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First Isolation and Identification of *Listeria monocytogenes* from Fresh Raw Dressed Broiler Chicken in Sudan

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ABSTRACT

Listeria spp. is 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 isolate and identify of *Listeria monocytogenes* from fresh raw dressed broiler chicken in Khartoum State, Sudan. A total of 500 fresh raw dressed broiler chickens were collected from five station chicken abattoir in Khartoum State, one hundred from each station, *Listeria* spp. were isolated by the conventional International Organization for Standardization method. The results showed that out of total 500 samples, 204 (40.8%) were found to be contaminated with *Listeria* species; *L. monocytogenes* (13.6%), *Listeria ivanovii* (19.8%), *Listeria grayi* (4.6%), *Listeria seeligeri* (1%) and *Listeria welshimeri* (2%). The results presented in this study indicate the contamination of fresh raw dressed broiler chicken products with *L. monocytogenes*. And this is the first isolation and identification of *Listeria monocytogenes* from fresh raw dressed broiler chicken in Sudan. These results point out for the importance of hygienic conditions described in Sudanese HACCP program to 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.

Key words: Chicken abattoir, *Listeria* sp., *Listeria monocytogenes,* raw dressed broiler chicken

INTRODUCTION

Listeria spp. is widely distributed in environment. The genus consists of six species i.e., *Listeria monocytogenes, Listeria ivanovii, Listeria seeligeri, Listeria innocua, Listeria welshimeri* and *Listeria grayi,* of which only *L. monocytogenes* is the primary human pathogen although there have been rare reports of illnesses caused by *L. seeligeri* and *L. ivanovii* (Perrin *et al*., 2003; Gasanov *et al*., 2005; Jeyaletchumi *et al*., 2010). In 2009, two newly identified species (*L. marthii* and *L. rocourtiae*) were reported (Graves *et al*., 2010; Leclercq *et al*., 2010). *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; 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. In mammals, *L. monocytogenes* can cause spontaneous abortions and is the cause of circling disease which is a

manifestation of basilar meningitis. Fecal-oral transmission is the probable means by which *Listeria* spp. is spread in animals. *Listeria* spp. can be transmitted directly from animals to humans and has been documented in veterinarians, farmers and abattoir workers. Vertical transmission from mother to neonate occurs transplacentally or through an infected birth canal. The approximate fatality rate is 30% that may increase up to 75% in high risk groups, such as pregnant women, neonates and immune compromised 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; McLauchlin *et al*., 2004). Most cases of listeriosis appear to be foodborne, including those acquired during pregnancy. Different food items can be contaminated by *L. monocytogenes* including raw vegetables, raw milk, soft cheeses, fish, poultry, processed chickens and beef. Approximately 15-70% of chicken hot dogs are reported to be contaminated with *Listeria* species (Posfay-Barbe and Wald, 2009). The important characteristics of *L. monocytogenes* contributing to foodborne transmission are the ability to grow as low as -0.4°C, resist heat, salt, nitrite, acidity, withstand osmotic stress and survive mild preservation treatment measures commonly used to control the growth of organisms in food (Jalali and Abedi, 2007). *L. monocytogenes* is a catalase positive, oxidase negative, facultative anaerobe with slight β-hemolysis on blood agar (Jeyaletchumi *et al*., 2010).

L. monocytogenes may cross-contaminate ready-to-eat (RTE) meat and poultry products during post-processing steps such as slicing, peeling and packaging (Murphy *et al*., 2005). As a facultatively anaerobic and psychrotrophic bacterium, *L. monocytogenes* can grow in vacuum-packaged and cold-stored RTE foods poultry products are widely consumed in Sudan. *L. monocytogenes* easily spreads by direct food contact with contaminated surfaces. 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). 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). The current standard methods for isolation are through enrichment and plating-based reference methods (Jeyaletchumi *et al*., 2010). Most regulatory agencies stipulate that isolation must be capable of detecting one *Listeria* organism per 25 g of food (Jeyaletchumi *et al*., 2010). This can only be achieved through enrichment methods that employ antimicrobial agents to suppress competing microflora, prior to plating onto selective agars and confirmation of cultures (Jeyaletchumi *et al*., 2010). The most commonly used culture reference methods for the detection of *Listeria* in foods are the ISO 11290 standards (ISO, 1996); FDA-BAM method to isolate *Listeria* spp. from dairy products, seafood and vegetables (Hitchins, 2003); USDA Standard method to isolate *Listeria* spp. for meat and poultry products as well as from environmental samples. In all the enrichment methods, other *Listeria* can grow faster while hiding the presence of *L. monocytogenes*. Thus, the use of isolation media that allows the identification of *L. monocytogenes* together with high numbers of other *Listeria* is recommended. Different chromogenic media have been developed to enable identification of pathogenic Listeria spp and *L. monocytogenes* based on enzymes produced by the pathogen and acids produced due to fermentation of sugars (Jeyaletchumi *et al*., 2010). In biochemical methods Listeria species vary in their ability to haemolyse horse or sheep red blood cells and produce acid from L-rhamnose, D-xylose and -methyl-D-mannoside. Conventional methods for the detection and identification of microbial pathogenic agents mainly rely on specific microbiological and biochemical identification (Velusamy *et al*., 2010).

In this study, the standard method for isolation of *L. monocytogenes* comprises selective enrichments (24 to 48 h) and isolation on selective media (48 h), followed by biochemical species-specific identification were examined. The recommended standard methods for isolation of *L. monocytogenes* take five days to confirm a negative result and up to 10 days to confirm a positive result (ISO, 2004). The food safety regulations of most countries required zero tolerance of *L. monocytogenes* in RTE food, especially food produced for specific subgroups of the population that are at risk (Rantsiou *et al*., 2008). Because the lack of surveillance about *L. monocytogenes* outbreaks in fresh raw dressed broiler chicken in Sudan, this research work was conducted to investigate, isolate and identify *L. monocytogenes* using conventional method.

MATERIALS AND METHODS

Samples: This study was conducted during October 2011-May 2012. Five hundred fresh raw dressed broiler chickens samples were used for detection of *L. monocytogenes*. Samples were collected from five stations for chicken abattoirs in Khartoum State. Fresh raw dressed broiler chickens samples were taken from chicken-neck skin. 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*: Fresh raw dressed broiler chickens 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 30°C. 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 37°C. 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 Listeriaceae 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 37°C for 24 h. The following tests were used for confirmation; Gram's staining, motility test, catalase reaction and oxidase test.

Confirmation of *Listeria* spp.: For confirmation, the suspected colonies were streaked on to Tryptone Soya Agar (Oxoid, CM0131) supplemented by 0.6% of Yeast Extract Powder (Oxoid, LP0021) and incubated at 37°C for 24 h and 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 (β-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 fresh raw dressed broiler chicken using conventional method: According to the growth on selective media, Gram stain reaction, oxidase test and catalase test, a total of 204 (40.8%) suspected *Listeria* spp. were isolated from 500 samples of raw fresh broiler chicken. The isolation was distributed between five stations chicken abattoirs in Khartoum State: station one (51%), station two (30%), station three (44%) and station four (42%) and station five (37%) (Table 1).

Confirmation of *L. monocytogenes* from fresh raw dressed broiler chicken using the Microbact™ Listeria 12L Kit System (Oxoid, MB1128A): Among 500 samples in five station chicken abattoirs in Khartoum State the isolated *Listeria* ssp. were distributed as follows:- *L. monocytogenes* (13.6%), *Listeria ivanovii* (19.8%), *Listeria grayi* (4.6%), *Listeria seeligeri* (1%), *Listeria welshimeri* (2%) (Table 1).

Figure 1 shows the percentage of each types of *Listeria* organism among 204 isolates of *Listeria* sp. These were as follows: *L. monocytogenes* (33.33%), *Listeria ivanovii* (48.52.8%), *Listeria grayi* (11.27%), *Listeria seeligeri* (2.45%) and *Listeria welshimeri* (4.90%).

Table 1: Occurrence of *Listeria* spp. in 500 samples of fresh raw dressed broiler in Khartoum, Sudan

		Listeria spp.		*L. monocytogenes*		*L. ivanovii*		*L. grayi*		*L. seeligeri*		*L. welshimeri*	
Location	No. of samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Station (1)	100	51	51	19	19	26	26	3	3	1	1	2	2
Station (2)	100	30	30	10	10	14	14	4	4	1	1	1	1
Station (3)	100	44	44	14	14	22	22	4	4	1	1	3	3
Station (4)	100	42	42	13	13	23	23	3	3	1	1	2	2
Station (5)	100	37	37	12	12	14	14	8	8	1	1	2	2
Total	500	204	40.8	68	13.6	99	19.8	23	4.6	5	1	10	2

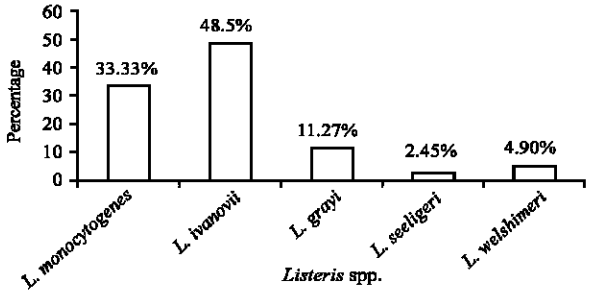

Fig. 1: The isolation percentage of different types of *Listeria* spp. from 204 isolates of *Listeria* spp. from fresh raw dressed broiler

DISCUSSION

Listeria monocytogenes is a gram positive, facultative intracellular pathogen with the capacity to cause food poisoning outbreaks as well as severe illness in vulnerable human population groups. It can cause a rare but serious disease called listeriosis with high fatality rates (20-30%) compared with other foodborne microbial pathogens (FAO/WHO, 2004). 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 in Sudan.

The first step to convince regulatory authorities and private industry about importance of *L. monocytogenes* in raw fresh dressed broiler chickens is to provide data on the isolation and distribution of the bacterium in these foods.

The standard method for isolation and detection of *Listeria* was used during this study confirms the findings of 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, this is because *L. monocytogenes* colonies on ALOA agar exhibited clear halo zone, this involves cleavage of the substrate, L-β-phosphatidylinositol 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.

Bailey *et al.* (1990) 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).

In present 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. Prevalence 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, 2007; 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

(Mena *et al*., 2004; Pesavento *et al*., 2010; Yucel *et al*., 2005) confirmed that *L. monocytogenes* was recovered in high percentages (36.1, 60, 11.5 and 21.6%) from raw chicken in Spain, Portugal, Turkey and Italy, respectively using conventional and serotyping methods.

This study has demonstrated that the highest incidence among *Listeria* spp. was *L. ivanovii* in tested sample (19.8%) followed by *L. grayi* (4.6%), *L. seeligeri* (1%), *L. welshimeri* (2%). This agrees with Osaili *et al*. (2011) who reported that *L. ivanovii* were the predominant isolates 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) 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. The highest numbers of *L. ivanovii* isolates could be attributed either to fecal contamination during evisceration, or to food handlers. *Listeria* spp. is 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 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.

In conclusion, this is the first isolation and identification of *Listeria monocytogenes* from fresh raw dressed broiler chicken in Sudan. These results point out for the importance of hygienic conditions described in Sudanese HACCP program to 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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