

OCCURRENCE OF *LISTERIA MONOCYTOGENES*
IN DAIRY PRODUCTS
IN SRI LANKA

By

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ABSTRACT

Listeria monocytogenes is a Gram positive rod, facultatively aerobic, non-spore forming, psychrotrophic bacterium which causes the disease listeriosis in mammals, birds, fish and insects. Listeriosis in man has been shown to be of food-borne origin. Dairy products have been suspected the primary vehicle.

The objective of the present study was to determine the occurrence of *Listeria monocytogenes* in local dairy products, to develop a routine test for its virulence testing and to study the antibiotic susceptibility patterns of the food isolates of *Listeria monocytogenes*.

A total of 96 milk samples including raw milk, pasteurized milk and ice cream were examined over a one year period for the presence of *Listeria monocytogenes*. All milk samples were obtained from MILCO industries - Narahenpita, Sri Lanka. The 64 pasteurized milk samples were obtained from different points of processing during the pasteurization.

Isolation of *Listeria monocytogenes* from milk was carried out using modified United States Food & Drug Administration (FDA) enrichment and *Listeria* Selective Agar (LSA)/Oxford agar and nutrient agar.

Differentiation of *Listeria monocytogenes* from the rest of the species in the genus *Listeria* was carried out using biochemical tests and results were compared with reference strain of *Listeria monocytogenes* (ATCC 19111).

The qualitative determination of listeriolysin production of food isolates was carried out by haemolysis on sheep blood agar and Modified CAMP reaction using prepurified cholesterol oxidase from *Rhodococcus equi* (Sigma). Also the presence of haemolysin of the food isolates of *Listeria monocytogenes* in the culture supernatants was tested for.

The antibiotic susceptibility of food isolates of *Listeria monocytogenes* for recommended antibiotics (viz. tetracycline, erythromycin, ampicillin, chloramphenicol, rifampicin and cloxacillin) was tested for, using two techniques (viz. macrodilution technique and microdilution technique) in accordance with National Committee Clinical Laboratory Standard (NCCLS - 1984) guidelines.

Out of the 96 milk samples 18 (18.75%) were positive for *Listeria monocytogenes*. Out of the 20 raw milk samples 5 (25%) were positive for *Listeria monocytogenes*. Out of the 64 pasteurized milk samples 9 (14.06%) were positive for *Listeria monocytogenes*. Out of the 12 ice cream samples 4 (33%) tested positive for *Listeria monocytogenes*.

Listeria monocytogenes was further detected in all sampling points down the processing line during pasteurization; viz. pasteurized vat samples (8.33%), buffer tank samples (16.67%) and the packets/final product (17.87%). Therefore an increase in the percentage of the occurrence of *Listeria monocytogenes* was seen along the processing line.

Haemolysis on sheep blood agar was undetectable except in the isolates A1 (from raw milk), B3 (from ice cream) and H1 (from pasteurized milk) which gave marked zones of β -haemolysis. All isolates gave positive CAMP reactions with *Staphylococcus aureus* (CCM 6188) and negative CAMP reactions with *Rhodococcus equi* (Cor 91/98).

In the Modified CAMP reaction test clear zones of β -haemolysis were detected in all isolates except in B2 (pasteurized milk), F1 (raw milk) and G1 (raw milk) isolates. In the detection of haemolysin in the culture supernatants of food isolates of *Listeria monocytogenes* all tested isolates produced haemolysin in the supernatant except for B2 (pasteurized milk), F1 (raw milk) and G1 (raw milk) isolates. Both the tests used to detect the virulence factor of *Listeria monocytogenes* gave similar results.

All *Listeria monocytogenes* isolates were sensitive to tetracycline, ampicillin, erythromycin, chloramphenicol. The following MIC90's were obtained; tetracycline - 0.8 $\mu\text{g/ml}$,

ampicillin - 0.250 µg/ml, erythromycin - 1.6 µg/ml, chloramphenicol - 6.25 µg/ml, cloxacillin - 0.8 µg/ml, and rifampicin - 0.0156µg/ml. The microdilution method can be recommended as a routine test for the detection of antibiotic susceptibility of *Listeria monocytogenes*.

It was concluded that: *Listeria monocytogenes* is prevalent in milk and milk products of Sri Lanka. The presence of *Listeria monocytogenes* in pasteurized and ice cream can be due to improper pasteurization or post processing contamination. The Modified CAMP reaction test can be used as a routine test in the detection of virulence of *Listeria monocytogenes* isolates. The haemolysin production of the *Listeria monocytogenes* can be further verified by the detection of haemolysin in the culture supernatants. The *Listeria monocytogenes* food isolates of the present study were sensitive to the recommended antibiotics tested for. The microdilution technique can be used in the routine testing of the antibiotic sensitivity of *Listeria monocytogenes*.