JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 895-897

Pathogenicity of *Listeria monocytogenes* Isolated from Human Clinical Cases and Foods of Animal Origin

Shakoor Ahmad Bhat* and Malik Mehboob Willayat

Division of Veterinary Public Health, Faculty of Veterinary Sciences & Animal Husbandry
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir,
Shuhama, Alusteng, Srinagar - 190 006, India.

(Received: 10 February 2011; accepted: 12 March 2011)

The purpose of the present study was to compare the pathogenicity of *Listeria monocytogenes* isolated from human clinical cases and various foods of animal origin (mutton, fish and cheese). The isolates were tested by various *in-vitro* and *in-vivo* pathogenicity tests like hemolysis in 5% sheep blood agar plates, CAMP test, development of kerato-conjunctivits and monocytosis in healthy rabbits. *L. monocytogenes* recovered from human clinical cases produced more prominent narrow zone of beta-hemolysis on blood agar plates compared to the food isolates. The development of kerato-conjunctivits and monocytosis were marked in rabbits with human isolates than those of food isolates confirming the increased pathogenicity of the isolates recovered from humans as compared to the food isolates.

Key words: Pathogenicity, *Listeria monocytogenes,* Monocytosis, Kerato-conjunctivits.

Human and animal listeriosis was first recognised as infection caused by a bacterium in 1920s, subsequently named *Listeria monocytogenes* (Rocourt & Buchrieser, 2007). It is one of the eight species of the genus and the only one that is considered as a major pathogen. The first conclusive link of the organism to be food-borne outbreak in 1981 stimulated research and survey work to determine ubiquity of the organism and its methods of transmission. Listeriosis is a public health problem of low incidence but high mortality, requiring prompt diagonosis and adequate antibiotic therapy (Aureli *et al.*, 2003). Over the last two decades a high number of food-borne listeriosis outbreaks have occured, some with high mortality rates (Ben-Embark, 1994; Waak *et al*., 2002).

* To whom all correspondence should be addressed.
E-mail: bhatshakoor@gmail.com

MATERIAL AND METHODS

Bacteria strains

The strains of *L. monocytogenes* used in the present study were isolated from human clinical cases and various foods of animal origin using a standard procedure (McClain & Lee 1988). The *Listeria* isolates were confirmed by standard biochemical tests like catalase, oxidase, motility at 22°C and acid production from mannitol, rhamnose and xylose, nitrate reduction, urea hydrolysis, gelatin liquification, methyl red test and Voges-Proskauer test as per Cowan & Steel (1993). The CAMP test with *S. aureus* was used to distinguish *L. monocytogenes* from other *Listeria* spp. *L. monocytogenes* isolates identified biochemically were tested for hemolytic activity by blood agar plate method.

EXPERIMENTAL

L. monocytogenes grown in brain heart infusion broth (BHIB) at 22°C for 24 h was inoculated intra-peritoneally in healthy Newzealand white rabbits. Approximately 10^8 bacteria/ml/animal was inoculated as determined by McFarlands opacity standard. Two groups of rabbits were taken and each group consisted of three rabbits (Group-1 and Group-2). Group-1 rabbits were given *L. monocytogenes* infection isolated from humans and Group-2 rabbits were given *L. monocytogenes* infection isolated from various foods. The blood was drawn from the animals prior and following the inoculations using disposable syringes fitted with 22 gauge needles from the marginal ear vein of each rabbit. Blood smears were made at regular intervals of 24 h upto 120 h post inoculation and stained with Giemsa's stain. The slides were then observed under microscope for development of monocytosis.

The isolates from both human clinical cases and various foods of animal origin were also tested for development of kerato-conjunctivitis in the eyes of two healthy Newzealand white rabbits. The rabbits were observed for development of kerato-conjunctivitis upto eight days following instillation.

RESULTS AND DISCUSSION

All the isolates in the present study were tested for their pathogenicity by various *in-vitro* and *in-vivo* tests. The isolates were tested for the degree of beta-hemolysis on 5% sheep blood agar plates. *L. monocytogenes* isolated from human patients produced a prominent zone of beta hemolysis, while the isolates from foods produced only moderate to narrow zones of hemolysis on blood agar plates depicting higher virulence of the human isolates which could be due to passaging of the organism in the definitive hosts resulting in increased virulence. In his findings, Zaidi (1998) reported that a 6th passaged culture of *L. monocytogenes* 7973 resulted in reduced dose of LD_{50} compared to the unpassaged culture. The relationship between hemolysin production and pathogenic property of *Listeria* has been widely investigated. Ivanov *et al.* (1982) described a positive correlation between hemolytic activity and pathogenicity. Portonoy *et al.* (1988) linked the production of hemolysin (*Listeriolysin O*) to the virulence of the organism. The importance of *Listeriolysin O* as a virulence factor has been clearly established (Geoffroy *et al.*, 1987). The spontaneous loss of hemolysin production has been observed to result in the loss of virulence (Hof, 1984).

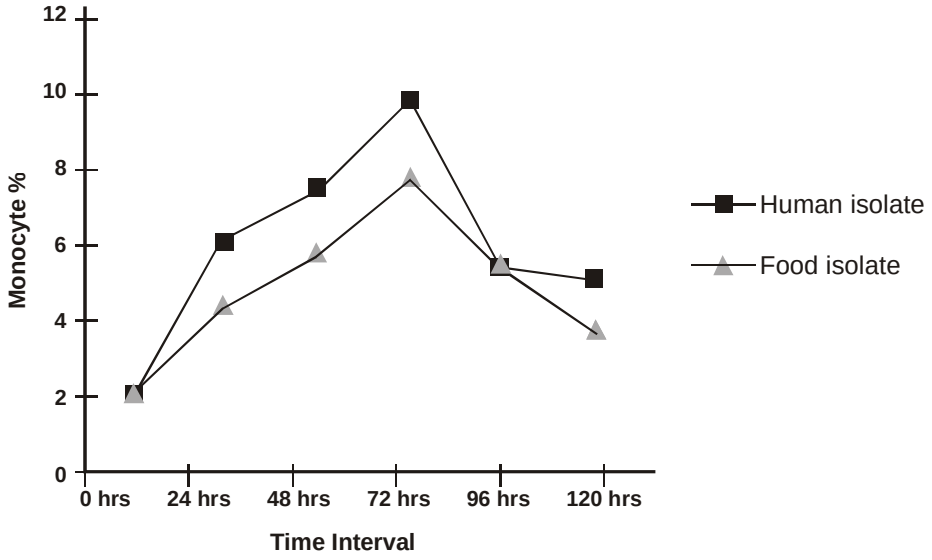

Fig. 1: Time course of percentage monocyte increase and decrease following inoculation of 10^8cells/ml/animal of *L. monocytogenes* isolated from humans and various foods of animal origin

Pathogenic nature of *L. monocytogenes* has been linked to the development of kerato-conjunctivitis in laboratory animals like rabbits following instillation of live culture into the conjunctival sac. In the present study, an 18 h old broth culture produced kerato-conjunctivitis in healthy rabbits within 2-5 days by intra-occular route. However, the human and the food isolates differed in their intensity with respect to the development of kerato-conjunctivits in experimental animals. The human isolates produced severe kerato-conjunctivits starting from 2nd day and becoming more severe on 5th day post-inoculation. The food isolates on the contrary produced only mild to moderate kerato-conjunctivits on 5th day post-inoculation suggesting an increased virulence of the human isolates. Severe kerato-conjunctivitis leading to fatal purulent meningitis in rabbits has been reported by some workers (Seeliger and Finger, 1976).

In the present study, monocytosis was demonstrated in all the rabbits following intra-peritoneal inoculation of live cultures of *L. monocytogenes*. The human isolate produced an increase in the monocyte count from 2% (Pre-inoculation level) to 10% (72 h post-inoculation). However, in case of food isolate the monocyte count increased upto 8% only (Fig 1). Similar observations have been made previously (Radostitis *et al*., 1994). A substance called monocytosis producing agent (MPA) has been found to be responsible for producing monocytosis in these animals (Galesworthy, 1987). Some workers have linked the monocytosis producing factor to virulence on the plea that only the virulent strains of *L. monocytogenes* were able to produce monocytosis in rabbits (Galsworthy *et al*., 1977), while as less virulent/avirulent strains of *Listeria* spp. like *L. ivanovii* failed to produce monocytosis (Hany *et al.*, 1995).

REFRENCES

1. Aureli, P., Ferrini, A.M., Mannoni, V., Hodzic, S., Wedell-Weergaard, C., Oliva, B.. Succeptibility of *Listeria monocytogene*s isolated from foods in Italy to antibiotics. *Int. J. Food Microbiol*., 2003; **83**: 325-330.
2. Ben-Embark, P.K. Presence, detection and groth of *Listeria monocytogenes* in seafoods: a review. *Int. J. Food Microbiol*., 1994; **23**: 17-34.
3. Cowan and Steel. Manual of identification of medical bacteria Ed. Cowan. Cambridge University Press, 1993; pp 317.
4. Galsworthy, S.B. Role of cell surface in the virulence of *Listeria monocytogenes*. *Annales de Institut de Pasteur Microbiologie.*,1987; **138**: 273-276.
5. Galsworthy, S.B., Gurofsky, S.M., Morray, R.G. Purification of monocytosis producing activity from *Listeria monocytogenes*. *Infect. Immunol.*, 1977; **16**: 500-509.
6. Geoffroy, C., Gaillard, J.L., Alouf, J.E., Berche, P. Purification, characterization, and toxicity of the sulfhydryl-activated hemolysin listeriolysin O from *Listeria monocytogenes*. *Infect. Immunol.*, 1987; **55**: 1641-1646.
7. Hany, Omme, Siddique, R., Khan, M.A., Atta-ur-Rehman., Zaidi, S.A.H. 1995. Virulence evaluation of pathogenic and less pathogenic *Listeria* spp. in mice. Poster: In proceedings of XII ISOPOL (1995), Perth. W. Australia.
8. Hof, H. Therapeutics activities of antibiotics in listeriosis. *Infect.*, 1984; **19**: 229-233.
9. Ivanovi, L., Masalski, N., Marsnova, A. Decarboxylase and hemolytic activity if *Listeria*. *Vet. Med. Nauki*., 1982; **19**: 45-50.
10. McClain, D., Lee, W.H. Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. *JAOAC.*, 1988; **71**: 660-664.
11. Portnoy, D.A., Jacks, P.S., Hinrichs, D.J. Role of haemolysis for the intracellular growth of *Listeria monocytogenes. J. Exp. Med.*, 1988; **167**: 1459-1471.
12. Radostitis, O.M., Blood, D.C., Gay, C.C. 1994. Veterinary Medicine. 8th Edition. Bailliere Tindall, London.
13. Rocourt, J., Buchrieser, C. The genus *Listeria* and *Listeria monocytogenes*: phylogenic position, taxonomy and identification. (Ryser E.T and Marth E.H. ed) *Listeria*, listeriosis and food safety New York: Marcel Dekker, 2007; pp. 1-20.
14. Seeliger, H.P.R., Finger, H. Listeriosis. In: *Infectious Diseases of the Foetus and the Newborn Infant* (Remington J.S. and Klein Saunders J, ed). 1976; pp 333-365.
15. Waak, E., Tham, W., Danielsson-Tham, M.L. Prevalence and fingerprinting of *Listeria monocytogene*s isolated from raw whole milk in farm bulk tanks and dairy plants receiving tanks. *Appl. Environ. Microbiol*. 2002; **68**: 3366-3370.
16. Zaidi, S.A.H., Studies on histopathological changes at various stages of experimental listeriosis. Thesis submitted to Department of Microbiology, University of Karachi. 1998; 189.