D-27: Determination of haemolysin production by food isolates of *Listeria monocytogenes*

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One of the significant characters used in identification of *Listeria monocytogenes* is haemolysis due to production of haemolysin. Most strains of *Listeria monocytogenes* do not show a visible zone of haemolysis and as a result they may be discarded from clinical specimens.

The objective of the present study was to perform an alternative method (Microhaemolysis test) with food isolates of *L.monocytogenes* which did not show a distinguishable zone of haemolysis.

L. monocytogenes strains isolated from chicken, vegetables, and dairy products were subjected to the Microhaemolysis test which was reported from Spain.

Two loopfuls from a 24 h culture of *L. monocytogenes* on sheep blood agar was mixed with 0.5 ml of 1% saline solution. Two fold dilutions of this suspension was made in a microtiter plate with U-form wells in which 50 mm^3 of saline solution had been placed. To each bacterial dilution, 100 mm^3 of a 3% suspension of washed human erthyrocytes (to which 10 ml of a 10% gelatine solution with 0.43% sodium azidel was added. The plates were incubated at 37°C for 6 to 8 h.

Of the 41 isolates of *L. monocytogenes* strains from different types of food products; raw chicken (fresh & frozen), vegetables (green leaves, cabbage & lettuce) and dairy products (hard & soft cheese, fresh cream, raw mild & pasteurized milk) 38 isolates did not show any haemolysis on sheep blood agar although the other biochemical reactions were in accordance with *L. monocytogenes*.

The Microhaemolysis test indicated haemolysis of human red blood cells clearly and based on the Minimal Haemolysis Unit (MHU) which is the reciprocal of the highest dilution at which haemolysis was observed, a clear distinction between *Listeria* species could be obtained.

According to literature the MHU of *L. onocytogenes* ranged between 12 to 96. *L.invanovii* and *L. seeligeri,* having the same sugar acidification patterns, displayed very intense (384 MHU) and very low (3 & 6 MHU) haemolytic activities respectively compared to *L. monocytogenes*.

In this study MMU of isolated *L. monocytogenes* strains ranged from 16 to 64. The mean MHU was 38.85. Control strain of *L.monocytogenes*. (ATCC 19111) gave a haemolytic activity titer of 16 MHU.

This is the first report of quantitative haemolytic test for *L. monocytogenes* isolates obtained in Sri Lanka. The MHU of the tested isolates were within the range of MHU of *L. monocytogenes* strains tested by Dominguez Rodriguez (1986). This test provides a convenient and simple method of confirming *L. monocytogenes* strains which do not show visible haemolysis on sheep blood agar.