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Research Article

Isolation and Identification of *Listeria monocytogenes* from Retail Broiler Chicken Ready to Eat Meat Products in Sudan

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Abstract: *Listeria* species are widely distributed in environment and *L. monocytogenes* are the causal agent of *Listeriosis*, the disease that can be serious and fatal to human and animals. The objectives of this study were to detect, isolate and identify *Listeria monocytogenes* from retail broiler chicken ready to eat meat products in restaurants-Khartoum state, Sudan. A total of 250 retail broiler chicken ready to eat meat products were collected from restaurants in Khartoum State, 50 sample from frozen chicken burger, 50 sample from frozen chicken sausages, 50 sample from frozen chicken meat balls (kofta), 50 sample from chicken shawerma and 50 sample from chicken mortedella, *Listeria* spp. were isolated by the conventional International Organization for Standardization method and *L. monocytogenes* identified by biochemical test. The results showed that out of total 250 samples, 95 (38%) were found to be contaminated with *Listeria* spp. the isolation rate was as follows: *L. monocytogenes* (13.6%), *L. ivanovi* (20.8%), *L. grayi* (1.6%), *L. seeligeri* (0.8%) and *L. welshimeri* (1.2%). The results presented in this study indicate the contamination of retail broiler chicken ready to eat meat products with *L. monocytogenes.* This study reported the occurrence and distribution of *L. monocytogenes* and other *Listeria* species in retail meat products (frozen chicken burger, frozen chicken sausages, frozen chicken meat balls (kofta), chicken shawerma and chicken mortedella), purchased from restaurants in Khartoum state Sudan.

Keywords: Broiler, *Listeria* spp., meat balls, mortedella, shawerma

INTRODUCTION

Listeria species are ubiquitous in the environment. *Listeria monocytogenes* is significant for cold-stored, Ready-To-Eat (RTE) foods and can grow at refrigerated temperatures. Isolation of *L. monocytogenes* from chicken meat was recorded previously by many investigators (Miettinen *et al*., 2001; Akpolat *et al*., 2004; Lekroengsin *et al*., 2007; El-Shabacy Rasha, 2008; Keeratipibul and Lekroengsin, 2009; Ahmed and El-Atti, 2010).

Listeria monocytogenes has been recognized as a human pathogen for several years (Gellin and Broome, 1989; Takarada *et al*., 2004). It causes illness in pregnant women, newborns, elderly persons and immunocompromised persons (Cossart and Mengaud, 1989; Pong and Bradley, 1999; Smith, 1999; Takarada *et al*., 2004).

L. monocytogenes is pathogenic for human, while *L. ivanovii* is rarely pathogenic for humans (Cummins *et al*., 1994) *L. monocytogenes* are the causal agent of *Listeriosis*, the disease that can be serious and fatal to human. It is a halo tolerant, Gram-positive, facultative anaerobic, non-spore forming rod bacterium (Larson *et al*., 1999). It can grow in a wide pH range from 4.6 to 9.5 and can grow in low water activity environments as 0.90 (FAO/WHO, 2004; Buchanan *et al*., 2004; Gandhi and Chikindas, 2007). *Listeria* spp. are considered as an important cause of zoonoses infecting many types of animals such as domestic pets, livestock, avian species, rodents, amphibians, fish, and arthropods. The approximate fatality rate is 30% that may increase up to 75% in high risk groups, such as pregnant women, neonates, and immunocompromised adults (Jalali and Abedi, 2007; Mead *et al*., 1999; Low and Donachie, 1997). *Listeriosis* is unique disease that represents a considerable public health concern because of its high mortality rate that reaches 20-40% (Wan Norhana *et al*., 2010a, b; Liu, 2006; McLauchin *et al*., 2004). Most cases of *Listeriosis* appear to be foodborne, including those acquired during pregnancy. *L. monocytogenes* may cross-contaminate RTE meat and poultry products during post-processing steps such as slicing, peeling, and packaging (Murphy *et al*., 2005). RTE meat and poultry products are widely consumed in Sudan. To our knowledge no published data has been found concerning isolation and characterization of *L. monocytogenes* recovered from RTE meat and poultry products in Sudan. The nature of strain persistence is unknown but biofilm formation in food-processing facilities could be one of the important reasons (Bresford *et al*., 2001). High the prevalence of *L.*

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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 and Garcia-Jalon, 2004; Jalali and Abedi, 2007; 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* (CDC, 1999; Mena *et al*., 2004; Vitas and Garcia-Jalon, 2004); another study in Australia also reported low prevalence rate where, only 0.2% prevalence of *L. monocytogenes* was reported in these products (Ross *et al*., 2009). Published information on the status of food borne listeriosis is very limited in the Sudan. The objectives of this study were to detect, isolate and identify *Listeria monocytogenes* from retail broiler chicken ready to eat meat products (frozen chicken burger, frozen chicken sausages, frozen chicken meat balls (kofta), chicken shawerma and chicken mortedella) in restaurants-Khartoum state, Sudan.

MATERIALS AND METHODS

Samples: Two hundred fifty retail broiler chicken ready to eat meat products samples were collected from restaurants in Khartoum state were used for detection of *L. monocytogenes*, fifty samples from frozen chicken burger, 50 samples from frozen chicken sausages, 50 samples from frozen chicken meat balls (kofta), fifty samples from chicken shawerma and 50 sample from chicken mortedella. All Samples were transported to Sudan University of Science Technology College of Veterinary Medicine Microbiology laboratory under aseptic and refrigerated conditions in portable insulated cold-boxes. Samples were kept at 4°C and analyzed within 24 h.

Isolation and identification of *L. monocytogenes*: Retail broiler chicken ready to eat meat products samples were tested for the presence of *L. monocytogenes* following the procedure recommended by using the International Organization for Standardization (ISO, 1996, 2004) procedure. A 25 g representative portion from each sample was introduced aseptically into a sterile stomacher bag containing 225 mL of Half Fraser Broth (Oxoid, Ltd., Basingstoke, UK, CM0895) (primary enrichment medium) to obtain a 1:10 sample dilution. The samples were then homogenized for 1 min at 260 rpm in a stomacher circulator unit 400 (Seward, UK) followed by incubation for 24 h at 30ºC. After incubation period, 0.1 mL sub-sample from each Half Fraser Broth culture was added to 10 mL of Fraser Broth (Oxoid, CM0895) (secondary enrichment medium), and incubated for 48 h at 37°C. A loopful of the Fraser Broth enrichment culture was streaked on the surface of Chromogenic Listeria Agar (Oxoid, CM1084) and on Listeria Selective Agar (Oxford Formulation) (Oxoid, CM0856). These selective agars were then incubated for up to 48 h at 37ºC. Selective agars were observed for suspected colonies at 24 and 48 h of incubation. Suspected colonies were those that appeared grayish colonies surrounded by black halos and sunken centers with possible greenish sheen on Oxford agar or green-blue colonies surrounded by an opaque halo zone on Chromogenic *Listeria* agar. Whenever possible, up to 5 suspected colonies showing typical morphology of *Listeriae* on these isolation media were streaked onto Tryptone Soya Agar (Oxoid, M290) supplemented by 0.6% of Yeast Extract Powder (Oxoid, LP0021) (TSYEA) and incubated at 37ºC for 24 h. The following tests were used for confirmation; Gram's staining, motility test, catalase reaction, and oxidase test.

Confirmation of *L. monocytogenes*:

Haemolysis test: An inoculating needle was used to stab the Sheep Blood Agar Base (Oxoid, CM0854), supplemented with 7% sterile sheep blood, with a culture taken from a typical colony on TSYEA and incubated at 37°C for 24 h (ISO, 1996). After incubation positive test cultures show narrow, clear and light zones (ß-haemolysis).

Carbohydrate utilization: The Microbact™ *Listeria* 12L Kit System (Oxoid, MB1128A) for rapid biochemical testing. Microbact™ Listeria 12L Kit System (Oxoid, MB1128A) is a standardized micro-substrate system designed to stimulate conventional biochemical substrates. Each identification strip consists of 12 tests, (11 sugar utilization tests (Esculin, Mannitol, Xylose, Arabitol, Ribose, Rhamonse, Trehalose, Tagatose, Glucose-1-Phosphate, Methyl-D-Glucose, and Methyl-D-Mannose) plus a rapid haemolysis test. The reactions occurring during the incubation period is demonstrated through either a color change in the sugar utilization tests or in the lyses of sheep red blood cells in the haemolysis test. The results were analyzed by Microbact Software (Oxoid, MB1244A) to determine the *L. monocytogenes* with percent probability number.

A statistically the difference between percentages of contaminated samples of various food groups with examined pathogen microorganisms was calculated.

RESULTS

Isolation of *Listeria* spp. from retail broiler chicken ready to eat meat products using of conventional method: According to growth on selective media,

Table 1: Occurrence of *Listeria* spp. in retail broiler chicken ready to eat meat products in Khartoum State, Sudan

Type of food	No. of samples	*Listeria* spp No. (%)	*L. monocytogenes* No. (%)	*L. ivanovii* No. (%)	*L. grayi* No. (%)	*L. seeligeri* No. (%)	*L. welshimeri* No. (%)
Frozen chicken burger	50	22 (44)	8 (16)	14 (28)	-	-	-
Frozen chicken sausages	50	11 (22)	6 (12)	4 (8)	-	-	1 (2)
Frozen chicken meat balls (kofta)	50	27 (54)	9 (18)	15 (30)	2 (4)	1 (2)	-
Chicken shawerma	50	9 (18)	2 (4)	6 (12)	-	-	1 (2)
Chicken mortedella	50	26 (52)	9 (18)	13 (26)	2 (4)	1 (1)	1 (2)
Total	250	95 (38)	34 (13.6)	52 (20.8)	4 (1.6)	2 (0.8%)	3 (1.2)

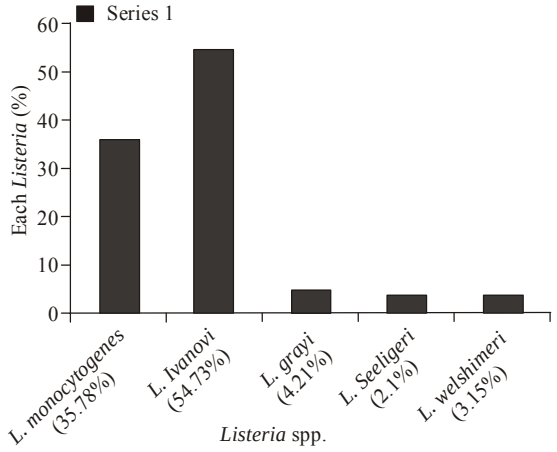

Fig. 1: The isolation percentage of different types of *Listeria* spp., from 95 isolates of *Listeria* spp., from retail broiler chicken ready to eat meat products

Gram stain, oxidase test and catalase test; a total of 95 (38%) suspected *Listeria* spp., were isolated from 250 samples of retail broiler chicken ready to eat meat products. Frozen chicken burger 22 isolates (44%) were detected, frozen chicken sausages 11 isolates (22%), frozen chicken meat balls (kofta) 27 isolates (54%), chicken shawerma 26 isolates (52%) and chicken mortedella 9 isolates (18%) (Table 1).

Confirmation of *L. monocytogenes* from retail broiler chicken ready to eat meat products using the Microbact™ *Listeria* 12L Kit System (Oxoid, MB1128A): Among 250 samples collected from restaurants in Khartoum State and distributed as follows: *L. monocytogenes* (13.6%), *L. ivanovi* (20.8%), *L. grayi* (1.6%), *L. seeligeri* (0.8%) and *L. welshimeri* (1.2%) (Table 1).

Figurer 1 shows the percentage of each types of organism among 95 isolates of Listeria spp. These were as follows: *L. monocytogenes* (35.78%), *L. ivanovi* (54.738%), *L. grayi* (4.21), *L. seeligeri* (2.10%) and *L. welshimeri* (3.15%).

DISCUSSION

The real situation of *listeriosis* in Sudan is unknown, and no information is available on the presence of *L. monocytogenes* in retail broiler chicken ready to eat meat products in Sudan.

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 and Kusumaningrum (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.

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.

Although conventional method of selective culture media are confirmed by the Microbact™ Listeria 12L identification System (Oxoid, MB1128A) were designed to detect the type of *Listeria* spp., Bailey *et al*. (1990) had examined the factors of colonization of broiler chickens with *L. monocytogenes* (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 farm healthy carrier chickens in the processing plant (Genigegorgis *et al*., 1989; Bailey *et al*., 1990).

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, 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% (Wilson, 1995; Bersot *et al*., 2001; Eleftheriadou *et al*., 2002; Soultos *et al*., 2003; Mena *et al*., 2004; Vitas and Garcia-Jalon, 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 spread of *L. monocytogenes* in food processing plants (Jemmi and Stephen, 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 (Bresford *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).

In present study (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. Prevalence of *L. monocytogenes* in retail broiler chicken ready to eat meat products was (13.6%).

The demonstrated highest isolation rate 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 of low presence in processed meat products. The present results are not in agreement 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., 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 (Bresford *et al*., 2001). 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.

L. grayi, *L. seeligeri* and *L. welshimeri* isolates were recovered from raw chickens 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 RTE meat products could be attributed either to improper hygienic practice during processing or to food handlers Fenlon *et al*. (1996). These findings are also reported by Gibbons *et al*. (2006) who found *Listeria* spp., in (34.4%) of raw chicken and RTE meat products, in Trinidad.

CONCLUSION

In conclusion; this study reported the occurrence and distribution of *L. monocytogenes* and other *Listeria* species in retail meat products (frozen chicken burger, frozen chicken sausages, frozen chicken meat balls (kofta), chicken shawerma and chicken mortedella), purchased from restaurants in Khartoum state Sudan. The study recommended that the importance of hygienic conditions described in Sudanese HACCP program should be enforced in order to minimize presence of *L. monocytogenes* in raw dressed broiler chickens during manufacturing, handling and storage process at plant and retail stores level.

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