Table 69: Summary of minimum inhibitory concentration (MIC) (µg/ml) of selected antibiotics against *L. monocytogenes* isolates.

Antibiotic	Breakpoint*			MIC range (µg/ml)	MIC (µg/ml) ATCC (7644)	Susceptibolity of *L. monocytogenes* ATCC (7644)	Number of isolats (%)		
	S	I	R				S**	I	R
Amoxycillin	≤ 4	> 8, ≤16	>16	0.13-0.5	0.25	S	97(100%)	0	0
Benzylpenicillin	≤ 0.25	> 0.25, ≤16	>16	0.03-0.5	0.125	S	93(95.8%)	4(4.2%)	0
Ciprofloxacin	≤ 1	> 1, ≤ 2	>2	1-2	1	S	89(91.7%)	8(8.3%)	0
Clindamycin	≤ 2	> 2, ≤ 2	>2	0.25-8	0.25	S	50(51. 6%)	2(2%)	45(46.4%)
Chloramphenicol	≤ 8	> 8, ≤16	>16	1- 4	2	S	97(100%)	0	0
Doxycycline	≤ 4	> 4, ≤8	>8	0.03-4	0.06	S	97(100%)	0	0
Fosfomycin	≤ 32	> 32, ≤32	>32	8-128	128	R	0	0	97(100%)
Fusidic Acid	≤ 2	> 2, ≤16	>16	0.5-32	8	I	19(19.6%)	77(79.3%)	1(1.1%)
Imipenem	≤ 4	> 4, ≤8	>8	1-16	2	S	97(100%)	0	0
Teicoplanin	≤ 4	> 4, ≤16	>16	0.5-32	0.5	S	97(100%)	0	0
Oxacillin	≤ 2	> 2, ≤2	>2	0.25-4	1	R	0	9 (8.7%)	88(91.3%)
Rifampicin	≤ 4	> 4, ≤16	>16	0.5-32	0.5	S	97(100%)	0	0
Streptomycin	≤ 8	> 8, ≤16	>16	1-16	2	S	85(88.5%)	8 (7.7%)	4 (3.8%)
Tetracycline	≤ 4	> 4, ≤8	>8	0.13-32	0.25	S	67(71%)	8(7.7%)	22(21.3%)
Trimethoprim	≤ 4	> 4, ≤8	>8	0.06-0.13	0.25	S	97(100%)	0	0
Vancomycin	≤ 4	> 4, ≤16	>16	0.25-1	0.5	S	97(100%)	0	0

*soussy et al., (1994)
**S: Sensitive, I: Intermediate.R: Resistance

Chapter Five

Discussion

The real situation of listeriosis in Sudan is unknown, and no information is available on the presence of *L. monocytogenes* in raw fresh dressed broiler chickens, frozen dressed broiler chickens and RTE poultry products in Sudan. There are no criteria for routine microbiological monitoring plan, including analysis of raw fresh dressed broiler chickens, frozen dressed broiler chickens and RTE poultry products for the presence of *L. monocytogenes* in Sudan. The eating habits of Sudanese people are also different from those of westerns. Aside from some western foods, a significant variety of locally produced and traditional foods such as shawerma are commonly consumed. The first step to convince regulatory authorities and private industry about importance of *L. monocytogenes* in raw fresh dressed broiler chickens, frozen dressed broiler chickens and RTE poultry products are to provide data on prevalence of the bacterium in these food products.

The standard method for isolation and detection of *listeria* is the use of enrichment procedure followed by selective media, are in agreement with Vlaemynck et al. (2000) and Beumer et al. (2003). ALOA medium has proved to be a useful and significantly better assay than other media (Oxford agar, UVM agar, and PALCAM agar) for the isolation and differentiation of *L. monocytogenes* from non-pathogenic *Listeria* species, because *L. monocytogenes* colonies on ALOA agar exhibited clear halo zone that gave same positive results by using PCR technique. The detection of pathogenic *L. monocytogenes* by this media involves cleavage of the substrate, L-α-phosphatidyl-inositol by the virulence factor phosphatidylinositol-phospholipase-C (PI-PLC) and phosphatidylcholin-phospholipase-C (PC-PLC) produced by pathogenic *L. monocytogenes* resulting in the formation of a white precipitation zone (halo) around the colony.

The results of *L. monocytogenes* showd that the prevalence rate was 7.4% in Fresh raw dressing broiler chicken meat, 7% in frozen raw dressing broiler chicken meat, 5.6% processed meats and 3.6% in frozen broiler chickens and shocked frozen broiler chickens, using PCR Molecular Technique. The results could be explained by presence of *L. monocytogenes* is naturally in gastrointestinal tract.

Although conventional method of selective culture media and the Microbact™ System were designed to isolate and characterize *L. monocytogenes*, however (6.25%) of the isolates with typical characters for *L. monocytogenes* was proved to be as *L. monocytogenes* using primers targeting the (*actA*) gene, (*hlyA*) gene and the (*iap*) gene by PCR technique. The other PCR unconfirmed isolates cannot be completely excluded as *L. monocytogenes* unless other primers targeting (*prfA*, *inlA*, *inlB*, and *inlAB*) genes have been used. These findings are also reported by Kalliopi et al. (2008) who found that the results of RTE meat products obtained by the real time PCR using IGS 1, IGS 2 primers did not agree with the cultural method.

Bailey et al., (1990) had examined the factors of colonization of broiler chickens with *L. monocytogenes*, when (orally inoculated) did not colonize chickens as easily as did *Salmonellae* or *C. jejuni*. Younger birds were more susceptible to colonization than older birds, and there was a dose-related colonization response. It is evident that poultry can become contaminated either environmentally during production or from healthy carrier chickens in the processing plant (Genigeorgis et al., 1989; Bailey et al., 1990). In present study 217 *L. monocytogenes* isolates were recovered from raw fresh dressed broiler chickens, frozen dressed broiler chickens and RTE poultry products using the conventional methods for isolation.

In first experimental study (19%, 10%, 14%, 13% and 12%) *L. monocytogenes* isolates were recovered from raw dressed broiler chickens in station 1, 2, 3, 4 and 5 respectively using the conventional methods for isolation. Presence of *L. monocytogenes* in raw dressed broiler chicken was only 13.6 %. Similar rate (9.4%) was reported by Osaili et al., (2011) .Also similar rate (11.5%) was reported by Al-Tahiri et al., (2008) for *L.*

monocytogenes in raw sheep milk in Karak district (south of Jordan). Other researchers (Jalali and Abedi, 2008; Peckrovi et al., 2008) reported that *L. monocytogenes* from raw chicken samples was not detected by either conventional method or PCR technique using primers targeting the (*hlyA*) gene in Iran and Croatia, respectively. Other studies (Vitas et al., 2004; Mena et al., 2004; Yücel et al., 2005) confirmed that *L. monocytogenes* was recovered in high percentages (36.1%, 60% and 11.5%) from raw chicken in Spain, Portugal and Turkey respectively using conventional and serotyping methods.

This study has demonstrated that the highest incidence among *Listeria* spp. was *L.ivanovii* in testd sample (19.8%) followed by *L. monocytogenes* (13.6), *L. grayi* (4.6%), *L. seeligeri* (1%), *L.welshimeri* (2%). this agrees with Osaili (2011)who reported that *L. ivanovii* were the predominant isolate among *Listeria spp*. in fresh dressed broiler chicken samples (30%) that followed by *L. grayi* (5%), *L. seeligeri* (2.5%), *L.welshimeri* (0.6%). Awaisheh (2010) who found that the prevalence rate of isolated *L. innocua* and *L. welshimeri* where the most and least frequently isolated from 56 beef and 36 poultry samples. The highest numbers of *L. ivanovii* isolates could be attributed either to fecal contamination during evisceration, or to food handlers. *Listeria* spp. are widely present in plant, soil, silage and processing environment. Chicken might be colonized by *Listeria* spp. due to consumption of contaminated feed and water (Beresford et al., 2001).

L. grayi, L. seeligeri, and *L. welshimeri* isolates were recovered from raw chickens. These might enter the processing plant via the animals harboring *Listeria* spp. in the intestinal tract or as part of pharyngeal microflora. Incidence of *Listeria* spp. in chicken could be attributed either to improper hygienic practice during processing or to food handlers (Fenlon et al., 1996). Similar findings were also reported by Gibbons et al., (2006) who found *Listeria* spp. in (90.9%) of raw chicken in Trinidad.

Awaisheh (2010) reported that *L. monocytogenes* was isolated from 41 samples (17.1%); 23 from beef and 18 from poultry samples using PCR method. Additionally, Bunci (1991) found that 69% of minced fresh meat found to be contaminated with *L. monocytogenes*.

A total incidence rate of *L. monocytogenes* in fresh meat samples was 12.5%, 13.7%, 4.3% and 17.3% in New Zealand, Belgium, Korea and Spain, respectively. It has been demonstrated that conventional cooking processes are effective in the destruction of *L. monocytogenes* (Nørrung, 2000). Therefore, the presence of *L. monocytogenes* in raw chicken cannot be considered as important as in RTE products since these raw products are normally cooked before consumption. .

At the rate of (16%, 12%, 18%, 4 % and 18%) *L. monocytogenes* isolates were recovered from retail broiler chicken ready to eat meat products in frozen chicken burgar, frozen chicken sausages, frozen chicken meat balls (kofta), chicken shawerma and chicken mortedella respectively using the conventional methods for isolation. Presence of *L. monocytogenes* in retail broiler chicken ready to eat meat products was only (13.6%).

The demonstrated highest incidence among *Listeria* spp. were *L. ivanovii* (20.8%) and *L. monocytogenes* (13.6%) but, *L. grayi* (1.6%), *L. seeligeri* (0.8%) *and L. welshimeri* (1.2%) were low incidence in processed meat products. The presented results disagreed with Awaisheh (2010) who found that the prevalence rate of isolated *L. innocua* and *L. welshimeri* were the most and least frequently isolated from 56 beef and 36 poultry samples. Osaili et al., (2011) reported that *L. ivanovii* were the predominant isolates among *Listeria* spp. in RTE chicken-shawerma samples (66.7%). RTE meat product samples were free of *L. innocua. Listeria* spp. Are distributed widely in the environment and vegetables, processed foods, silage and soil. These results could be explained by fecal contamination during evisceration, or to food handlers (Beresford et al., 2001).

Processed meats are meat products produced from raw meat and other ingredients, stored under refrigeration or frozen conditions that may need further cooking to stop *L. monocytogenes* or could be consumed directly without cooking (Tsutomu et al., 1990). In current study, the presence of *L. monocytogenes* in processed meats could be explained by the inadequate heat treatment to destroy the growth of *L. monocytogenes* or as a result of post process contamination. Many studies have reported that the prevalence rate in RTE meats ranged from 1.8% to 48% (Ryu et al., 1992; Wilson, 1995; Bersot et al.,

2001; Eleftheriadou et al., 2002; Soultos et al., 2003; Mena et al., 2004; Vitas et al., 2004).

The growth of *L. monocytogenes* in meat is highly dependent on the temperature, the pH of the meat, the type of tissue and the type and amount of background micro flora present. Glass and Doyle (1989), found that the growth of *L. monocytogenes* in meat was highly dependent on product type and pH. The organism was able to grow well in meat products with a pH value near or above 6.0 while it grew poorly or did not grow in meats that have pH 5.0.

L. monocytogenes is able to contaminate RTE meat products by transmitting through post-processing steps including slicing, packaging and freezing.Thus, these foods needs a further heat cooking at homes, hotels, restaurants to eliminate *Listeria* spp. especially *L. monocytogenes.* In addition, cross-contamination between raw materials, equipments, utensils, humans, rodents, insects, animals and birds could contribute to the spreadity of *L. monocytogenes* in food processing plants. (Jemmi and Stephan, 2006). In addition, the ability of *L. monocytogenes* to form biofilms inside food facility and on the surfaces of food production lines that is quite difficult to be removed and cleaned (Beresford et al., 2001). For example, *Listeria* spp. especially *L. monocytogenes* is able to transfer through vacuum and gas- packaged products due to their ability to grow and survive at low temperature (Duffy et al., 1994; Huss et al., 2000).

This besides that previous study had also demonstrated that colonization of refrigerators by *L. monocytogenes* is a potential source of contamination of food samples (Sergelidis et al., 1997). The nature of strain persistence is unknown but biofilm formation in food-processing facilities could be one of the important reasons (Beresford et al., 2001).

To our knowledge the present study reports for the first time the detection of *L. monocytogenes* in RTE frozen chicken burgar, frozen chicken sausages, frozen chicken meat balls (kofta), chicken shawerma and chicken mortedella in Sudan.

The prevalence of *L. monocytogenes* in RTE food is commonly reported in high rates in different parts of world. *L. monocytogenes* was detected in Spanish-style sausage (3.7%), blood sausage (11.1%), cooked meat samples (8.8%), different RTE foods (7.3%) and in

in-store-packaged deli meat products (8.5%) (Mena et al., 2004; Vitas et al., 2004; Jalali and Abedi, 2008; Garrido et al., 2009). A USDA-FSIS survey published in 2001 showed that 1-10% of retail RTE meat and poultry products were contaminated with *L. monocytogenes* (Levine et al., 2001). Although RTE are commonly encountered *L. monocytogenes* (Anonymous, 1999; Mena et al., 2004; and Vitas et al., 2004); another study in Australia also reported low prevalence rate where, only 0.2% prevalence of *L. monocytogenes* was reported in these products (Tom et al., 2009).

Since *L. monocytogenes* was isolated from raw dressed chickens, it appears that RTE products are generally subjected to potential cross-contamination during processing and handling. Therefore, it is generally accepted that efficient preventive measure has to be taken to limit the contamination of RTE products. Two crucial factors are generally suggested to control contamination with *L. monocytogenes*.

Firstly, is the implementation of Hazard Analysis and Critical Control Point (HACCP) systems and Sanitation Standard Operating Procedures (Sanitation SOPs). Other food safety programs at the retail level, as well as new disinfection strategies to control persistent strains (Holah et al., 2004), will contribute to prevent cross-contamination and exposure to the pathogen. Secondly, storage time and temperature are other important factors in controlling the growth of *L. monocytogenes*. Improved consumer education concerning refrigerator temperature control is needed, together with more concise and informative labeling of all RTE may be effective for controlling the pathogen (National Advisory Committee on Microbiological Criteria for Foods, 2005; Kosa et al., 2007).

At the rate of (19%, 8%, 12%, 13% and 12%) *L. monocytogenes* isolates were recovered from frozen raw dressed broiler chickens in station 1, 2, 3, 4 and 5 respectively using the conventional methods for isolation. Presence of *L. monocytogenes* in frozen raw dressed broiler chicken was only 12.8 %.

The demonstrated highest incidence among *Listeria* spp. were *L. ivanovii* (19.4%) and *L. monocytogenes* (12.8%) but, *L. grayi* (4 %), *L. seeligeri* (1 %) and *L. welshimeri* (1.8%) were at low incidence in processed meat products.

While (17%, 15% and 7%) *L. monocytogenes* isolates were recovered from fresh raw dressed broiler chickens, frozen raw dressed broiler chickens and shock frozen raw dressed broiler chickens station using the conventional methods for isolation. Presence of *L. monocytogenes* was only 13 %. There is marked effect of temperature on *L.monocytogenes* between fresh dressed broiler chickens and frozen dressed broiler chickens 17% and 15 % respectively, thus *L.monocytogenes* has ability to survive freezing and frozen storage at -18°C. (Novak & Juneja, 2003) reported that, the freezing and frozen storage of foods do not induce a marked inactivation of *Listeria monocytogenes* Scott A in under-cooked ground beef. Flessa., et al,(2005), reported that, the *L.monocytogenes* can survive on frozen strawberries at - 20º C±2º C for periods at least 4 weeks.

Palumbo and Williams, (1991) studied the ability of *L. monocytogenes* to survive freezing and frozen storage at -18°C in ground beef, ground turkey, frankfurters, canned corn, ice-cream mix, and tomato soup. Then the results showed that *L. monocytogenes* survived freezing and frozen storage well in five of the examined foods, was not injured, and was quantitatively recovered on Listeria-selective media. In contrast, the organism showed a decline in viable count after extended frozen storage in tomato soup, was injured, and could not be quantitatively recovered on Listeria-selective media.

El-Kest and Marth, (1992), reported that the higher (-18º C) freezing temperatures were more lethal than lower (-198 º C) temperatures, and freeze–thaw cycles were more lethal with freezing to (-18º C) than with freezing to (-198 º C). also freezing at (-198 º C) and storage at (-18º C) was more lethal than freezing and storing at (-18º C). (Palumbo and Williams, 1991) reported that there are variability among strains of *L. monocytogenes* in susceptibility to freezing. In another study using five foods with a pH of 5.8 or above, and one with a pH of 4.74, there was little effect of freezing and frozen storage at (-18º C) on *L. monocytogenes in* the pH 5.8 foods, but declines in viability, and evidence of injury during storage in the pH 4.74 foods.

listeria monocytogenes cells respond to a decrease in temperature by inducing a set of proteins, called cold shock proteins (csps). These proteins are thought to play a role in the protection of cells against damage caused by freezing. (DO et al, ١٩٩٦).

It has been shown that cold-shocked (4 h at -10ºC) cells of *Listeria monocytogenes* synthesised cold-shock proteins and became much more resistant to either freezing or to pressurisation at 200MPa . Wemekamp et al, (2002).

The highest incidence among *Listeria* spp. was in frozen raw dressed boiler chicken meat, these included *L. ivanovii* (18%) and *L. monocytogenes* (13%) but, *L. grayi* (3.6 %), *L. seeligeri* (1 %) and *L. welshimeri* (1.3%) is low incidence in processed meat products.

In the study, the rate of (17%, 15% and 7%) *L. monocytogenes* isolates were recovered from fresh raw dressed broiler chickens, frozen raw dressed broiler chickens and shock frozen raw dressed broiler chickens showed 7% lower than fresh and frozen this may be due to decrease in temperature suddenly at - 40 º C lead to injury of *L. monocytogenes.* Chou , (1999) studied the inactivation and injury of *Escherichi coli* O157:H7 and *Listeria monocytogenes* in Tryptic soya broth stored at -5 º C , -18 º C and -28º C the results were population reduction of E. coli O157:H7 determined with TSA was ca 1.72 log CFU/ml. On the other hand, a population reduction of only 0.64 log CFU/ml was noted with *L. monocytogenes*. Besides, the surviving population of *E. coli* O157:H7 contained a larger proportion of injured cells than *L. monocytogenes*.

In the presented study, artificially infected meat chicken with *listeria monocytogenes*, there is no effect to frozen (-18 º) and shock freozen (- 40 º C) temperatures for one year. *L. monocytogenes*. stay a live. El-Kest and Marth, (1992), reported that, the higher (-18º C) freezing temperatures were more lethal than lower (-198º C) temperatures. sublethal damage may occur in microorganisms that have undergone a freeze thaw cycle, and selective agents in dilution, enrichment or plating media may render the injured cells unable to multiply. This could lead to a false conclusion about lethality, since the damaged microorganisms, if placed in a menstruum that permits repair (resuscitation), may become fully viable (and possibly infectious). For example, in studies on the effects

of freezing various strains of *L. monocytogenes* in tryptose phosphate broth, apparent greater losses of viability for thawed cells that had been held at (-18º C) for 14 days were observed if the plating medium contained 8% NaCl than if the medium was without NaCl (Golden et al, 1988).

Among 100 samples in breeding and Slaughter house (abattoir) chicken abattoir in Khartoum State the (41%) isolated *listeria* ssp. were distributed as follows:- *L. monocytogenes* (12%) .*Listeria ivanovi* (23%). *Listeria seeligeri* (5 %), *Listeria seeligeri* (5%), *Listeria welshimeri* (11%) .

From 40 samples in breeding house 21 (52.5%) isolated *listeria* ssp. were distributed as follows:- *L. monocytogenes* 6 (15%) .*Listeria ivanovi* 11 (27.5%). *Listeria seeligeri* 4 (10 %), and *Listeria welshimeri, listeria grayi* were absent. .

Listeria monocytogenes was recovered from 4 (40%) of a total 10 cotton-swabbed samples collected from the surfaces of water line and from 2 (40 %) of a total 5 cotton-swabbed samples collected from the surfaces of worker's hands these .

From 60 samples in slaughter house (abattoir) 20 (33.3%) isolated *listeria* ssp. were distributed as follows:- *L. monocytogenes* 6 (10%) .*Listeria ivanovi* 12 (20%). *Listeria seeligeri* 1(1.66 %), 1(1.66 %) *Listeria welshimeri and listeria grayi* was absent.

Listeria monocytogenes was recovered from 6 (24%) of a total 25 cotton-swabbed samples collected from the surfaces of worker's hands {3 (60%) of a total 5 hand of workers, 1 (20%) of a total 5 Hand of Slaughter worker, 1 (20%) of a total 5 Chicken head cutting worker hand and 1(10%) of a total 10 hand of worker cutting neck}.

These results similar to Dimitrijevíc et al, (2011) reported that *Listeria monocytogenes* was recovered from 10 (6.06%) of a total 165 cotton-swabbed samples collected from the surfaces of equipment and worker's hands at two separate processing facilities. The results are similar to Loura et al, (2005).who reported that workers hands were contaminated by *L. monocytogenes* ,*L. innocua* and *L. Grayi.*

Listeria contamination of food contact surfaces can lead to cross-contamination of ready-to-eat foods in delicatessens. Recognizing that variations in Listeria biofilm-forming ability exist,

The study of Keskinen LA, et al (2008) was to determine whether these differences in biofilm formation would affect the Listeria transfer rate during slicing of delicatessen turkey meat.

He used six previously identified strong and weak biofilm-forming strains of *Listeria monocytogenes* and inoculated onto flame-sterilized AISI grade 304 stainless steel knife blades that were subjected to 6 and 24 h of ambient storage at approximately 78% relative humidity.

Tested, these blades were used to obtain 16 slices of retail roast turkey breast. Listeria populations decreased 3 to 5 log CFU per slice after 16 slices. Overall, total transfer to turkey was significantly greater for strong (4.4 log CFU total) as opposed to weak (3.5 log CFU total; $P < 0.05$) biofilm formers.

Ma´rcia et al (2007) studied to establish the occurrence of *Listeria* spp., especially *L. monocytogenes* and its main serotypes, in beef and processing plants. The occurrence of *Listeria* spp. 51.4% were from equipment, 35.4% from installations and 30.2% from products. The identified species were: *L. monocytogenes* (12.6%), *L. innocua* (78.4%), *L. seeligeri* (1.2%), *L. welshimeri* (7.2%) and *L. grayi* (0.6%).

The detection of *L. monocytogenes* by conventional methods was higher compared to molecular techniques. *Listeria* spp. was detected in ١٣,٦ % of raw, 12.8 % of frozen, 13.6 % of processed meat products, 13 % effect of temperature on broiler and 12% of chicken breeding-slaughter house using cultural methods compared to 7.4 % of raw, 7 % of frozen, 5.6 % of processed meat products, 3.6 % effect of temperature on broiler and zero % of chicken breeding-slaughter house using PCR method. However, even conventional culturing method considered as standard method for detection of *Listeria* spp. in food, biological and environmental samples, it could lead to false deviation results. The

obtained results in the present study could support this conclusion since the total confirmed isolates through using PCR was 97 compared to 217 by conventional methods. In fact, the isolation and characterization of *L. monocytogenes* in biological, environmental and food samples by conventional methods was limited by survival of *L. monocytogenes* in low numbers as a result of existence of high number of competing microorganisms, the growth of non-pathogenic *Listeria* spp. and the interference of inhibitory food components (Norton , 2002).

Chromogenic medium as ALOA is considered as the most useful culture media for isolation of *Listeria* spp. from different samples compared to Oxford formulation and other conventional selective media. This could be attributed to its easy preparation and interpretation as well as less time consuming for development of presumptive *L. monocytogenes* cells (Reissbrodt , 2004).

The chromogenic Listeria media used to differentiate between pathogenic and nonpathogenic *Listeria* spp. through a process that depends on cleavage of substrates by the virulence factor phosphatidylinositol-phospholipase C (PI-PLC) and phosphatidylcholinphospholipase C (PC-PLC) (Reissbrodt , 2004). The Microbact™ Listeria 12L system and CAMP- test were used in this study to differentiate between Listeria ssp. based on the fermentation of sugars and its heamolysis activity. In the current study the total identified isolates by Microbact™ Listeria 12L System were 217 compared 97 isolates confirmed by PCRThe difference in the detection of *L. monocytogenes* between Microbact™ Listeria 12L and PCR could be explained that the primers used in current study were not effective to detect all *L. monocytogenes*, therefor, other primes sush as *inlA, plcA, prfA* and *inlB* may be used.The primers used in current study to detect *actA, hylA*, and *iap* were described to be specific for *L .monocytogenes* detection in food. The presented results agree with Kalliopi et al., (2008) who found that the prevalence rate of RTE meat products collected by Real Time- PCR using *IGS 1, IGS 2* primers did not agree with the cultural method. In contrast, Barocci et al., (2008) reported that the prevalence rate of *L. monocytogenes* in meat samples by conventional methods agree with PCR molecular method using *LL5* and *LL6* primers. Preliminary

studies conducting out the lab showed that primers targeting the *inlA* gene are more accurate in detection *L. monocytogenes* than other primers such as that targeting the *actA, hlyA* and *iap* genes. Liu et al. (2007) who showed that the *inlA* gene was very accurate in confirmation of *L. monocytogenes* isolates.

Although PCR has become the most common method for the detection of *L. monocytogenes* in biological, environmental and food samples, it has been hampered by external factors as the presence of inhibitory substances as organic and phenol compounds, glycogens fats and Ca+2 that found in food sources or humic and fulvic acids, heavy metals in water, sludge, soil and compost. It is interfering with complex composition of starting materials which have inhibitors for PCR system (Rossen et al., 1992; Bickley et al., 1996).

The ability of both PCR and ISO culturing methods todetect *L. monocytogenes* from salmon samples, gave similar results in spiked samples if culture enrichment is used prior to PCR to lower the detection limit for *L. monocytogenes.* (Wan et al., 2003).

All *L. monocytogenes* isolates were sensitive to nine antibiotics; ampicillin, Chloramphenicol, Doxycycline, Imipenem, Teicoplanin, Rifampicin, Trimethoprim and vancomycin, and more than 88% of isolates were sensitive to three antibiotics: benzylpenicillin, ciprofloxacin and Streptomycin.

All the isolates were resistance to fosfomycin and 91.3% of the isolates were resistance to oxacillin. Therefore, ampicillin, Chloramphenicol, Doxycycline, Imipenem, Teicoplanin, Rifampicin, Trimethoprim and vancomycin could be a choice for treatment of listeriosis in Sudan. Navratilova et al. (2004) reported that isolated *L. monocytogenes* from different meat products were sensitive to tested antibiotics (ampicillin, neomycin, erythromycin, gentamycin, cephalotin, clindamycin, norofloxacin, oxacillin, penicillin, streptomycin, tetracycline and vancomycin).

Arslan and Ozdemir (2008).reported that all isolates of *L. monocytogenes* sensitive to imipenem, teicoplanin and vancomycin.

Vancomycin and erythromycin are also used to treat bacteraemia and pregnant women diagnosed with listeriosis, respectively, (Charpentier and Courvalin, 1999). However, (Abrahim et al., 1998) reported that the Doxycyclin and Amoxicillin tested antibiotics had great impact on *L. monocytogenes* isolates from Greece where a low prevalence of acquired resistance in *L. monocytogenes* isolated from sausages. In contrast some clinical isolates exhibited multidrug resistance (Tsakris et al., 1997). At the same trend one *L. monocytogenes* isolate from beef displayed resistance to tetracycline with a MIC > 32µg/ml (George et al., 2009). *L. monocytogenes* is considered a slowly becoming antibiotic resistance microbe (Conter, et al., 2009) and that probably justified the sensitivity of the majority of *L. monocytogenes* to antibiotics. All of *L. monocytogenes* isolates (97) showed resistance for fosfomycin, and 91.3% of isolates were resistance to oxacillin. These results are in agreement with the results of Conter et al. (2009) who reported that 96.8% and 97.6% of *L. monocytogenes* isolates were resistance to fosfomycin and oxacillin, respectively.

The results of antibiotic resistance toward fosfomycin and. 91.3% of the isolates were resistance to oxacillin. Could be explained by genetic factors such as ability of environmental bacteria to transform resistant genes to human- food bacteria.The ability of isolated *L. monocytogenes* strains to adapt adverse environmental conditions such as wrong using of drug therapy for infected animals, is an important factor in the development of resistance (Sorum and L'Abee-Lund, 2002).

Also resistance towards antibiotic could be explained by genetic factors. The coexistence of resistance genes with plasmids, transposons, and integrons enhance the rapid transform of antibiotic resistance genes among bacteria (Sunde and Nordstrom, 2006). On the other hand, any mutation in bacterial cell under stressed condition such as defects in the DNA repair systems increases the possibility of gaining resistance (Alonso et al., 1999).

Conclusion

- The presence of *L. monocytogenes* has been demonstrated for the first time in wide varieties of raw fresh dressed broiler chickens, frozen dressed broiler chickens and RTE chicken products sold in Khartoum-Sudan.
- The Presence rate of *L. monocytogenes* in fresh, frozen and processed poultry meat (RTE) products that sold in Sudan was 7.4%, 7%, and 5.6%, respectively.
- There are no large effect of temperature (-18°C) on *L.monocytogenes* between fresh dressed broiler chickens and frozen dressed broiler chickens 17% and 15 % respectively because *L.monocytogenes* has ability to survive freezing and frozen storage at -18°C.It may be difficult to avoid *L. monocytogenes* in one or more steps of the food chain from production to distribution because the organism is so widespread in food plant environments.
- The shock - frozen temperature (- 40°C) for six hours showed insignifecante reduction in the presence of *L. monocytogenes.*
- *L. monocytogenes* in artificially infected meat chicken stayd a live in frozen temperature (-18°C) and shock frozen temperature (- 40°C) for one year.
- The Presence rate of *L. monocytogenes* in chicken breeding and slaughter house was 15% and 10% respectively.
- The potential risk of eating RTE food or undercooked foods is emphasized.
- In current study, PCR method showed lower Presence rate than conventional method. This may be due to the small number of target genes used.
- The Genotypes which coud be detected from the isolated *L. monocytogenes* using PCR molecular technique are *act A, hly A* and *iap* genes.
- Amoxicillin, Doxycyclin, Chloramphenicol, Imipenem, Teicoplanin, Rifampicin, Trimethoprim and Vancomycin are the best drugs among the tested antibiotics to be used against *L. monocytogenes*.

Recommendations

- The Sudanese Standard Specification lack regulation concerning *L. monocytogenes* in RTE meat products, therefore, there is an urge to establish a national control program for detecting *L. monocytogenes* in RTE meat products.
- Establishment of national record and documentation system for outbreaks of food poisoning concerning *L. monocytogenes*.
- Consumers' education is needed to avoid eating undercooked and raw RTE meat products.
- Using multiple primers for detection of multiple virulence-associated genes of *L. monocytogenes* by multiplex PCR is recommended.
- *Listeria* spp. is ubiquitous in the environments of farms and food processing plants and therefore the control of *L. monocytogenes* during food process is not an easy task. Food safety programs at all stages in the food chain such as ISO 22000 and HACCP, GMP, and Good Personal Hygiene should be enforced in order to reduce the incidence of *L. monocytogenes* in poultry meat products. The controls at both the retail and consumer levels include prevention of cross-contamination, and maintaining chill cabinets and refrigerators at as low a temperature as possible.
- Molecular techniques have proven to be more beneficial when working with food borne pathogens because of their high specificity and sensitivity. Therefore, it can be proposed for further investigation of foods for a preliminary screening of negative samples.
- The results of this study can be of valuable information for government entities in order to develop a risk assessment for *L. monocytogenes* in these products.
- A continued surveillance of emerging antimicrobial resistance of this pathogen is important to ensure effective treatment of human listeriosis.

- More studies on the occurrence of *L. monocytogenes* in other products are needed.
- Prevention of cross contamination between raw materials and RTE meat products should be under taken through application of prerequisite programs such as good manufacturing practices (GMP's).
- Educational programs and training are needed to keep away from eating fresh meat and RTE meat products and further cooking.
- Correct drug at correct dose should be used for treatment of listeriosis.

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Appendix

A. Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes.*

The International Standard ISO 18593 which describes surface sampling methods to detect or enumerate viable micro organisms does not give sufficient guidance or advice specific to *L. monocytogenes* detection. Wipe sampling methods (swab and sponge/ cloth method) are the only appropriate methods to use for *L. monocytogenes*. The ISO standard does not describe when sampling should be performed or what areas should be sampled. The present guidelines aim to compensate for this gap when implementing Article 5.2 of EC Regulation 2073/2005. Furthermore, it was chosen not to address how to enumerate *L. monocytogenes* on surfaces for the following reasons. First swabbing does not detach all bacterial cells and the proportion of detached cells is unknown and variable. Secondly *L. monocytogenes* cells are not evenly distributed on a surface and comparisons of results from large and small areas would thus be invalid.

Choice of sampling locations.

L. monocytogenes can be found on visually clean surfaces but it is most frequently found at wet and soiled places where the bacterium is able to grow and persist (Carpentier ,2011;Chasseignaux et al,.2002). Hard to reach places such as holes or crevices in fibrous, porous, rusting and hollow materials, poorly cleanable equipment are potential harbourage sites that should be sampled. It can be difficult to sample unreachable areas where food debris can collect. These areas should be sampled after dismantling the equipment with the maintenance team. It is not recommended to take a sample by rinsing such areas as rinsing does not have the same efficiency as wiping for detaching the microorganisms from the surfaces.

Sampling should be done frequently in areas where the food product is exposed to contamination, but it may be interesting to also sample, less frequently, in areas where it is not (storage areas).

The choice of sampling location has to be chosen according to historical data linked to each factory and after step-by-step examination of the process. A non-exhaustive list of places to choose sampling locations is given below (FDA, 2008; Tompkin et al., 1999; NSW, 2008).

The time at which sampling should be performed.

The detection of *L. monocytogenes* can be difficult if samples are taken immediately or soon after cleaning and disinfection. Cells, because of the injury caused by the chemical agents used for cleaning and disinfection, can be still alive but non-culturable, therefore not easily detectable (NSW, 2008). Furthermore cells remaining in harbourage sites despite cleaning and disinfection can also be undetected, while they are more accessible to sampling once dislodged during processing because equipment vibrates and/or because foods and liquids come in contact with harbourage sites (Tompkin RB, 2004).

Therefore, to increase the probability of detecting a persistent strain, sampling should be performed during processing, after at least two hours of production or at the end of production runs i.e. before cleaning and disinfection.

B: No. PCR profile of the isolated *L.monocytogenes.*

Serial No	Sample No	Primer		
		actA	*hylA*	*iap*
1	2 A1	+	-	-
3	12 A1	-	+	-
5	17 A1	+	-	-
6	18 A1	-	-	+
7	20 A1	-	+	-
8	23 A1	-	-	+
9	25 A1	+	-	-
13	49 A1	-	+	-
17	57 A1	+	-	-
18	62 A1	-	-	+
20	6 A2	-	+	-
21	8 A2	-	-	+
23	17 A2	-	-	+
25	47 A2	+	-	-
27	68 A2	-	+	-
30	5 A3	-	+	-
31	8 A3	+	-	-
33	20 A3	-	-	+
35	23 A3	-	+	-
36	26 A3	-	-	+
39	83 A3	+	-	-
41	94 A3	-	-	+
42	97 A3	-	+	-
43	99 A3	+	-	-
44	1 A4	-	-	+
50	42 A4	+	-	-
52	49 A4	-	+	-
55	76 A4	-	-	+
57	6 A5	-	+	-
58	9 A5	-	-	+
59	17 A5	+	-	-
60	21 A5	-	-	+
61	22 A5	-	+	-
64	32 A5	-	-	+
65	40 A5	-	+	-
66	46 A5	+	-	-
68	73 A5	+	-	-
69	2 B1	+	-	-
71	12 B1	-	+	-

73	17 B1	+	-	-
74	18 B1	-	-	+
75	20 B1	-	+	-
76	23 B1	-	-	+
77	25 B1	+	-	-
81	49 B1	-	+	-
85	57 B1	+	-	-
86	62 B1	-	-	+
88	6 B2	-	+	-
89	8 B2	-	-	+
90	17 B2	-	-	+
92	47 B2	+	-	-
96	5 B3	-	+	-
97	8 B3	+	-	-
98	20 B3	-	-	+
100	23 B3	-	+	-
103	83 B3	+	-	-
105	94 B3	-	-	+
106	97 B3	-	+	-
107	99 B3	+	-	-
108	1 B4	-	-	+
114	42 B4	+	-	-
116	49 B4	-	+	-
119	76 B4	-	-	+
121	6 B5	-	+	-
122	9 B5	-	-	+
123	17 B5	+	-	-
124	21 B5	-	-	+
125	22 B5	-	+	-
128	32 B5	-	-	+
129	40 B5	-	+	-
130	46 B5	+	-	-
132	73 B5	+	-	-
133	5 C1	+	-	-
137	26 C1	-	+	-
139	43 C1	-	-	+
140	49 C1	-	+	-
141	12 C2	-	-	+
142	25 C2	+	-	-
143	31 C2	-	-	+
145	41 C2	-	+	-
147	3 C3	+	-	-

148	11 C3	+	-	-
150	26 C3	-	+	-
154	45 C3	+	-	-
157	17 C4	+	-	-
162	39 C5	+	-	-
172	21 D1	+	-	-
175	36 D1	-	+	-
180	59 D1	+	-	-
181	69 D1	+	-	-
183	95 D1	-	+	-
191	36 D2	-	+	-
195	59 D2	+	-	-
196	69 D2	+	-	-
198	95 D2	-	+	-
203	59 D3	+	-	-
205	95 D3	-	+	-

(-): negative; (+): positive

A1: first experimente, fresh raw dressed broiler. Station one
A2: first experimente, fresh raw dressed broiler. Station two
A3: first experimente, fresh raw dressed broiler. Station three
A4: first experimente, fresh raw dressed broiler. Station four
A5: first experimente, fresh raw dressed broiler. Station five
B1: Second experimente, frozen raw dressed broiler. Station one
B2: Second experimente, frozen raw dressed broiler. Station two
B3: Second experimente, frozen raw dressed broiler. Station three
B4: Second experimente, frozen raw dressed broiler. Station four
B5: Second experimente, frozen raw dressed broiler. Station five
C1: Third experimente,, frozen chicken-burger
C2: Third experimente, frozen chicken-sausages
C3: Third experimente, Chickens meat balls (Kofta).
C4: Third experimente, Chicken- shawerma
C5: Third experimente, Chicken- mortadella
D1: Four experimente, fresh broiler chickens
D2: Four experimente, frozen broiler chickens
D3: Four experimente, Shock frozen broiler chickens