LISTERIA MONOCYTOGENES: Another conspicuous food pathongen in marine seafoods.

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Nowadays *Listeria monocytogenes* creates an increasing attention as an agent affecting human health through foodborne infection. Initially it was regarded as a main disease of sheep but during the bruccella irradiation compaign in Britain, listeria infections were reported more frequently in cattle than in sheep (Gitter 1985). The organism gets its specific name to the fact that infection produces monocytosis in the blood. The disease listeriosis is now recognized to have world-wide distribution affecting many species of wild and domestic animals, birds and fishes. It was regarded as a zoonic but transmission from animals to man was not properly established. In nature, *Listeria monocytogenes* is widely distributed in nature and is most commonly found in soil, in marine sediments and in water (Weis 1975) and has ability to survive in the environment (Welshiwer 1960). Natkins and Sleath (1981) reported the clear association of this organism with animals and man and in the guts. However it is not necessarily to find in the feacal materials.

Knowledge of pathogensis and clinical manifestation are very essential requirement for correct diagnosis. Listeriosis, in animals shows a wide variety of clinical manifestations, such as infection of the pregnant uterus with subsequent abortion, still birth, or neonatal death, sepicaemia with miliary lesions mostly in unweaned animals, encephalitis and mastitis etc,. In human beings the clinical manifestations that typically include neonatal sepsis or meningitis, sepsis or meningitis in the immuno depressed patients and puerperal sepsis or 'fluelike' illness during pregnancy. Eventhough the occasional outbreaks were reported the mortality rate is about 30% (Doyle and Scheeni 1986).

Listeria monocytogenes was not generally recognized as foodborne pathogen, but now information available on the occurence of this dreaded organism on three recent food associated outbreaks of listeriosis has increased an awareness, concerned and necessiated for detection in food and environments. Normally we become attentive to a problem when it

culminate in some human tragedy in one way or other. The first incidence occured in Nova Scotia, Canada in 1981 (Schlech *et al* 1983). They found that cole slaw was infected. The infection was traced out to be the cabbage used for the preparation of cole slaw which was heavily infected by this organism from contaminated manure. Several sheep had died of listeriosis prior to addition of this manure to the soil. The second out break was reported during June and August 1983. In this 49 patients in Massachusetts were hospitalised with septicemia or meningitis. The source of contamination was whole or pasteurized milk (Fleming *et al* 1985). The third occurrence reported in Los Angels, and California during 1985. The contaminated source was linked to Mexican-style cheese (James *et al* 1985). *L. monocytogenes* had been also isolated from rice soup, ice creams, lettuce and meat products. But there is no information about the listeriosis outbreak from marine fishes and fishery products. However recently an incidence was reported of this organism in frozen and canned lobster from the processing plants in Prince Edward and New Brunswick (Quality control Tit-Bits No. 3. 1989). This indicates that a wide variety of foods can serve as vehicle for transmission of listeriosis (Conner *et al* 1986). Several surveys on the incidence of *Listeria monocytogenes* in foods not involved in outbreaks of listeriosis have been published, e. g. in raw milk; Fernandez Garayzabal *et al* (1987), soft cheese; Beckers *et al* (1987), raw meat and raw delicatessen; Nicoles *et al* (1987). The incidence of *L. monocytogenes* in these products varied between 4% to 45% (W. Tham & M. L. D. Tham 1988). However it is very sensitive to heat treatment and does not survive in pasteurization process. Freezing also adversely affects *Listeriae* (Larsen 1969). But meat pickling and salting process does not destroy the organism for a definite period (Farchmein 1963, Beganovic *et al* 1971).

To isolate *L. monocytogenes* it is comparatively easier from blood samples, lesions or spinal fluids than from contaminated foods and environs. It has the ability to multiply at 4°C and this undoubtedly played for suitable development for isolation method. This low temperature is used as an effective source of enrichment from the samples contaminated heavily with other microbial flora which may result in failure to isolate *L. monocytogenes* by direct plating. Sometimes the contaminated materials have to incubate for a period upto 12 months to enumerate this organism. Due to this long time enrichment procedure it is not possible to use routine laboratory testing procedure for food and other environment samples. So there is indeed a need for a shortened procedure for isolating *L. monocytogenes*. The methodolgoy consists of using suitable pre-enrichment medium which is very useful to resuscitate and allow multiplication of severely damaged low numbers of organisms before going for selective enrichment medium. The technique used for isolating *Listeriae* includes pre-enrichment in a nutrient medium, at a low temperature and further subcultured in nutrient selectivo medium with incubation at 35°C for 24 to 48 hrs. The above two steps allow the organism to recover and multiply rapidly so as to make indentification easier. Further the enrichment broths are enumerated on a suitable non selective agar medium of tryptose or tryptic soy agar. The method most frequently used for detecting *Listeriae* in food (mainly dairy products) was described by Lovett *et al* 1987 and this method is also

followed by U. S. F. D. A, In this method trypticase soy broth is used for enrichment and incubated at 30°C for 24 hrs and 7 days respectively. Further a loopful of culture is streaked onto Modified Mc-Bridge Agar (Lovett *et al* 1987) plates. The plates are incubated at 35°C for 24 hrs. The colonies of *L. monocytogenes* can be identified by examining the plates by obliquely transmitted light (Henri 1933). Colonies of *L. monocytogenes* can be recognised by characteristic lilac to blue-green or blueish grey colouration. The colonies which are white or pink in colour can be discarded. The periphery of the colony when viewed by reflected light with microscope shows the characteristic green colour.

Smith and Archer (1987) indicated that several selective media including those recommended by U. S. F. D. A. failed to support colony formation of heat injured *L. monocytogenes*. Dominiquez Rodriquez et al (1987), Hayes *et al* (1987) and Hao *et al* (1987) recommended two stage enrichment procedure which increases the isolation of *Listeriae*. The recommended procedure allows primary cold enrichment (4°-5°C for 24-48 hrs or longer as the case may be) in a non selective medium followed by a secondary enrichment in a selective broth incubated at 22°-35°C for 24 to 48 hrs and further plating on a selectlve agar medium plates for isolation. In this method a routine testing for food material like frozen fish and fishery products the cold enrichment period must be kept as short as possible. Otherwise it is time consuming procedure and the products may not be able to export with in the prescribed period.

Morphologically *L. monocytogenes* is Gram positive straight or slightly curved nonsporing rods, appears often in pairs and sometimes elongated filaments may be observed particularly in solid medium at room tempcrature. It is feebly motile at 37°C but in low temperature of 25°C it is very active (characteristic tumbling motility). The colonies are smooth and transparent and become opaque after 24 hrs. In blood agar they produce a narrow zone of haemolysis (Beta hemolytic), gelatin is not liquified. Catalase reaction positive and in glucose and maltose sugar acid is produced without any gas. Lactose and sucrose fermented slowly and not mannitol. Finally *Listeriae monocytogenes* is confirmed by slide agglutination using polyvalent 'O' antiserum Colonies which do not form uniform suspension or which auto agglutinate are not *L. monocytogenes*.

In fish processing plants the source of contamination for *Listeriae* is floors, processing tables, water drains, water hoses, contaminated plastic containers, utensils, nonchlorinated water supply and also transoprted by aerosols such as water vapour and steam from other places to processing environs.

L. monocytogenes is very sensitive and destroyed by the contact of chlorine iodine and other quarternary ammonium, compounds when used in the normal recommended levels.

The following points are to be considered for the effective removal of *Listeriae* contamination in the processing environs and the products.

—The water supplied for the fish processing plants should be adequately chlorinated according to the standards prescribed.

—Removal of all stagnant waters and solids (Fish wastes) from sewage drains

must be carried out properly and frequently.

—Frequent washing and cleaning of floors with recommended levels of chlorinated water.

—Provide adequate hand washing, soap and clean towel (sanitizing) facilities for the plant personnel.

—Effectively screen all the doors, windows and ventilators and keep them closed always so that to eliminate flies, birds, rodents and other insects into the processing plants.

—Clean properly the utensils, grading and processing tables, equipments, plastic containers, contact surfaces and work area of the processing plants.

—Avoid cross contamination of the material.

—Do not allow personnel to enter any area, where unprotected unfinished and finished fishery products are exposed, without proper cleaning and sanitizing their hands and clothes.

Listeria monocytogenes will create a public health problem and therefore should be absent in marine seafoods. The U. S. Food and Drug Administration has specified a zero level of *Listeria monocytogenes* in seafoods.

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