. monocytogenes* and preliminary pattern of its susceptibility to commonly used antibiotics. The data will be useful for food producers and for epidemiological and public health studies concerning the antibiotic susceptibility of *L. monocytogenes*.

Although exposure to *Listeria monocytogenes* cannot be avoided completely, proper food preparation and storage decrease the risk. Pregnant women and immune-compromised individuals should be advised to avoid consumption of ready-to-eat meat products and other farm products that do not requires cooking before consumption because they can be contaminated

at a high level. Avoiding cross-contamination is also an important protective strategy; all utensils and surfaces should be washed well after preparation of meat or cutting of prepared foods. And finally, good sanitary measures among individuals, home, food industries and food vendors will enhance in reducing the risk of listeriosis in Nigerian population.

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