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Thermal Adaptation and Inactivation of Vegetable and Environmental isolates of *Listeria monocytogenes* in Fish Soup Model

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Abstract: All over the world, vegetables are routinely eaten raw or partially exposed to heat as part of food garnishing. Used in these ways, these vegetables can become sources of infection with various food borne pathogenic organisms. This study assessed the contamination of vegetables and related environmental materials with *L. monocytogenes* and the thermal adaptive responses, heat resistance (D_{values}) and antibiotic resistance profile of the isolates. The bacterium was present in 92% of 250 vegetable and 65.52% of 290 environmental samples. Highest incidence occurred in leafy vegetables (100%) which also bore the heaviest load (>5log cfu g^{1}). Among the environmental samples, highest incidence was found in soil (100%) with contamination load greater than 6log cfu g^{-1}. These indicate that raw vegetables are of public health interest for transmission of *L. monocytogenes* and genesis of listeriosis. The mean thermal death times (*D*-value) of representative isolates were 1.82 min, 1.27 min and 56.4 sec at 60°, 65° and 70°C respectively in fish soup. Heat shocking *L. monocytogenes* cells at 45°C / 30 min resulted in significant increase in *D*-value at 60°C but not 65° and 70°C. Antimicrobial susceptibility profile of the isolates showed resistance to two or more antibiotics suggesting prior exposure of the organisms to antimicrobial agents. The public health implications of the findings are discussed.
Keywords: *Listeria monocytogenes*, *D*-value, heat shock, soup model, vegetable, Antibiotic susceptibility

INTRODUCTION

Listeria monocytogenes is the only human pathogen and most virulent of the ten *Listeria* species (Swaminathan, 2010). It causes a disease condition known as listeriosis which can be very severe, particularly in immuno-compromised individuals (Pagadala *et al*., 2012). Although relatively rare, mortality of human listeriosis can approach 50% (Swaminathan, 2001). This is attributed to the invasive nature of *Listeria* which has been isolated from normally sterile sites such as cerebrospinal fluid, bone or joint fluid, blood and products of conception (CDC, 2011). In Nigeria, sporadic cases of listeriosis have been reported (Chukwu *et al.*, 2006). Although no major outbreaks or epidemics of human listeriosis have been reported it is known that *L. monocytogenes* causes febrile gastroenteritis in healthy individuals, a symptom that is similar to disease conditions caused by such organisms as *Shigella, Campylobacter* and *Salmonella* spp and so can be easily overlooked under conditions where isolation of disease agents is hardly pursued as part of routine diagnostic and treatment procedures (Dogbe, 2010).

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The organism is widespread in food and environment, including soil, water, food processing plants, and raw foods including vegetables, meat, poultry, dairy products, sea foods and ready-to-eat foods (Locatelli *et al.,* 2013*)*. It is of great importance in food safety because of its ability to resist environmental stresses commonly encountered in food. Although it cannot form spores, it is known to be very tough, tolerating high concentrations of salt, relatively low pH and refrigeration temperatures (ILSI, 2005). Studies have shown that *L. monocytogenes* responses to heat stress in food and processing environment are heterogenous; as inactivation, survival and persistence or even growth during storage (Lianou and Koutsoumanis, 2011; Sant'Ana *et al*, 2012). Like many other pathogens, *L. monocytogenes* has been reported to show enhanced tolerance when exposed to sub lethal stress such as heat. This phenomenon is attributed to their ability to produce heat shock and stress proteins (Sergelidis and Abrahim, 2009; Porta *et al.*, 2010) that protect them from ordinarily destructive temperatures and stress conditions upon secondary exposure. This is important in food processing because prior exposure of pathogens to sub-lethal temperatures can increase the likelihood of their survival, making it difficult to meet pathogen reduction targets (Juneja and Marks, 2003; Wood *et al.*, 2015).

The consumption of fresh vegetables has been on the increase worldwide owing to enhanced awareness of their nutritional benefits (Adeshina *et al*., 2012). This is particularly the case in Nigeria where, besides home harvests, over 10% of household expenditure on food goes to fresh vegetables particularly tomatoes, cabbage, onions, lettuce, cucumber, salad cream, okro and garden eggs (NBS, 2012; Irene *et al*., 2013). Majority of the vegetables enter the home without any decontamination process except for gross removal of dirt by the seller, yet these vegetables are either consumed raw or following only minimal heat treatment. The presence of *Listeria* on vegetables can therefore present considerable public health challenge. This paper, presents information on the contamination of *L. monocytogenes* in vegetables consumed in raw form or following only minimal exposure to heat. In addition it explores heat resistance behavior of this organism in nutritionally rich, cosmopolitan and cultural foods in which raw vegetables may be used as garnishing. To determine if the isolates have had prior exposure to humans and animals, their antibiotic resistance characteristics are also investigated.

MATERIALS AND METHODS

Sample Collection, Isolation and identification of *L. monocytogenes*

Two hundred and Fifty (250) vegetable samples were collected from ten (10) local markets in Nsukka, Enugu State and Abuja, Federal Capital Territory areas of Nigeria. The vegetables include; *Gongronema latifolium* ('Utazi')(50), *Gnetum africanum*('Uziza') (50), *Solanum macrocarvum*(Garden egg) leaf (50) and fruit (50), *Brassica oleracea*(Cabbage) (25), and *Daucus carota*(Carrot) (25). Two hundred and ninety (290) environmental samples comprising garden soil (100), retail table swabs (100) and fish (*Clarias gariepinus*) pond water (90) were also collected aseptically by standard methods from both areas. For detection and isolation of *L. monocytogenes,* a modification of the method described by U. S. Food and Drug Administration (FDA, 2003) was used. The vegetable samples were homogenized using Salton®food processor which was sterilized using 70% ethanol before and between samples. The soil samples were collected at depth not exceeding 2.0cm and suspended in conical flasks containing nine parts of sterile distilled water and homogenized for thirty minutes in wrist action shaker at 120 rpm. 10g of homogenized vegetable samples were placed in conical flasks containing 90 ml of Fraser broth (containing Fraser broth base and Fraser selective supplement (Oxoid, England)), while 1 ml of liquid samples (soil suspension, fish pond water and Ringer solution with swabs which had been used to swab approximately $10cm^2$ of vegetable retail table top) were suspended and shaken thoroughly in 9 ml of Fraser broth in Falcon tubes. These were incubated in orbital shaker at 130 rpm and 30°C for 24 hrs. Thereafter, a micropipette was used to transfer 10 µl of the sample to Petri dishes containing Oxoid Chromogenic Listeria Agar (with Brilliance Listeria Agar Base), Brilliance Listeria Selective Supplement and Brilliance Listeria Differential Supplement (Oxoid, England). Samples were evenly spread across the plates using a sterile disposable spreader and the plates were incubated at 30°C for 48 hrs. The plates were prepared in duplicates. Serially diluted samples (10^{-1} and 10^{-2}) were also plated for ease of counting. The resulting colonies were identified using cultural, morphological and biochemical tests. Working cultures were maintained on OCLA slants at 4°C with biweekly sub-culturing while stock were maintained in vials in liquid nitrogen.

Determination of Decimal Reduction (D-value) Time and Response to Sub-Lethal Heat

Inoculum preparation: Five confirmed isolates were selected for determination of *D*-values at 60°, 65° and 70°C. To prepare the cultures, Bijou bottles containing the stored *L. monocytogenes* isolates were warmed to room temperature in a water bath and 1.0 ml of the content transferred to 9 ml of tryptic soy broth in 50-ml Falcon tubes. The tubes were incubated for 24hrs at 37°C and 120 rpm. The contents of the tubes were plated onto tryptic soy agar (TSA) and incubated aerobically at 37°C for 18 hrs. The colonies were harvested into tryptic soy broth and incubated in an orbital shaker for 24 hrs. Each culture was then centrifuged at 18000 g for 15mins at 4°C and the cell pellets washed twice in 0.1 % peptone water. After decanting, the cells were suspended in peptone water to a target population of 8 – 9logcfu ml^{-1} determined by serial dilution and plate count in duplicate on TSA plates at 37°C for 18 hrs.

Determination of Decimal Reduction Time (D-value) A modification of the process described by Juneja and Marks (2003) was used to determine the D-values of isolates at different temperatures. The heating menstrum was freshly prepared fish (*Clarias gariepinus*) soup (pH6.6) strained of fish and solids. It was prepared using 1.4kg of cat fish, 20g of *Gongronema latifolium* leaf, two medium size onion bulbs, four seeds of calabash nutmeg, three maggi(seasoning) stock cubes, and one tablespoon of dry *Piper guineense* seeds, chilli pepper and salt to taste (All Nigerian Food Recipes, 2013). Equal volume of bacteria broth was added to the heating menstrum in Erlenmeyer flasks and 10 ml of the mixture were dispensed into a set of eight test tubes prepared in duplicate for specific time intervals for each isolate. A similar arrangement was set up for sub-lethal heat shock treatment to evaluate the effect of sub-lethal heat treatment on ability of *L. monocytogenes* to withstand lethal heat treatment. Erlenmeyer flasks containing 100 ml of prepared bacterial broth were first heat shocked at 45°C by immersing the flasks in a water bath for 30mins and cooling to room temperature in a water bath for 30mins. The contents of the flasks were plated on both OCLA and Brain heart infusion agar (BHI) which is a non-selective agar to estimate the level of injury in the cell populations. Equal volumes of heating menstrum were added to the flasks, stirred and dispensed into test tubes as described earlier. Both the sub-lethally heated and untreated samples were then heated in fish soup samples that had been pre-equilibrated to 60°, 65° and 70°C for 7 min. At 30 seconds or one minute intervals, samples were removed from the water bath, cooled by plunging into ice packs and plated in duplicate onto non selective OCLA following appropriate dilution. The plates were incubated at 37°C for 24 hrs and the colonies counted. The log of survivors was plotted against time and the slope determined. The *D*-values were calculated as the negative reciprocal of the slope using linear regression analysis with R^2 between 0.90-0.99 and also mathematically for comparison and control. Distilled water and 0.1 molar phosphate buffer (pH=7.4) were also prepared and used as heating menstrum for comparison (using isolates UT1 and CB1) and normal cells to evaluate the effect of heating menstrum on the *D*-values of isolates.

Antimicrobial susceptibility of isolates

Susceptibility of isolates to antibiotics was conducted using Kirby-Bauer antibiotic disc diffusion techniques (Bauer *et al.,* 1966; Parihar*et al,* 2010) on Mueller-Hinton agar incubated at 37°C for 24 hours. Overnight cultures of the isolates were sub-cultured in Mueller Hinton (MH) broth and incubated for 18 to 24hrs until the turbidity equaled 0.5% McFarland. The following panel of antimicrobial agents was used: perfloxacin (10µg), gentamycin (30µg), ampiclox (30µg), zinnacef (20µg), amoxicillin (30µg), rocephin (25µg), ciprofloxacin (10µg), streptomycin (30µg), septrin (10µg), and erythromycin (10µg). Sensitivity of the isolates was tested in triplicates and the means taken. Results were interpreted using CLSI antimicrobial susceptibility testing standards (CLSI, 2006).

Statistical analysis

The survival curves were fitted using Microsoft Excel and the equation of the curve generated. Descriptive and statistical analyses of data obtained were performed using SPSS 16.0. One-way Analysis of Variance (ANOVA) was used to test significant difference between variable means across different groups. LSD and Duncan's multiple range tests were used to separate the sample means at 5% level of significance.

RESULTS AND DISCUSSION

Incidence of *Listeria monocytogenes*

Macroscopic examination of *L. monocytogenes* on Oxoid chromogenic listeria agar showed colonies that were greenish blue and surrounded by an opaque white halo. Results of morphological, physiological and biochemical characterization of the presumed *L. monocytogenes* isolates obtained from vegetable and environmental samples are shown on Table 1. Table 2 shows the frequency of occurrence of *L. monocytogenes* in the food and environmental samples. Of the 250 vegetable samples tested, 230 or 92% percent were positive for *L. monocytogenes* with counts ranging from 40 to 1.12×10^6 cfu g^{-1}. The prevalence of *L. monocytogenes* in samples of vegetables (Table 2) is quite high for an organism of considerable public health concern.

However, it was in the range reported in similar products in several locations in Nigeria and other parts of Africa (Ikeh *et al.*, 2010; Irene *et al.*, 2013; Jamali*et al.*, 2013a). In contrast, lower incidence and concentrations have been reported in other countries including Chile (Cordano and Jacquet, 2009), Malaysia (Ponniah *et al.*, 2010) and China (Yan *et al.*, 2010; Yu *et al.*, 2014). The differences may be attributed to a variety of reasons including livestock management practices and use of farm house wastes and effluents and sewage for irrigation and other related agricultural practices (Yan *et al.*, 2010). The highest prevalence was detected in leafy vegetables including cabbage (Table 2). This is similar to the report by Irene *et al.* (2013). The broad leafy form of this vegetable could provide greater opportunity for attachment of microorganisms. Besides, its growth close to the ground means that when irrigated with contaminated water the leaves could easily get contaminated. High levels of fermentable sugars in leaf exudates of cabbage (Dogbe *et al*., 2010; Irene *et al*., 2013) and the micro-architectural structure of cabbage which aids growth of *L. monocytogenes* (Ongeng *et al*., 2007) have been suggested to be reasons for easy association of *L. monocytogenes* with cabbage leaves. Carrot, a root vegetable in contact with the soil had low prevalence. Similar incidence has been reported in other locations (Cordano *et al*., 2009; Irene *et al*., 2013); and this was attributed to inhibitory effect of raw carrot on *L. monocytogenes* (Beuchat and Brackett, 1990; Irene *et al.,* 2013). Besides, carrots unlike leafy vegetables are washed prior to sale and this can reduce gross contamination. Carrots are also harvested from underground; growing at a level where the survival of organism may be challenged by environmental and biotic pressures. Enumeration of *L. monocytogenes* in vegetables showed mean counts between 3-5log cfu g^{-1} (Table 3). This is high relative to what has been reported in other locations (Ponniah *et al,* 2010). Since this study was done during rainy season, the high humidity may have enhanced multiplication of organism, while splashing of rain water on vegetables can also enhance contamination. There was greater contamination of vegetables sampled in Nsukka area. This may be as a result of relatively high average rainfall in Nsukka compared to Abuja (Akintola, 1986), besides differences in farm management practices between these locations.

In the case of environmental samples, out of 290 samples, 190 or 65.52% were positive for *Listeria* with counts ranging from 20 cell g^{-1} of soil to 7.3 X 10^6 cells $10cm^{2-1}$ of retail table area. The frequency of isolation of *L. monocytogenes* in environmental samples (Table 2) was similar to reports from other locations. Garden soil had highest frequency (100%) as well as very high *L. monocytogenes* count (> 6log cfu g^{-1}).This is similar to the report by Ikeh *et al*, (2010). Isolation frequency of 66.7% was observed in fish pond water with *L. monocytogenes* count between 3-4log cfu ml^{-1}. This is similar to the report by Miettinen and Wirtanen, (2006). Irrigation of vegetable farms with fish pond effluent is now a common practice and it is not surprising that 61 out of the 90 fish farms sampled in this study had vegetable gardens beside them. The least frequency (30%) was observed in samples of retail tables. Comparable data on other food processing surfaces have been reported (Gudbjornsdottir *et al*., 2004; Kells and Gilmour, 2004; Pagadala *et al*., 2012). Although this organism is hardy, retail table surfaces like other fomites is unlikely to support survival of these organism over considerable periods. Notwithstanding the low count obtained, the presence of this organism on retail tables is of considerable concern because of its ability to serve as source for contamination of otherwise uncontaminated vegetables brought in for sale, particularly if quality of sanitation is low.

Effect of Sub-lethal Heat Shock on Population of *L. monocytogenes*

Sub-lethal heat shock at 45^{o}C for 30 min significantly induced injury in the cell population. Injured cells appear as the differences between the number of cells recovered from OCLA (a selective agar) and BHI (a non-selective agar which can support growth of injured cells). This is shown in Table 3. The percentage injury in the cells varied considerably between isolates and ranged between 4.2% (isolate UT_1) and 18.4% (isolate FP_2).

D-values of Isolates

The *D*-values for normal cells and heat (sub-lethal) shocked cells of the isolates in fish sauce at different temperatures are shown in Table 4. Following heat shock, mean *D*-values of the isolates in fish soup increased significantly. Mean $D_{60°C}$ increased from 1.82 min to 2.25 min, an increase of 23%, while $D_{65°C}$ and $D_{70°C}$ increased from 1.27 minutes to 1.45 minutes and 0.94 minutes to 1.0 minutes representing increase of 14% and 6% respectively.

However, the specific impact of heat shock on the *D*-values of *L. monocytogenes* appeared to vary between isolates. Isolate CB_1 showed the most increase in resistance to heat following sub-lethal treatment with increase in survival rate of 64.54, 32.32 and 26.19% at 60°, 65°C and 70°C respectively. For isolate UT_1 there was no protection at 65°C and 70°C while for isolate FP_2 heat shock appeared to cause increase in sensitivity of the organism above 70°C. In general, the protection induced by sub-lethal treatment decreased with increase in temperature except for isolate GEL_2 for which peak protection was recorded at 65°C. The differences in the *D*-values of the isolates showed variability in heat resistance between strains of this organism. The mean $D_{60°C}$ value obtained in this study was 1.82 min. At 65° and 70°C the *D*-values were found to be 1.27 min and 56.4 sec respectively. When cells were exposed to sub-lethal heat shock prior to lethal treatment the *D*-values increased by 23%, 14% and 6% at 60°, 65° and 70°C respectively. This increase in heat resistance has been attributed to development of protective heat shock proteins following exposure to sub-lethal temperatures and is common in the microbial world. In the case of the organisms studied here the protection seemed to decrease with increase in the temperature of inactivation.

The differences in heat resistance due to heating medium is important in food environment where different heating media may differentially protect organisms from inactivation. When isolates UT_1 and CB_1 were used to study the effect of heating menstrum on *D*-values the result showed that the medium had mixed impact on the death of organisms (Table 5). At 60°C isolate UT_1 was most resistant in water and fish soup but this was reversed at 70°C with the most resistance occurring in fish soup and buffer, while resistance was highest in soup and buffer at 65°C. Isolate CB_1 was more resistant in water and buffer at 60°C, 65°C and 70°C than in soup. The pH of fish soup at room temperature was 6.6. The results indicate that the impact of heating medium varied with isolate in a manner that was not clearly defined or predictable.

Antimicrobial Susceptibility

The pattern of susceptibility / resistance of five *L. monocytogenes* isolates to ten antimicrobial agents examined are shown in Table 6. All five isolates were resistant to ampiclox and amoxicillin while three isolates (UT_1, CB_1 and FP_2) were resistant to zinnacef. Two isolates UT_1 and FP_2 were partially resistant to cotrimoxazole and erythromycin. The five isolates were 100% susceptible to the rest of the antimicrobial agents (pefloxacin, gentamycin, rocephin, ciprofloxacin, and streptomycin). Yu *et al*, (2014) reported susceptibility to ciprofloxacin (83.1%), gentamycin (100%) and streptomycin (98.3%) while Jamali *et al*, (2013), Korsak *et al.,* (2012) and Wang *et al.*, (2013) reported susceptibility to gentamycin, ciprofloxacin and streptomycin ranging between 78.8% and 100%. Two isolates (UT_1 and FP_2) showed partial resistance to cotrimoxazole and erythromycin while isolate GEL_2 showed partial resistance to zinnacef. All five isolates were resistant to ampiclox and amoxicillin. This is comparable to Ohue*et al* (2015) who reported high resistance (93%) of Listeria isolates to ampiclox. It is in contrast to what has been reported by other authors in other countries. All isolates were resistant to more than one antimicrobial agent, which is consistent with the pattern of multidrug resistance reported by many authors. Antibiotic resistance profile of the isolates suggests that they are not entirely saprophytic or environmental and may have had prior exposure to human or veterinary environments and therefore, these antibiotics (particularly ampiclox and amoxicillin) which are widely used.

CONCLUSION

Significance of the work: In most countries, vegetables are eaten raw or shredded into fish or meat dishes as garnishing when the food temperatures are already too low to constitute critical control point and so destroy microorganisms, but high enough to induce heat shock. This poses significant public health risks. The vegetables examined in this study were all heavily contaminated with antibiotic resistant *L. monocytogenes.* The isolates survived exposure to heat at temperatures that would routinely be expected to obtain in the many foods in which the vegetables are used as garnishing. Exposure to sub-lethal temperatures, as obtained in this study, induced increased heat resistance in the surviving isolates, and this varied with the temperature of exposure. Antibiotic resistance pattern of the isolates suggest that they were not entirely environmental isolates, and that they may have been prior exposed to antibiotic substances. In many middle income countries it is not known whether people routinely come down with listeriosis,

often because definitive diagnoses of food borne diseases are rarely achieved. People who eat raw or partially cooked vegetables, particularly in culturally important delicacies which are garnished and then stored for later use must take steps to achieve good sanitation, where no critical control point stages precede consumption.

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Table 1: Morphological and biochemical properties of presumed *L. monocytogenes* isolates

Isolate	Gram Reaction	Catalase	Motility	Haemolysis
UT_1	+ rod	+	+	β
UT_2	+ rod	+	+	β
OK_1	+ rod	--	+	β
OK_2	+ rod	+	+	--
GEL_1	+ rod	+	+	β
GEL_2	+ rod	--	+	β
GEF_1	+ rod	--	+	β
GEF_2	+ rod	+	+	--
CB_2	+ rod	+	+	β
CA_2	+ rod	+	+	β
GS_1	+ rod	+	+	β
GS_2	+ rod	+	+	--
FP_1	+ rod	+	+	β
FP_2	+ rod	+	+	β
RT_1	+ rod	--	+	β
RT_2	+ rod	+	+	β

*Isolates with subscript 1 were from Nsukka samples, while those with subscript 2 were from Abuja samples.

Table 2: Frequency of occurrence of *L. monocytogenes* in examined samples

Sample Category	Sample type	No. examined	Positive Samples (%)	Mean Listeria count cfu g^{-1} ml^{-1}	
				Nsukka	Abuja
Vegetable Samples	*Gongronema latifolium*	50	50 (100)	5.08×10^5	3.5×10^4
	Gnetum africanum	50	50 (100)	4.36×10^5	6.06×10^4
	S. macrocarpum leaf	50	50 (100)	3.6×10^3	2.614×10^3
	S. macrocarpum fruit	50	35 (70)	7.2×10^5	1.53×10^5
	Brassica oleracea	25	25 (100)	-	1.435×10^5
	Daucuscarota	25	20 (80	-	4.34×10^3
Environmental samples	Garden soil	100	100 (100)	40	20
	Fish pond	90	60 (66.7)	2.84×10^4	1.05×10^3
	Retail table	100	30 (30	7.32×10^6	3.61×10^6

Table 3: Effect of sub-lethal heat injury on recovery of *L. monocytogenes* in selective and non-selective media

Isolate	BHI (log cfu/ml)	OCLA (logcfu/ml)	Injury (logcfu/ml)	% injury
UT_1	8.83	8.46	0.37	4.2
GEL_2	8.47	7.32	1.15	13.6
CB_1	10.01	8.97	1.04	10.4
GS_1	8.65	8.08	0.57	6.6
FP_2	9.42	7.69	1.73	18.4

Table 4: The *D*-values of normal and heat shocked isolates at different temperatures in fish soup

Isolate	Process Temperature					
	60°C		65°C		70°C	
	Normal	Heat Shock	Normal	Heat Shock	Normal	Heat Shock
UT_1	1.62 (1.59)	1.87	1.30 (1.27)	1.29	1.12 (1.22)	1.11
GEL_2	2.33 (1.94)	2.41	1.42 (1.46)	1.77	0.98 (0.93)	1.13
CB_1	1.41 (1.44)	2.32	0.99 (1.02)	1.31	0.84 (0.88)	1.06
GS_1	2.20 (2.08)	2.69	1.30 (1.31)	1.31	0.82 (0.88)	0.86
FP_2	1.53 (1.45)	1.96	1.32 (1.16)	1.59	0.94 (0.97)	0.84
Mean	1.82±0.17	2.25±0.15	1.27±0.07	1.45±0.1	0.94±0.05	1.00±0.06

Figures in parentheses are calculated D-values

Table 5: Impact of heating media on the D-vales of two *L. monocytogenes* isolates

Isolate	D-*values* in minutes and seconds								
	Water			Buffer			Soup		
	60°C	65°C	70°C	60°C	65°C	70°C	60°C	65°C	70°C
UT_1	1.63	1.09	57sec	1.25	1.44	1.0	1.62	1.30	1.12
CB_1	1.79	1.31	56sec	1.62	1.29	55sec	1.41	59sec	50sec

Table 6: Susceptibility profiles of *Listeria monocytogenes* isolates.

Antimicrobial agent	Susceptibility Pattern				
	UT_1	GEL_2	CB_1	GS_1	FP_2
Pefloxacin	S	S	S	S	S
Gentamycin	S	S	S	S	S
Ampiclox	R	R	R	R	R
Zinnacef	R	I	R	S	R
Amoxicillin	R	R	R	R	R
Rocephin	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S
Streptomycin	S	S	S	S	S
Septrin	I	S	S	S	I
Erythromycin	I	S	S	S	I

*(S) susceptible; (R) resistant; (I) intermediate