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Prevalence of *Listeria monocytogenes* in Poultry Meat, Poultry Meat Products and Other Related Inanimates at Faisalabad

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Abstract: Present study was performed on three hundred and twenty samples of poultry meat and poultry meat products, 40 samples each from fresh poultry meat, fresh chicken boneless, frozen poultry meat, frozen chicken nuggets, frozen chicken burgers, chopping boards, mincing machines and cleaning cloths collected from different poultry meat sale centers, supermarkets and shops at Faisalabad. Listeria species could be isolated from all the examined samples at different percentages ranging from 10 to 37.5%. *Listeria monocytogenes* (*L. monocytogenes)* was differentially identified from other Listeria species by colony characteristics, "beta haemolysis", "cold enrichment procedure", "Anton test", "aesculin test", characteristic "tumbling motility", sugar fermentation tests and serotyping. *L. monocytogenes* could be isolated at variable percentages ranging from 2.5 to 17.5% of the examined samples from poultry meat and poultry meat products. From the 31 isolates of *L. monocytogenes* isolated from examined samples, 23 strains belonged to type 1 and 8 strains belonged to type 4. Study revealed that the incidence of *L. monocytogenes* was much higher in frozen poultry meat products and other related inanimates as compared to fresh poultry meat samples. The public health hazards as well as suggestive measures to reduce human Listeriosis have been discussed.

Key words: Poultry meat, poultry meat products, *L. monocytogenes*

Introduction

L. monocytogenes is present in soil, water, vegetables, and intestinal contents of a variety of birds, fish, insects and other animals. Human listeriosis is a sporadic disease, which is associated with consumption of contaminated milk, soft cheese, under-cooked meat, unwashed raw vegetables and cabbage (Schuchat *et al*., 1992). Meat and meat products have frequently been contaminated with *L. monocytogenes* and may serve as vehicle of other pathogenic organisms. The frequent occurrence of *L. monocytogenes* in meat and salad may pose a potential risk for consumers, particularly for immunocompromised people. In human, the illness may range from mild flu-like sickness to severe manifestations. The severe forms of human listeriosis are present as meningoenceohalitis followed by septic infections and occasionally isolated organ involvement. Groups at highest risk are pregnant women, neonates, adults with underlying disease (cancer, AIDS, diabetes, chronic hepatic disorder, transplant recipients), the elderly (>65 years old) and other immuno-compromised individuals. Death is rare in healthy adults but can occur at a rate as high as 30% in persons at highest risk (Demetrios *et al*., 1996). Because of its ability to survive and proliferate at refrigeration temperature, *L. monocytogenes* may cause disease through frozen foods (Schillinger *et al*., 1991). Due to its ubiquitous character, *L. monocytogenes* easily enters the human food chain and may multiply rapidly (Farber and Peterkin, 1991). The present study was designed to determine the incidence of *L. monocytogenes* in raw and frozen poultry meat and meat products along with chopping board, mincing machine and cleaning cloths.

Materials and Methods

A total of 320 meat and meat products from different poultry shops and supermarkets of Faisalabad were collected for examining the presence of *L. monocytogenes*. Samples from chopping boards, mincing machines and cleaning cloths with chickens slaughter shops were also examined. The samples comprised 40 of each from the fresh poultry meat, fresh boneless, frozen poultry meat, frozen chicken nuggets, frozen chicken burgers, chopping boards, mincing machines and cleaning cloths.

The samples were inoculated/cultured onto blood agar plates containing 0.05% potassium tellurate, which is reported to be inhibitory medium for Gram-negative bacteria and is mainly recommended for isolation of Listeria by Cassiday and Bracket (1989). Blood agar plates with samples were incubated at 37 $^{\circ}$C for 24 hrs. A "cold enrichment" was applied by inoculating the growth in nutrient broth and placing the broth suspension at 4 $^{\circ}$C in refrigerator. Broth was sub-cultured onto blood agar plates once weekly for 10 weeks as reported by Pearson and Marth (1990). This method was adopted to select *L. monocytogenes*, which is able to grow at refrigeration temperature.

Identification of *L. monocytogenes:* Colonies were

examined for their size, color, consistency and shape. Culture on blood agar was noted for presence of haemolysis and type of haemolysis. Smears made on glass slides were Gram-stained adopting Jensen's modification. The stained slides were examined microscopically for shape, arrangement of bacteria and its staining reactions. Organism was cultured onto semisolid media to observe any motility as discussed by Quinn *et al.*,1994. Hanging-drop method was also applied to confirm the type of mortality adopting Quinn *et al.*, 1994. "Aesculin test" to see hydrolyses of Aesculin and "anton test" were performed to confirm the organism following Quinn *et al.*, 1994. For further confirmation of *L. monocytogenes* the isolates were inoculated into 10% aqueous stock solution of Mannitol, L-Rhamnose and D-xylose as described by Collee and Miles (1989).

Serotyping: Rapid slide agglutination technique was adopted using L. *monocytogenes* O antisera types 1,4 and poly (Difco, USA).

Results

Colonies of *L. monocytogenes* were small 1-2 mm in diameter and transparent with smooth borders. Narrow zones of Beta haemolysis, which were almost under the colonies, were seen. Microscopically, these were Gram-positive and oval rods. When cultured on semisolid media, the organism gave umbrella shaped growth. By "hanging-drop method" a typical "tumbling motility" was observed. The organism hydrolyzed aesculin. *L. monocytogenes* fermented L-rhamnose but mannitol and D-xylose were not fermented. As a result of "Anton test" on inoculation of the organism into conjunctiva of rabbits, the organism produced keratoconjunctivitis within 36 hrs. Based on growth on medium under cold enrichment, fresh poultry meat and meat products showed 10-12.5% prevalence of listeria species whereas frozen poultry meat products showed 17.5-25%. In case of inanimates (chopping board, mincing machine and cleaning cloths) prevalence ranged from 30 to 37.5% as shown in Table 1. On the basis of Anton test, aesculin test and sugar fermentation tests *L. monocytogenes* was found in 2.5-5% of fresh poultry meat samples, in 7.5 to 12.5% of frozen poultry meat samples and in 10-17.5% of other inanimate samples as shown in Table 2. Serotyping identified that 7.18% out of 9.68% *L. monocytogenes* belonged to serotype 1 and 2.5% belonged to serotype 4 as shown in Table 3.

Discussion

Results given in Table 1 revealed that Listeria could be isolated from (23.75%) 76 out of 320 samples of poultry meat and its products. The Listeria could be isolated from fresh poultry meat, fresh chicken boneless, frozen poultry meat, frozen chicken nuggets, frozen chicken burgers, chopping board, mincing machine and cleaning cloth at the percentage of 12.5, 10, 17.5, 25, 22.5, 37.5, 35 and 30, respectively.

Table 1: Incidence of Listeria species in poultry meat and poultry meat products

Type of sample	No. of samples examined	Positive samples for Listeria species	
		No.	% age
Fresh poultry meat	40	5	12.5
Fresh poultry boneless	40	4	10
Frozen poultry meat	40	7	17.5
Frozen chicken nugget	40	10	25
Frozen chicken burgers	40	9	22.5
Chopping board	40	15	37.5
Mincing machine	40	14	35
Cleaning cloth	40	12	30
Total	320	76	23.75

Lower incidence of Listeria species in fresh poultry meat was recorded by Gohil *et al.*, 1995), while higher incidence was obtained by Hassouba (1997). At the same time higher incidence of Listeria in frozen meat and frozen sausage were recorded by Elgazzar and Salam (1997).

It was clear that the incidence of Listeria species was higher in frozen meat than in fresh meat. This may be due to the reason that frozen meat and meat products are more liable to be contaminated during their preparation and storage. While fresh poultry meat was slaughtered and quickly offered to the customer thereby decreasing the possibilities of contamination (Wang *et al.*, 1992). Highest incidence of Listeria was noticed with the samples collected from dead stock i.e. chopping board, mincing machine and cleaning cloth, which a chicken sales person keeps in the shop for an indefinite period. Moreover no good methods of sterilization or disinfection are adopted to keep these usables hygienically clean. Therefore, higher incidence of Listeria in chicken nuggets and chicken burgers than fresh poultry meat could be attributed to contamination caused by chopping board, mincing machine, knives, cleaning cloth, other working surfaces and more human contact (Lowry and Tiong, 1985). It is evident from Table 2 that 31 out of 320 samples were found positive for *L. monocytogenes* at the percentage of 9.68. The positive samples revealed 2 of fresh poultry meat, 1 of fresh boneless, 3 of frozen poultry meat, 5 of frozen chicken nuggets, 3 of frozen chicken burgers, 6 of chopping boards, 4 of mincing machine and 7 of cleaning cloths. Fathi and Saad (1992), in which incidence of *L. monocytogenes* in frozen meat products was higher than fresh raw meat, support these results. McClain and Lee (1988); Casolari *et al.* (1994) reported also higher incidences of *L. monocytogenes* in minced meat. Although chopping board, mincing machine and cleaning cloths are not primarily meant to be the host of *L. monocytogenes* but these are important contaminating steps in the chicken processing chain.

Table 2: Incidence of *L. monocytogenes* in poultry meat and poultry meat products with its percent occurrence in Listeria species

Type of sample	No. of samples examined	No. of positive samples for Listeria Species	No. of positive samples for *L. monocytogenes*	% age
Fresh poultry meat	40	5	2	5.00
Fresh poultry boneless	40	4	1	2.50
Frozen poultry meat	40	7	3	7.50
Frozen chicken nugget	40	10	5	12.50
Frozen chicken burgers	40	9	3	7.50
Chopping board	40	15	6	15.00
Mincing machine	40	14	4	10.00
Cleaning cloth	40	12	7	17.50
Total	320	76	31	9.68

Table 3: Serotyping of confirmed isolates of *L. monocytogenes* from poultry meat and meat products by using polyvalent and monovalent antisera type 1 and type 4

Type of sample	No. of samples examined	No. of positive samples for *L. monocytogenes*	Polyvalent type		Monovalent types			
			No.	%	Type 1		Type 4	
					No.	%	No.	%
Fresh poultry meat	40	2	2	5	1	2.5	1	2.5
Fresh poultry boneless	40	1	1	2.5	1	2.5	0	0
Frozen poultry meat	40	3	3	7.5	3	7.5	0	0
Frozen chicken nugget	40	5	5	12.5	4	10	1	2.5
Frozen chicken burgers	40	3	3	7.5	2	5	1	2.5
Chopping board	40	6	6	15	4	10	2	5
Mincing machine	40	4	4	10	3	7.5	1	2.5
Cleaning cloth	40	7	7	17.5	5	12.5	2	5
Total	320	31	31	9.68	23	7.18	8	2.5

Once a positive carcass is processed, the usables would be contaminating all the meat being processed through this chain as stated by Lowry and Tiong (1985). Serological typing of isolated *L. monocytogenes* strains revealed that all isolated strains (31) belonged to *L. monocytogenes*. Further serotyping of the identified strains revealed that 1(2.5%), 1(2.5%), 3(7.5%), 4(10%), 2(5%), 4(10%), 3(7.5%) and 5(12.5%) proved to belong to *L. monocytogenes* type 1 in fresh poultry meat, fresh chicken boneless, frozen poultry meat, frozen chicken nuggets, frozen chicken burgers, chopping board, mincing machine and cleaning cloth respectively. While 1(2.5%), 0(0%), 0(0%), 1(2.5%), 1(2.5%), 2(5%), 1(2.5%) and 2(5%) in fresh poultry meat, fresh chicken boneless, frozen poultry meat, frozen chicken nuggets, frozen chicken burgers, chopping board, mincing machine and cleaning cloth respectively belonged to *L. monocytogenes* type 4 (Table 3).

It was seen that the majority of isolates were serotype 1 with a less proportion of serotype 4 as recorded by Qvist and Liberski (1991) and Sharif and Tunail (1995). However both the serotype 1 and 4 are reported to be pathogenic to man and animals (Donnelly, 1992).

The ability of *L. monocytogenes* to multiply at refrigeration temperatures could be considered of a significance in food intended for consumption without further cooking and foods which have received cooking presumed sufficient to eliminate listeria, but nevertheless intended be received further cooking prior to consumption where the potential competitive microflora has been largely eliminated and thus even low numbers could pose a potential hazard if proper storage conditions are not adhered to. The Listeria in foods which have received minimal or no processing could be considered as a source for cross contamination occurring at the food chain (Schuchat *et al*., 1992).

In 1989, a case of human listeriosis which was contracted through consumption of a poultry product was dead. A cancer patient died after developing listerial meningitis and the source of *L. monocytogenes* was reported as turkey frankfurters (Wanger *et al*., 1990).

In humans, the illness can range from a mild flu like sickness (some times leading to a carrier state) to severe manifestations. The severe forms of human listeriosis present as meningoencephalitis followed by septic infections and occasionally isolated organ involvement. Groups at higher risk are pregnant women,

neonates, adults with underlying diseases like cancer, AIDS, diabetes, chronic hepatic disorder, transplant recipients, old age, and other immunocomporised individuals. Death is rare in healthy adults but can occur at as high as 30% in persons at highest risk (Demetrios *et al*., 1996). In order to minimize human listeriosis, foods should be cooked to an internal temperature of 70 °C for more than 20 minutes to ensure destruction of *L. monocytogenes*. Reheat cooked food thoroughly (70 °C), immediate aseptic packaging of the finished product to avoid post processing environmental contamination. Proper cold storage of meat and meat products (freezing - 18 °C) and proper personal hygiene of food handlers is advisable.

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