

Case Report

A fatal case of *Listeria monocytogenes* sepsis in a newborn

Zaklina Cekovska^{1*}, Ana Kaftandzieva¹, Nikola Panovski¹, Milena Petrovska¹, Vesna Stojkova¹, Gordana Jankoska¹ and Aspazija Sofijanova²

¹Institute of Microbiology and Parasitology, Faculty of Medicine, Ss. Cyril and Methodius University, 50 Divisija, 6, 1 000 Skopje, R. of Macedonia

² University Clinics for Child Diseases, University Hospitals, Skopje, R. of Macedonia

Accepted 06 February, 2012

***Listeria monocytogenes* most often causes infection in the neonates, pregnant women, elderly and immunosuppressed persons. We report a case of *Listeria monocytogenes* sepsis, also with signs of endophthalmitis and respiratory tract infection in a newborn. The microorganism was isolated from blood culture, from eye's swab and from tracheal aspirate. The infection was treated with crystalline penicillin and gentamicin, but despite the treatment, the newborn baby boy had developed a severe sepsis and died after 24 hours of life.**

Keywords: *Listeria monocytogenes*, blood culture, sepsis, newborn.

INTRODUCTION

Neonatal sepsis may be categorized as early or late onset. Onset is most rapid in premature neonates. Early onset sepsis syndrome is associated with acquisition of microorganisms from the mother (Liewen MB, 2005). Transplacental infection or an ascending infection from the cervix may be caused by organisms that colonize mother's genitourinary tract, with acquisition of the microorganism by passage through a colonized birth canal at delivery (Liewen MB, 2005; Entis P and Lerner I, 2000). The microorganisms most commonly associated with early-onset infection include group B *Streptococcus* (GBS), *Escherichia coli*, coagulase-negative *Staphylococcus*, *Haemophilus influenzae* and *Listeria monocytogenes*.

Early - onset listeriosis in newborns can manifest either early within the first 2 days after birth. Newborns which are infected in utero generally present with acute sepsis and also have disseminated disease with pustular skin lesions (Liewen MB, 2005; Entis P and Lerner I, 2000; Liu D, 2006). The mortality rate of this type of listeriosis is high. Late-onset listeriosis can manifest clinically usually more than 3 to 5 days of age, with disease appearing 7 to 14 days after birth. *Listeria monocytogenes* is being

found in the brain, liver, blood, lung, kidneys and spleen. The placental tissues usually show evidence of acute chorioamnionitis. Late-onset listeriosis usually presents as neonatal meningitis, with signs and symptoms of fever, irritability and bulging fontanelles (Entis P and Lerner I, 2000; Feil EJ and Spratt BG, 2001; Didelot X and Falush D, 2007).

The aim of this study is to emphasize the importance of regular control of pregnant women, (especially in the third trimester of pregnancy) in order to detect carriage of *Listeria monocytogenes* in the urogenital tract as a potential danger of developing serious life-threatening infections in babies.

Case report

In this case the mother had an uncomplicated pregnancy controlled regularly, until the third trimester when the mother showed signs of infection such as mild flu-symptoms, headache and muscle pain. Symptoms were not serious and personal doctor didn't prescribe any therapy.

A baby boy was born at 38 weeks, with BW = 2300 g, BL = 48 cm, with apgar 8 / 9, in the Department of Gynaecology and Obstetrics in Medical Centre in Veles (60 km far from Skopje). After the spontaneous and regular delivery baby immediately started crying and

*Corresponding Author E-mail: zcekovska@yahoo.com; fax: 00 389 02 3 214 317; Phone: 00 389 02 3 109 829

didn't become blue. The baby did not develop physiological jaundice. But after 24 hours from delivery, the baby was transferred to University Clinic for Child Diseases in Skopje with signs of sepsis. It had been unconscious, febrile, hypotrophic, with signs of bradycardia and tachypnea, the skin and other mucous membranes were cyanotic. Lymph nodes accessible to palpation were not enlarged. BW of admission was 2220 gr. There had been visible infection of purulent blepharoconjunctivitis, characterized as violent acute conjunctival inflammation, great swelling of lids and copious secretion of pus.

Because the boy couldn't breathe properly, he was intubated and set on an artificial ventilation system in an incubator with oxygen mask with O₂ 2.5 L/min. IV fluids with antibiotics (imipenem, gentamicin, amoxicillin) were given as well as additional symptomatic therapy. Throughout the infant's hospitalization it was in severe septic condition with signs of bradycardia, dyspnea and cyanosis. Twenty four hours after admission baby suffered from cardiac arrest and the resuscitation and adrenaline application didn't help. The newborn died from connatal sepsis, connatal bronchopneumonia, acute renal insufficiency and cardiorespiratory insufficiency.

Microbiological investigation

Because there were clinical signs of sepsis present in the newborn, 2 ml blood distributed into Pedi-Bact blood culture bottle was sent for blood culture analysis to the Institute of Microbiology and Parasitology in Skopje. Due to the eye secretions and signs of inflammation of the respiratory tract, eye's swabs and tracheal aspirate were sent also for analysis.

The samples from the swabs were immediately cultured on proper selective and nonselective agar plates and incubated according to standard conditions. Pedi-Bact blood culture bottle (containing supplements that support growth of wide range of different microorganisms), was placed in the Bact/Alert system (BioMérieux), which is designed to rock each bottle during incubation in ambient air at 35°C for period of 5 days or until the results were indicated to be positive.

After 24 h, computer connected to the Bact/Alert system gave positive signal. Gram staining was performed and short Gram-positive bacilli with tendency to palisade (diphtheroid-like) were observed (Figure 1, A).

In the same day (after 24h incubation of the plates at 37°C), from all 5% sheep blood agar plates (from previously cultured eye's swabs and tracheal aspirate) were obtained pure culture of gray-white colonies with subtle β-hemolysis directly beneath the colonies closely resemble to those of group B β-hemolytic streptococci (Figure 1, B). The colonies were catalase reaction positive. Gram staining was also done and the same Gram-positive bacilli were seen.

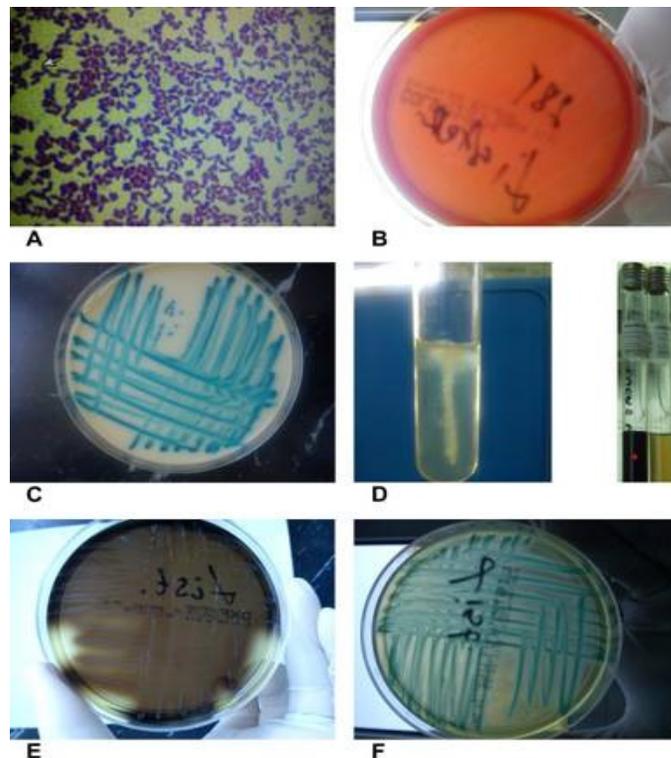


Figure 1. Microbiological findings. (A) Gram stain slide shows Gram positive bacilli. (B) *L. monocytogenes* culture on 5% sheep blood agar plate. (C) *L. monocytogenes* culture on CHROMagar plate. (D) Umbrella like motility and esculin reaction (positive and negative control). (E) Culture on selective agar medium for *Listeria* (Oxford). (F) Culture on selective agar only for *Listeria monocytogenes*.

In the CHROMagar plate, colonies were small and blue coloured resembling to those of *Enterococcus spp* (Figure 1, C). In the selective agar medium for *Listeria* (Oxford), the typical colonies for this microorganism were observed (*Listeria monocytogenes* grows as brown-green colored colonies with a black halo (esculin splitting) (Figure 1, E and F).

The next day series of biochemical reactions from isolated colonies were performed to confirm the diagnosis of *Listeria monocytogenes*, (esculin and Voges-Proskauer reaction were positive - typical for this organism), CAMP test was also positive and umbrella like motility performed in 0,4% agar media incubated in room temperature was seen (Figure 1, D).

The definitive confirmation of *Listeria monocytogenes* by VITEK 2 system (BioMérieux) was done. All biochemical details showed this organism. Analysis time was only 7 hours with probability of 99%. (Confidence was sign with excellent identification).

Antibiotic susceptibility was determined in accordance with the recommendations from the National Committee for Clinical Laboratory Standards using a disc diffusion test. The susceptibility towards investigated antibiotics was as follows: susceptible to penicillin G, ampicillin,

Table.1 Value of laboratory investigations

Laboratory tests	Normal values	Our results
White blood cell count (WBC)	5.5 -17.5 X 10 ⁹ /L	9.1 X 10 ⁹ /L
Red blood cell count (RBC)	2.7 - 4.9 X 10 ¹² /L	3.44 X 10 ¹² /L
Platent count (Plt)	160-550 X 10 ⁹ /L	66 X 10 ⁹ /L
Hematocrit (Hct)	50 - 62%	39.6%
CRP (C reactive protein)	<6 U/L	130 U/L
Blood glucose	(3.6-6.1 mmol/L)	38.9 mmol/L
Urea	(2.5-7.1 mmol/L)	12.4 mmol/L
Creatinine	(62-133 mmol/L)	232 mmol/L
Sodium (Na)	(137-145 mmol/L)	123 mmol/L
Potassium (K)	(3.6-5.0 mmol/L)	6.2 mmol/L
Calcium (Ca)	(2.10-2.55 mmol/L)	1.26 mmol/L
Magnesium (Mg)	(0.7-1.0 mmol/L)	1.1 mmol/L
Phosphate (HPO ₄ ²⁻)	(0.81-1.45 mmol/L)	2.09 mmol/L
Total proteins	(63.0 – 82 g/L)	29 g/L
Albumin	(35-50) g/L	15 g/L
Total bilirubin	0-3 day 34-170 µmol/L 1 month to adult 0-26 µmol/L	62 µmol/L
Aspartate transaminase (AST)	infants 18-74 units/L children 15-46 units/L adults 5-35 units/L	137 U/L
Alanine transaminase (ALT)	0-2 month 8-78 units/L >2 month 8-36 units/L	42 U/L

amoxicillin, amoxicillin clavulanic acid, vancomycin, amikacin, gentamicin, erythromycin, ciprofloxacin and trimetoprim sulphamide, intermediate susceptible to oxacillin and clindamycin and resistant to cephalosporines (ceftriaxon, cefotaxime, cefuroxime, cefixime, cefepime, cefadroxil and cefpodoxime).

Two mounts after delivery, the mother went to regular gynecology control where vaginal swabs were taken. After culturing the swabs in the Institute of Microbiology and Parasitology in Skopje there weren't any signs for *Listeria monocytogenes* growth. The patient was consulted again, and after more detailed re-taken history she provides evidence that she took antibiotic therapy (ampicillin). before tacking recent gynecological swabs. *Listeria monocytogenes* is sensitive to this antibiotic and because of that there was no growth on the microbiological plates. Serology was not performed.

Clinical investigation

The initial clinical signs for septicemia in this infant began to appear within a few hours after birth. It had fever, body temperature of 38 - 39° C, with lower activity and with other signs and symptoms of septicemia.

There were changes in many Laboratory tests. The results are presented in the Table. 1.

Clinical investigation confirmed sepsis in the newborn.

According to performed chest X-ray (Figure 2) there are diffuse bilateral patchy changes of the lungs which in some parts form bigger consolidation mass, also there is subtotal subsequent atelectasis of the middle lobe and retraction of the right hemi-thorax. The detection of the right phrenico-costal sinus is unclear, which does not exclude smaller pleural effusions. Endotracheal tube is seen with the distal end of the Th3 level.

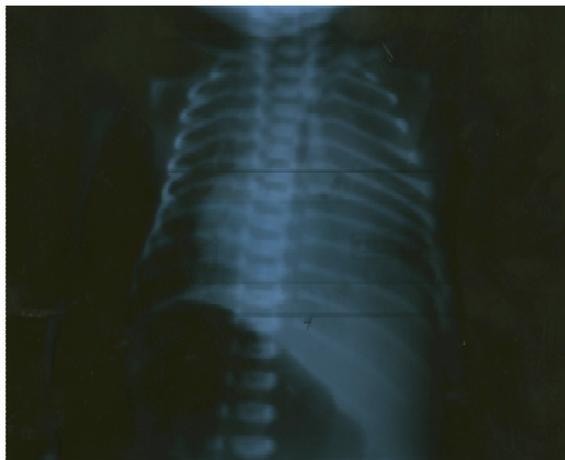


Figure 2. Chest X-ray shows bilateral bronchopneumonia changes of the lungs.

Because of this lung changes and other signs and symptoms, the infant was admitted to the intensive care unit and placed on assisted mechanical ventilation.

DISCUSSION

Listeria monocytogenes was first described by Murray et al., in 1926 (Nightingale KK et al., 2006). This organism is found in water and soil. Vegetables can become contaminated from the soil, and animals can also be carriers. *Listeria* has been found in uncooked meats, uncooked vegetables, unpasteurized milk foods made from unpasteurized milk, and processed foods. *Listeria* is killed by pasteurization and cooking. There is a chance that contamination may occur in ready-to-eat foods such as hot dogs and deli meats because contamination may occur after cooking and before packaging. In most human cases, the mode of infection is unknown, though it probably involves ingestion of infected soil or foodstuffs or contact with infected animals (Liewen MB, 2005; Nightingale KK et al., 2006; Sramova et al., 2000; Gelfant SM, 2007 a,b).

Although asymptomatic carriage of *L. monocytogenes* is observed, most systemic, invasive listerial infections occur in individuals with one or more predisposing conditions. These include pregnancy, corticosteroid therapy, other immunocompromised hosts, and age. Corticosteroid therapy is the most important predisposing factor in nonpregnant patients. Other immunosuppressive medications, organ transplantation and collagen vascular diseases are risk factors for listeria infection. *Listeria* is the most common cause of bacterial meningitis in patients with underlying neoplastic disease, especially lymphoma, in organ transplant recipients, and in those receiving corticosteroids for any reason. (Sramova et al., 2000; Gelfant SM, 2007a,b; Koneman's, 2006; Doumith

M et al., 2005). If the infection spreads to the nervous system it can cause stiff neck, disorientation or convulsions. (A 47 year old man with history of alcoholism and diabetes presents in the Clinic of Infection Disease in Skopje with 2 week old history of convulsions and other CNS signs. From his blood and CSF fluid sample *Listeria monocytogenes* was isolated. But despite the treatment with IV antibiotics - Penicilin G and aminoglycosides, he died after a week of his admission to the clinic).

Pregnant women, especially in the third trimester, are particularly susceptible to *Listeria* and account for up to one-third of reported cases. This bacterium primarily affects the pregnant uterus (abortion or bacteremia and meningitis in the newborns), the central nervous system (meningitis) or the blood system (septicemia) (Liu D, 2006; Feil EJ and Spratt BG, 2001; Sramova et al., 2000). Symptoms of listeriosis in mother may show up 2-30 days after exposure. Symptoms in pregnant women include mild flu-like symptoms, headaches, muscle aches, fever, nausea and vomiting. Our patient had similar symptoms (flu-like symptoms, headaches, muscle pains) several weeks before delivery, but personal gynaecologist hadn't thought for possible infection with *Listeria monocytogenes*. Listeria infection in pregnant women can lead to fetal death, premature birth, or infected newborns (like this case).

Ocular listeriosis is rare, with conjunctivitis being the most frequent manifestation. Keratitis, endophthalmitis and acute chorioretinitis have also been reported. Infective endophthalmitis is a potentially devastating disease that may lead to vision loss (Gelfant SM, 2007a). Exogenous endophthalmitis occurs after ocular surgery or penetrating ocular trauma. Endogenous endophthalmitis results from the hematogenous spread of bacterial infection to the eye. It is a rare entity that accounts for 2 to 8% of all cases of endophthalmitis

(Gelfant SM et al., 2003).

Purulent conjunctivitis is characterized as violent acute conjunctival inflammation, great swelling of lids, copious pus secretion (like our case) and a marked tendency to corneal involvement and even possible loss of the eye.

When listeric meningitis occurs, the overall mortality may reach 70%, from septicemia 50%, and from perinatal/neonatal infections greater than 80%. In infections during pregnancy, the mother usually survives (in this case also).

Interesting is the fact that listeria is a food born infection, and intrauterine fetal transmission occurs. How does it occur?

L. monocytogenes is a facultative intracellular pathogen that is able to invade and survive within mammalian cells, including macrophages and several human tissue culture cell lines (Doumith M et al., 2005; Gombas DE et al., 2003; Pesavento G et al., 2010; Steve H et al., 2009).

The bacterium is phagocytosed by the host cell, using cell adhesion proteins. It escapes the vacuole by secreting a pore-forming toxin, listeriolysin, and the action of phospholipases. Subsequent to its escape from the vacuole, *Listeria monocytogenes* acquires actin based motility, which enables it to form filopods and spread from cell to cell. These mechanisms enable the organism to move directly from cell to cell without exposure to soluble immune factors like antibodies and complement (Steve H et al., 2009; Ebrahim R et al., 2010; Swaminathan B, 2001).

L. monocytogenes can act as a saprophyte or a pathogen, depending on its environment. When this bacterium is present within a host organism, quorum sensing causes the up regulation of several virulence genes (Didelot X and Falush D, 2007; Nightingale KK et al., 2006; Ebrahim R et al., 2010; Swaminathan B, 2001; Thévenot et al., 2006). Depending on the location of the bacterium within the host organism, different activators up regulate the virulence genes; SigB, an alternative sigma factor, up regulates Vir genes in the intestines, whereas PrfA up regulates gene expression when the bacterium is present in blood. Little is known about how this bacterium switches between acting as a saprophyte and a pathogen; however, several noncoding RNAs are thought to be required to induce this change (Swaminathan B, 2001; Thévenot D et al., 2006; Pagotto F et al., 2005; Pagotto et al., 2006; Vasilev V et al., 2009).

L. monocytogenes is susceptible to a wide range of antibiotics but not to cephalosporins. This organism produce enzymes - cephalosporinases which destroy antibiotics of this group (cephalosporins). Nevertheless, since the appearance of the first multiresistant strain of *L. monocytogenes* in France in 1988, occur strains resistant to one or several antibiotics, such as trimethoprim, erythromycin, streptomycin, tetracycline or chloramphenicol, have become more frequent (Vasilev et al., 2009; Lyytikäinen O et al., 2006; Swanson KMJ,

2005; Sakaridis I et al., 2011). The treatment of choice for systemic *L. monocytogenes* infection is the administration of ampicillin or penicillin G combined with an aminoglycoside such as gentamicin (Sakaridis I et al., 2011; Gasanov U et al., 2005). Trimethoprim-sulfamethoxazole alone or in combination with rifampin is considered as an alternative treatment for patients allergic to penicillin (Hofer E et al., 2006; Kaur S et al., 2007; Ramaswamy V et al., 2007).

In our case, beside application a combination of an aminoglycoside such as gentamicin and a beta-lactam such as penicillin (which has been the treatment of choice for early neonatal sepsis in many neonatal Intensive Care Units), sepsis was severe and newborn died after 24 hours. Unfortunately, organism was isolated and confirmed after newborn's death, because the time of incubation necessary is about 24 hours.

It can be concluded that regular control of a pregnant woman, to determine the carriage of certain microorganisms (*Listeria monocytogenes*, GBS, etc) may be crucial in delivering a healthy and normal baby.

ACKNOWLEDGMENTS

We wish to thank the following Institutions: University Clinic for Child Diseases and Clinic of Infection Disease of Faculty of Medicine, for their continued collaboration. We wish to thank also to other colleges from Institute of Microbiology and Parasitology, for their support.

REFERENCES

- Didelot X, Falush D (2007). Inference of bacterial microevolution using multilocus sequence data. *Genetics*. 175:1251–1266.
- Doumith M, Jacquet C, Gerner-Smidt P, Graves LM, Loncaveric S, Mathisen T, Morvan A, Salcedo C, Torpdahl M, Vasquez JA, Martin P (2005). Multicenter validation of a Multiplex PCR assay for differentiating the major *Listeria monocytogenes* serovars 1/2a, 1/2b, 1/2c and 4b: toward an international standard. *J. Food Prot.* 68:2648-2650.
- Ebrahim R, Mehrdad A, Hassan M (2010). "Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran." *Food Control*, vol. 21:1448-1452.
- Entis P, Lerner I (2000). Twenty-four-hour direct presumptive enumeration of *Listeria monocytogenes* in food and environmental samples using the ISO-GRID method with LM-137 agar. *J. Food Protec.* 63:354-363.
- Feil EJ, Spratt BG (2001). Recombination and the population structures of bacterial pathogens. *Annu Rev. Microbiol.* 55:561–590.
- Gasanov U, Hughes D, Hansbro PM (2005). Methods for isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiol. Rev.* 29: 851-875.
- Gelfant SM. Epidemiology and pathogenesis of *Listeria monocytogenes* infection. Uptodate. Com. 2007.
- Gelfant SM. Clinical manifestations and diagnosis of *Listeria monocytogenes* infection. Uptodate. com 2007.
- Gombas DE, Chen Y, Clavero RS, Scott VN (2003). Survey of *Listeria monocytogenes* in ready-to-eat foods. *J. Food Prot.* 66 (4):559-69.
- Hofer E, Reis CMF, Hofer CB (2006). Serovars of *Listeria monocytogenes* and related species isolated from human clinical specimens. *Rev. Soc. Bras. Med. Trop.* 39:32-37.
- Kaur S, Malik SV, Vaidya VM, Barbudde SB (2007). *Listeria monocytogenes* in spontaneous abortions in humans and its

- detection by Multiplex PCR. *J. Appl. Microbiol.* 103:1889-1896.
- Koneman's (2006). *Color atlas and textbook of diagnostic microbiology*. P 766-773.
- Liewen MB (2005). *Listeriosis*. Centers for Disease Control and Prevention. CDC, 2006.
- Liu D (2006). Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important foodborne pathogen. *J. Med. Microbiol.* 55:645-659.
- Lyytikäinen O, Nakari UM, Lukinmaa S, Kela E, Nguyen Tran MN, Siitonen A. (2006). Surveillance of listeriosis in Finland during 1995-2004. *Euro Surveill.* 11:82-85.
- Nightingale KK, Lyles K, Ayodele M, Jalan P, Nielsen R (2006). Novel method to identify source-associated phylogenetic clustering shows that *Listeria monocytogenes* includes niche-adapted clonal groups with distinct ecological preferences. *J. Clin. Microbiol.* 44:3742-3751.
- Pagotto F, Clark Ng LKC, Farber J and the Canadian Public Health Laboratory Network (2006). Canadian Listeriosis Reference Service. *Foodborne Pathogens and Disease*. 3 (1):132-137.
- Pagotto F, Corneau C, Scherf P, Clark LC, Farber JM (2005). Molecular Typing and Differentiation of Foodborne Bacterial Pathogens. In PM Fratamico, AK Bhunia and SL Smith (ed). *Foodborne Pathogens. Microbiology and Molecular Biology*. Caister Academic Press. 51-75.
- Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A (2010). "Prevalence and antibiotic susceptibility of *Listeria spp.* isolated from raw meat and retail foods." *Food Control*. vol. 21:708-713.
- Ramaswamy V, Cresence VM, Rejitha JS, Lekshmi MU, Dharsana KS, Prasad SP, Vijila HM (2007). *Listeria*: review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infec.* 40:4-13.
- Sakaridis I, Soultos N, Iossifidou E, Papa A, Ambrosiadis I, Koidis P (2011). Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated in chicken slaughterhouses in Northern Greece. *J. Food Prot.* 74 (6):1017-21.
- Sramova H, Benes C, Karpiskova R (2000). Listerioses in the Czech Republic and around the world (in Czech). *The Bulletin of Centre of Epidemiology and Microbiology Prague*. 9:363-365.
- Steve H, Imane S, Omar Z, Elias B, Elie B, Nisreen A (2009). "Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products." *Science of the Total Environment*. vol. 407: 4022-4027.
- Swaminathan B (2001). Foodborne pathogenic bacteria: *Listeria monocytogenes*. In: M.P. Doyle, L.R. Beuchat and T.J. Montville. Editors. *Food Microbiology. Fundamentals and Frontiers* (2nd ed.). ASM Press. Washington. DC. 337-352.
- Swanson KMJ (2005). *L. monocytogenes* Challenge Study "How To" Guidelines. *Food Safety Magazine*. June/July 2005.
- Thévenot D, Dernburg A, Vernozy-Rozand C (2006). An updated review of *Listeria monocytogenes* in pork meat industry and its products. *Journal of Applied Microbiology*. 101:7-17.
- Vasilev V, Japheth R, Andorn N (2009). A survey of laboratory-confirmed isolates of invasive listeriosis in Israel, 1997-2007. *Epidemiol. Infect.* 137:577-580.