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REVIEW ON OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FOODS OF ANIMAL ORIGIN AND ITS ANTIBIOTIC SUSCEPTIBILITY PROFILE

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ABSTRACT

Listeriosis is a disease of humans and animals, which is mostly caused by *Listeria monocytogenes*, as the result of zoontic infection and environmental contamination. In the world, this disease is becoming an emerging bacterial disease, with low incidence but high case fatality rate. Animal food products serve as important source of many food pathogens including *Listeria monocytogenes*. The *bacterium* is ubiquitous in the nature and which is rod shaped, Gram-positive, motile and psychotropic and has been isolated from a variety of foods and environmental sources like soil, dust, water, sewage, decaying vegetation, etc. In human, the organism primarily affects elders, pregnant women, newborns and adults with weakened immune systems. *Listeria monocytogenes* has also been assocated with meningoencephalitis, septicaemia, and abortions in animals. However reports as well as information regarding the occurrence, distribution and impact of *L. monocytogenes* and other *Listeria* species is very limited both in the veterinary and public health sectors in Ethiopia. This paper aims to reviews the epidemiology, means of transmission and possible recommendation pertaining to control *L.monocytogenes*. The control of *Listeria* in foods relies largely on a HACCP approach and the establishment of effective critical control points in the food process.

KEYWORDS: *Listeria monocytogenes*; Public health; Veterinary; Foods of animal origin; HACCP.

1. INTRODUCTION

Nowadays, Listeriosis is one of the important emerging bacterial zoonitic diseases that occur in humans from a variety of animals. It arises mainly from the consumption of contaminated food products (Acha *et al*., 2001). The disease in human primarily affects elders, pregnant women, newborns and adults with weakened immune systems. However, rarely, persons without these risk factors can also be affected. Among the different species of the genus *Listeria, L. monocytogenes* has been known to cause listeriosis in humans and animals (Schukken *et al*., 2003 and Pal, 2007). Beside this major veterinary importance animals used for food particularly in cattle, sheep and goats. It causes encephalitis, abortion and septicemia (Dhanashree *et al*., 2003).

Various studies have shown that people at greater risk are pregnant women, foetal children, alcoholics, a patient with corticosteroid therapy and AIDS patients. Infection acquired in early pregnancy may lead to abortion, still birth or premature delivery. When listeriosis is acquired late in pregnancy it can be transmitted transplacentally and lead to neonatal listeriosis (Malik *et al.,* 2002).

In animals, listeriosis usually occurs in five distinct clinical presentations, of which encephalitis is by far the most common form, followed by abortions, whilst neonatal septicemia, mastitis and keratoconjunctivitis/ uveitis occur quite rarely. These syndromes seldom overlap within the same animal or the same flock. Some authors speculate that encephalitis occurs as a distinct syndrome and more frequently than other clinical syndromes in farm ruminants because immunity acquired through ingestion of contaminated silage protects against septicemia and abortion but is not fully effective in protection against encephalitis (Muhammed *et al.,* 2013). Furthermore; ruminants may commonly be asymptomatic intestinal carriers of the organism (Oevermann *et al*., 2010).

The available current literature shows that *L. monocytogenes* and other *Listeria* species have been reported from awide variety of animal origin food items and clinical samples in various countries of the world (Cordano AM, 2001). Published information on the status of food borne listeriosis is very limited both in the veterinary and public health sectors in Ethiopia.

Among the 7 species of the genus *Listeria*: *L.monocytogenes* has been known to cause listeriosis; the other pathogenic species is *L. ivanovii*, which causes abortion in animals. *Listeria* species are ubiquitous in the environment and possess unique physiological characteristics that allow the *bacterium to* growth at refrigeration temperature that differ from most pathogenic food-borne bacteria. The organism can also tolerate a pH between 5.4 and 9.6 (Rocourt J *et al.*, 2001). Numerous reports implicated food types such as milk and milk products, meat and meat products, raw vegetables and sea foods as sources of food borne listeriosis (Farber JM *et al.*, 2000).

Therefore, the objectives of this paper are:-

- ✓ To summarize the epidemiology and means of transmission of *L.monocytogens.*
- ✓ To review effective diagnostic and characterization methods for *L. monocytogen* from food of animal origin.
- ✓ To forward possible recommendations pertaining to its control, reduction of transmission and effective detection methods.

2. CURRENT KNOWLEDGE ON *LISTERIA MONOCYTOGENES*

2.1 History and taxonomy

Listeria monocytogenes has attracted the attention of a diverse group of investigators, including clinicians, food microbiologists, immunologists and medical microbiologists after recognized as a human pathogen since1929, but the route of transmission was unclear until the 1980s when an outbreak in Canada indicated that *L. monocytogenes* was transmitted by food (White, 2010).

Listeria monocytogenes is a member of Genus *Listeria* which is a Gram-positive, short-bacillus and it is a facultative anaerobe, non-spore forming bacterium which measures 0.4µm in width and 1 to 1.5µm in length. The bacterium is motile being flagellated, especially below 33^0C via 1-5 peritrichous flagella (Fig 1) and grows with optimum temperature between $30\text{-}37^0$C and can grow at temperature of 0 to 5^0C (Jennifer *et al.*, 1980).

Figure 1: *Listeria monocytogenes* Scanning EM showing pertirichous Flagella *Adapted from* Todar's Online of Textbook of Bacteriology *(Todar's online, 2003).*

The reason for this broad degree of interest is due, in large part, to the fact that this facultative intracellular pathogen is highly amenable to experimental manipulation and has a broad range of relevant biologic activities ranging from its growth in the environment, infection of many different animal species, and as an important human pathogen (Uyttendaele *et al.*, 2008).

Increasing interest in this organism has resulted from foodborne outbreaks, concerns about food safety and the recognition that foodborne infection may result in self-limited febrile gastroenteritis as well as invasive disease. Separate from its immediate clinical relevance, the study of listeriosis has provided insights into bacterial pathogenesis and the role of cell-mediated immunity in resistance to infection with intracellular pathogens (Portney, 2007).

Taxonomy of the genus *Listeria* has been problematic. *L. monocytogenes* was previously in the family *Corynebacteriaceae* (Stuart and Pease, 1972) but in the 8th edition of Bergey`s Manual of Determinative Bacteriology, *Listeria* along with *Erysipelothrix* and *Caryophanon* were grouped as uncertain affiliation. On the basis of DNA-DNA hybridization, Stuart and Welshimer (1974) suggested a new family *Listeriaceae* to accommodate genera *Listeria* and *Morraya*. Today, the genus *Listeria* belongs to the *Clostridium* sub-branch together with *Staphylococcus, Streptococcus, Lactobacillus* and *Brochothrix. Listeria* includes six species, of which one is divided into two subspecies: *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. grayi and L. ivanovii subsp. ivanovii* and *L. ivanovii* subsp. *londoniensis* (Boerline *et al.*, 1992). Only *L. monocytogenes* causes disease in both animals and humans. However, occasional human infection with *L. ivanovii* and *L. seeligeri* has been reported (Gilot and Content, 2002). *L. ivanovii* is known to cause spontaneous abortions in sheep (Parihar, 2004).

2.2 Prevalence

Listeria species are found worldwide and everywhere, in animals, in foods, in humans, in soil, in the food processing environment, in contact surfaces and also in food containers (Peeler and Bunning, 1994; FDA/ CFSAN, 2003). Until about 1960 it was associated almost exclusively with infections in animals and less frequently in humans. However, in the last 30 years it has been known that it was widely distributed in nature (Vázquez-Boland *et al*., 2001).

L. monocytogenes has been reported several times from raw milk, in different countries; in USA, in 1987 (4.2%), in 1992 (4.1%), in 1997 (4.6%); in Canada, in 1988 (1.3%, and 5.4%), and in 1998 (2.7%); in South Africa, in 1990 (5.2%); in Ireland, in 1992 (4.9%) and in England and Wales (5.1%) (Siegman-Igra *et al.,* 2002). The source of *L. monocytogenes* in raw milk is mostly the gastrointestinal tract of animals and the environment, skin of the teats, in particular shedding of *Listeria* into

milk due to mastitis (O'Donnell, 1995). In cows with mastitis, *L. monocytogenes* may be shed at 10,000-20,000 cells per ml of milk, with the appearance of the milk being normal and there being no inflammation of the affected quarter. So that raw milk collection has the potential of delivering *L.monocytogenes* to the cheese making facility (Bunning *et al.,* 1988).

However, in most African countries, there are a few reports on listeriosis, when compared to the Europe and USA. This could be associated with lack of awareness or lack of diagnostic facilities and limited resources together with the presence of other disease epidemics that claim more priority than listeriosis in developing countries including Ethiopia. However, nowadays there are some reports on prevalence of *L.monocytogenes* in different samples. For example, in Addis Ababa, Ethiopia, study conducted in 2004 showed that over all prevalence of 32.6%.out of the total 316 examined samples (Molla *et al*., 2004). Also, of the samples examined (391) in Addis Ababa in 2010, (26.1%) were found to be positive for *Listeria. L. monocytogenes* was detected in 5.4% of the samples analyzed. It was isolated mainly from raw milk (13%) (Gebretsadik *et al.,* 2010).

In another study, reported that the carriage rate of *Listeria* in animal farms and showed that silage had the maximum prevalence rate of the organism (75%), followed by fodder (43.5%), feed (33.5%), fecal matter (20.5%), sewage (16.6%) and soil (4.8%). It was also isolated from 2-6% of fecal samples from healthy people (Rocurrt *&* Cossart*,* 1997), which shows, humans and animals can be asymptomatic excretors, thus introducing *L. monocytogenes* into the environment (Uyttendael *et al*., 1997).

The incidence of *listeriosis* in humans is very low, in the order of 3-8 cases per million inhabitants per year in the industrialized countries (Johan *et al.,* 2004*).* Most countries within the EU have an annual incidence between 2-10 reported cases per million populations per year (Valk *et al.,* 2005). However, because of its high case fatality rate, *listeriosis* ranks among the most frequent causes of death due to food borne illnesses: it ranks second, after salmonellosis, in the USA and France; and fourth in England and Wales (Opinions, 1999). The number of new illness cases, in comparison with other food borne pathogens, per year per 100,000 population in the EU and the USA, respectively, was reported recently as, *Salmonella* (42 vs. 14.5), *Campylobacter* (48 vs. 20), *Listeria* (48 vs. 20), E. coli (1.3 vs. 0.9), and *Yersinia* (2.4 vs. 3.9) (Gutler, 2006).

2.3 Reservoirs and Risk factors

The common risk factor for *Listeria* infection are food storing time, temperature, type of product, infective dose, immunity, and traditionally accepted consumption of raw foods. The following factors might contribute to the occurrence of high incidence of *listeriosis* in the future (Rohrbach *et al*., 1992); the increased proportion of susceptible people due to age, immuno-compromised diseases or treatment; increased use of cold storage to prolong the shelf life of foods; consuming of raw foods like raw milk or cheese from unpasteurized milk etc, especially which are known to harbor dangerous pathogens (Oliver *et al.*, 2005).

Listeria species are wide spread in nature and live naturally in plants and soil environments. It can grow in a wide range of temperature and pH, and they are relatively resistant to freezing, drying, and high salt concentration (Bhilegaonkar *et al.,* 2001). These adaptabilities enable *Listeria* to grow in refrigerated animal food origins with a pH>4.5 (Bunning et *al.,* 1988).

L. monocytogenes has been recovered from dust, soil, water, sewage, decaying vegetation, at least 42 species of wild and domestic mammals, and 17 avian species, crustaceans, pond trout, ticks, and flies. Among food sources milk and milk products, and uncooked vegetables, fish and shell fish, ready-to-eat meat products, ground beef, and poultry have all been found to contain the organism (Gellin and Broom, 2001). In addition, a human reservoir is suggested by isolation of the organism from human feces at rates ranging from 6% to 16% of the population at any given time.

2.4 Modes of Transmission

L. monocytogenes is transmitted mostly through ingestion of the organism with contaminated food. It was reported that, approximately 99% of human *listerioisis* appear to be food-borne. The contamination sources are animal or human fecal matters. However, animal hides are found to be the most important source of contamination than fecal matter (Jennifer *et al.,* 1980).

The most common route of infection of humans is consumption of foods contaminated by *L. monocytogenes* (Pal, 2007). Inadequately pasteurized milk (or milk contaminated post-pasteurization), soft cheeses, ice cream and other dairy products also are important sources of *L. monocytogenes* (MacDonald *et al.,* 2005; Pal *et al*., 2012a). Milk and milk products are considered as risk food stuffs for *L. monocytogenes* (Pal *et al.,* 2012b). Pregnant women can transmit the infection to their unborn fetuses in uterus (through hematogenous spread) or during birth (Siegman-Igra *et al*., 2002).

Mostly, the source of such major outbreaks was reported to be dairy products (Kerr *et al*., 1993), which were unpasteurized milk, Mexican style soft cheese and Swiss regional type soft cheese (Kerr *et al*., 1993; Molla *et al*., 2004). The outbreak of the Mexican style cheese associated listerial infection, occurred in California that resulted in 142 cases and 48 deaths. As a result of such environmental contaminations and poor sanitary conditions while handling of the food samples, during harvesting, processing, packing, preparing or cooking times; many people could be infected and would have *listeriosis* at any time.

In immuno-competent individual, *listeriosis* may occur mostly after being exposed to higher doses of the organisms. However, exposure of vulnerable individuals to even low doses may end up in acquiring *listeriosis* (Todar's, 2003). The clinical course of infection usually begins about 20 hours after the ingestion of heavily contaminated food in case of gastroenteritis, whereas the incubation period for the invasive illness is around 20 to 30 days or much longer. Similar incubation period have been reported in animals for both gastroenteritis and invasive disease (Vazquez-Boland *et al*., 2001).

2.5 Pathogenesis

Following ingestion of the bacteria with contaminated food, it reaches the digestive tract and start secreting invasin /Internalin (In1A & In1B), which enables the *Listeria* to penetrate the host non-phagocytic cells of the epithelial lining and binds with extracellular domain of E-cadherin (a transmembrane cell to cell adhesion molecule) and then disseminate into circulatory system to cause systemic diseases.

Listeria attaches host cells receptors and are then engulfed and remain as a membrane-bound vesicle, but the bacterium soon escapes into the cytosol (Rocourt Cossart, 1997). After phagocytosis or within parenchymal cells after induced phagocytosis *L. monocytogenes* is capable of multiplying extracellularly and intracellularly, within macrophage, and then spread to hepatocytes of the liver, causing cell disruption. It is internalized by phagocytic and non-phagocytic cells and can deliver antigen to both endogenous and exogenous antigen processing and presentation pathways. It is also able to penetrate the endothelial layer of the placenta and thereby infect the fetus (Todar, 2003). However, this will occur only if the immune system of the individual is compromised. This is because normally the immune system eliminates the organism before it spreads by producing T-lymphocytes.

Since *L. monocytogenes* multiplies intracellularly, the effective host response is cell mediated immunity, which involves both TH1 (CD4+) cells and Tc (CD8+) cells that stimulates production of TNF (Tissue Necrotizing Factor), interferon gamma, macrophage activating factors and a cytotoxic T cell response (Todar, 2003; Opinions 1999). In general, protection against *Listeria* is mediated via lymphokine activation of T cells on macrophages and by interleukin-18 (Vazquez-Boland *et al*., 2001).

Majority of *L. monocytogenes* strains are virulent (Opinion, 1999), by having different peculiar properties. For instance, they are able to multiply at low temperature, so that they multiply and accumulate in such refrigerator stored foods; and also are actively motile by means of pertrichous flagella at room temperature (20-25^{0}C), which is help them to spread in the environment. Surprisingly, they don't synthesize flagella at body temperature. Therefore in this case, the virulence is associated with the ability of the bacteria to move themselves into, within and between host cells, by polymerization of host cells actin (gene product Act A) at one end of the bacterium, that can propel the bacteria through cytoplasm (growing actin tails) and, helps for movement, attachment, penetration and cell to cell spread following phagocytosis and engulfment of such infected cells by adjacent cells.

L. monocytogenes causes both perinatal and adult *listeriosis* in humans. Perinatal human *listeriosis* may cause intrauterine infection resulting in intrauterine sepsis and death before or after delivery. Adults can develop meningo-encephalitis, bactermia and sometimes focal infections.

In pregnant women infection of the fetus is extremely common and can lead to abortion, still birth or delivery of *Listeria* infected infant. Granulomatosis infantiseptica is one of the syndromes, which is associated with feto-maternal or neonatal form of meningitis and is characterized by the presence of pyogranulomatous abscesses disseminated over the body. The neonatal form of infection is reclassified as early- onset of sepsis, which results in premature birth and late- onset meningitis, which is acquired through vaginal transmission.

In animal the disease has Meningoencephalitic and Visceral forms;

Meningoencephalitic form involves neurological signs, dullness and somnolence, drooling, lack of interest in food and mastication, lateral deviation of the head with a tendency to circle. Paralysis then recumbency and death from respiratory failure: Whereas Visceral form: involves abortion with retained placenta, Microabscesses occur throughout the brain, multiple foci of necrosis in the liver, spleen and heart. Placental lesions are characteristic with yellow necrotic foci and multiple granulomas in the fetal liver.Abscess formation in the eye can lead to blindness (Jenkins *et al*., 1964; Kingdom and Sword, 1970; Geoffroy *et al*., 1987).

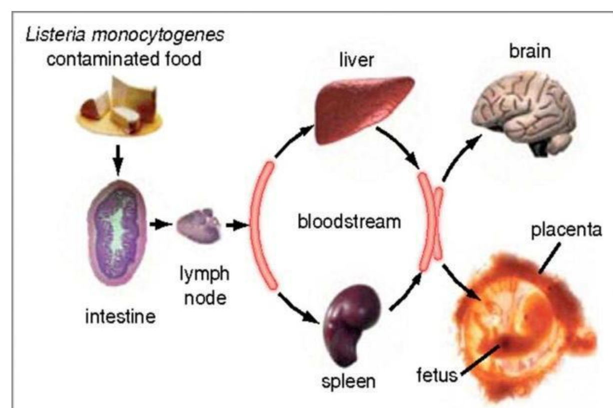

Figure 2: Spreading *Listeria monocytogenes* in the body. Source: Cossart, P. 2011.PNAS 108: 19484-19491.

In food infection, febrile gastroenteritis syndrome is the main clinical manifestation of *L. monocytogenes* infection. The potential entero-pathogenicity of *L. monocytogenes* has also been recognized in animals with outbreak of diarrhea and gastroenteritis having been reported in sheep. Another major clinical manifestation form of *listeriosis*, occurred in animals, is meningoencephalitis also known as a 'circling disease'. It was first described by Gill in 1933 in New Zealand. The syndrome is characterized by involuntary torticollis and walking aimlessly in circles as a result of brain stem lesions. In its severe form, animals lie on the ground with evident signs of uncoordination (paddling movement) and cranial nerve paralysis (strabismus, salviation.) In cows, it causes mastitis (Vazquez-Boland *et al*., 2001; Ryser and Donnelly, 2007).

3. ISOLATION OF *LISTERIA MONOCYTOGENES*

3.1 Conventional cultural method

A number of methods are available for the detection of *Listeria* species. However, culture is the best method, with detection power of about 10^2 CFU/ml. Colonies of *Listeria* species in general are small, smooth, translucent, and bluish gray when viewed in normal light, but blue green sheen is visible by oblique light. The most widely used selective media is OXA (Oxford agar) agar which was developed in 1989. Most literatures recommended to use OXA agar and either one of the following media; PALCAM (Polymixin Acriflavine, Lithium chloride, Ceftazidime Aesculine Mannitol), MOX (Modified Oxford Agar), and LPM (Lithium Chloride Phenylethanol Moxacalatam medium) with esculine and ferric iron (Kiiyukia, 2003). The principle of the media is the ability of esculine hydrolysis by *Listeria* species. Since all *Listeria* species utilize B-D-Glucosidase, they cleaved esculine (esculinase), evidenced by blackening of the medium. In addition, different antimicrobial agents like acriflavin, nalidixic acid, cyclohexamide are supplemented with those media to suppress the growth of other non-*Listeria* species of bacteria and other microbial agents.

The two-stage enrichment method for detection of *L. monocytogenes* with isolation on PALCAM agar and Oxford agar (ISO, 1996) are widely used. The CAMP reaction is useful for identifying *Listeria* species. This test uses horse blood agar and streaks of hemolytic *Staphylococcus aureus* and *Rodococcus equi* in combination with *Listeria* isolates. *L. monocytogenes* and *L. seeligeri* hemolytic reactions are enhanced in the zone influenced by the *S. aureus* streak, while the other species remain non-hemolytic in this zone. In contrast, the hemolytic reaction of *L. ivanovii* is enhanced in the zone influenced by *R. equi* (Hitchins, 2002). For confirmed and specification, different standard biochemical tests can be used. The biochemical test used for identification and confirmation of *Listeria species,* tests can be done by picking pure colonies and transferring into the following biochemical media and broths. These are motility test medium (motility), blood agar (haemolysis), mannitol, rhamnose, galactose, xylose, Hippurate hydrolysis and xylose broths for carbohydrate fermentation testing (James *et al*., 2005).

In general, *L. monocytogenes* is catalase positive, glucose fermenter with acid production but no gas formation, ferment lactose (after 3-5 days of incubation), urease negative, oxidase negative, methyl red and Voges Proskauer positive, indole test negative, express hemolysis which produces clear zone on blood agar (Beta hemolytic), do not utilize citrate, do not produce hydrogen sulfide and also do not hydrolyze urea, gelatin, and casein (Vazquez- Boland *et al.*, 2001; Ryser and Donnelly, 2001).

Table 1: Biochemical chart for *Listeria* species identification.

Species	Production of acid			Hemolysis	CAMP test	
	Xylose	Rhamnose	Mannitol		*S.aureus*	*R.equi*
L. onocytogenes	-	+	-	+	+	-
L .innocua	-	+/-	-	-	-	-
L .ivanovii	+	-	-	++	-	+
L. seeligeri	+	-	-	(+)	(+)	-
L. welshimeri	+	+/-	-	-	-	-
L.grayi subsp.Grayi	-	-	+	-	-	-
L.grayi subsp Murrayi	-	+/-	+	-	-	-

+/-: variable; (+): weak reaction; +: 90% positive reaction; -: no reaction (-). R.equi; *Rhodoccocus equi.*Source: (FDA/CFSAN, 2003; Jemmi and Stephan, 2006).

Hemolysis is an important characteristic which would seem to be directly related to the pathogenicity of *Listeria*, since non–haemolytic *Listeria* species can be considered as non–pathogenic (FAO/WHO/OIE, 2008) and the pathogenicity of the pathogen is highly correlated to the haemolytic factor (Yadav *et al.,* 2010). Considering haemolysis, only *L. monocytogenes* and *L. ivanovii* produced characteristic haemolysin like *listeriolysin–O* (LLO) and ivanolysin–O, respectively.

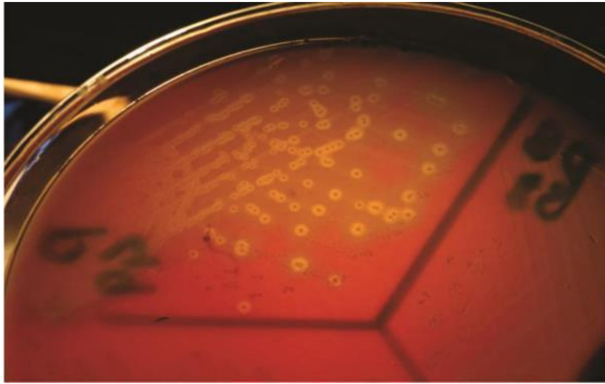

Figure 3: Haemolysin activity of *L. monocytogenes* isolates (FDA/CFSAN, 2003; Jemmi and Stephan, 2006).

In CAMP test, haemolysin acts synergistically with the β– haemolysis of *S. aureus* on sheep erythrocytes and gives synergistic zone of hemolysis towards *S. aureus* which is due to either phosphatidylinositol specific or phosphatidyl–choline specific C from *L. monocytogenes* and sphingomyelinase from *Staphylococcus aureus* (Farber and Peterkin, 1991; Anderson N.W. *et al.,* 2012). Evaluation of the pathogenicity of *Listeria* species by phosphatidylinositol specific phospholipase C (PI– PLC) based assay has been reported to be reliable indicator to discriminate pathogenic and non– pathogenic *Listeria species* (Soni D.K. *et al.,* 2013). Copious workers have been used CAMP test to charecterised *L. monocytogenes* isolates (Tasci *et al*., 2010; Mahmoodi, 2010; Gebretsadik *et al*., 2011).

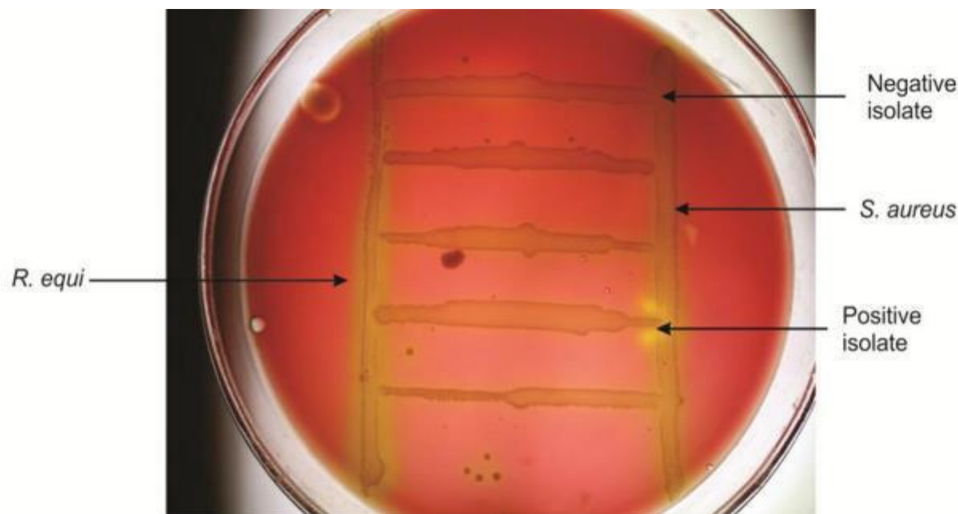

Figure 4: CAMP activity by *L. monocytogenes* isolates. Source: (FDA/CFSAN, 2003; Jemmi and Stephan, 2006).

All species of Listeria produce catalase though some catalase-negative strains of *L. monocytogenes* have been isolated (Stephan R. *et al*., 2011). *L. monocytogenes*, *L. seeligeri* and *L. ivanovii* hemolyse red blood cells on blood agar; *L. monocytogenes* and *L. seeligeri* produce a narrow zone of hemolysis while a wider zone surround colonies of *L. ivanovii*. Sugar fermentation tests are also important in differentiating between them. *L. monocytogenes* is the main human pathogen (Jones *et al*., 2008) even though *L .ivanovii* and *L. seeligeri* has at least once been associated with listeriosis (Cummins *et al.,* 1994).

3.2 Rapid *Listeria* test/Chromogenic Substrate

A more recent development is the production and commercial availability of chromogenic media. Rapid identification of bacterial enzymes is provided by the use of chromogenic substrates, which are incorporated into plating media to allow direct identification of colonies by their characteristic colour. Phosphatidylinositol-specific phospholipase C (PIPL-C) is an enzyme that is produced only by *L. monocytogenes* and *L. ivanovii* and activity of this enzyme is measured using chromogenic media. Many chromogenic media are commercially available and are gradually gaining acceptance by regulatory authorities.

In addition, alternative new chromogenic differential selective agars like BCM, ALOA, CHROM agar *Listeria* and Rapid *L.monocytogenes* can be used in parallel with one of the selective agars (Cox *et al*., 1998). FDA recommended and validated such chromogenic rapid kits with threshold of detection greater than 10^4 CFU/ml of enrichment culture and incubation period of 4-6 hours. The advantage of such tests, are their principle lies on identifying of a specific virulence factors like phosphatidylinositlol specific phospolipase and in enumeration of the organism in the food samples (FDA/CFSAN, 2003).

3.3 Serological methods

Listeria species possess group-specific surface proteins, such as somatic (O) and flagellar (H) antigens that are useful targets for serological detection with corresponding monoclonal and polyclonal antibodies. While there are 15 *Listeria* somatic (O) antigen subtypes (I–XV), flagellar (H) antigens comprise four subtypes (A–D) (Seeliger & Ho¨hne, 1979; Seeliger & Jones, 1986), with the serotypes of individual Listeria strains

being determined by their unique combinations of O and H antigens. Through examination of group-specific *Listeria* O and H antigens in slide agglutination, at least 12 serotypes (i.e. 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e and 7) have been recognized in *L. monocytogenes*, several (e.g. 1/2a, 1/2b, 3b, 4a, 4b, 4c and 6b) in *L. seeligeri,* one (i.e. 5) in *L. ivanovii*, and a few (e.g. 1/2b, 6a and 6b) in *L. innocua*, *L. welshimeri* and *L. grayi* (Seeliger & Jones, 1986; Kathariou, 2002). Since slide agglutination is not easily adapted for high-throughput testing, an ELISA has recently been developed to improve efficiency (Palumbo *et al.,* 2003).

3.4 Molecular methods

Identification of *Listeria spp*. and *L. monocytogenes* using molecular methods is becoming increasingly popular because these techniques are extremely accurate, sensitive and specific. Identification and differentiation of *L. monocytogenes* from other Listeria species to a sub-species level can be performed in the same time frame as ELISA-based assays.

As molecular methods are accurate, sensitive and specific, they are increasingly used in Identification of *L. monocytogenes* from foods. Detection by In vitro amplification of nucleic acid is a more recent addition to the genetic detection methods for pathogen identification and diagnosis. Among several elegant approaches to nucleic acid amplification, PCR was the first and remains the most widely applied technique in both research and clinical laboratories. PCR employs two primers (usually 20–30 nucleotides long) that flank the beginning and end of a specific DNA target, a thermostable DNA polymerase that is capable of synthesizing the specific DNA, and doublestranded DNA to function as a template for DNA polymerase. The PCR process begins at a high temperature (e.g. 94^0C) to denature and open the double-stranded DNA template into single-stranded DNA, followed by a relatively low temperature (e.g. 54^0C) to enable annealing between the single-stranded primer and the singlestranded template, and then a temperature of 72^0C to allow DNA polymerase copying (extension) of the template. The whole process is repeated 25–30 times so that a single copy of DNA template can turn into billions of copies within 3– 4 hrs. As PCR has the ability to selectively amplify specific targets present in low concentrations (theoretically down to a single copy of DNA template), it offers exquisite specificity, unsurpassed sensitivity, rapid turnover, and ease of automation for laboratory detection of *L. monocytogenes* from clinical specimens, in addition to its value for identifying both cultured and non-cultivable organisms. The amplified DNA products can be separated by agarose gel electrophoresis and detected with a DNA stain, or alternatively detected via labelled probes, DNA sequencing, microarray and other related techniques (Wang *et al*., 1993; Manzano *et al*., 2000; Volokhov *et al*., 2002).

Phenotypic methods such as serotyping and phage typing hold certain drawbacks owing to the existence of non-typable strains, and the low discriminative power of such techniques. Therefore, more discriminatory genotypic methods are need. To this end, ribotyping (Swaminathan *et al*., 1996), pulsed-field gel electrophoresis (PFGE) (Kerouanton *et al*., 1998), amplified fragment length polymorphism (AFLP) (Guerra *et al*., 2002), and random amplified polymorphic DNA (RAPD) (Vogel *et al*., 2001) have been developed. PFGE and RAPD-PCR are the techniques most often used to type *L. monocytogenes* strains (Cocolin *et al*., 2005). PFGE is one of the most discriminatory methods, but it is time consuming, the five to seven days are needed before results are available and it requires an expensive apparatus (Franciosa *et al*., 1998). However, the RAPD technique is appropriate for monitoring strains on a wide scale and for determining whole genome diversity (Stephan R *et al*., 2011). A previous report indicated that the genetic diversity of the *inlA* gene might be useful for discrimination among *L. monocytogenes* isolates from foods, animals and environmental samples by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis (Saito *et al*., 1998).

In RFLP analysis, bacterial genomic DNA is digested with restriction enzymes to yield hundreds of fragments, which are then separated by conventional agarose gel electrophoresis to form distinctive banding patterns for individual strains. Given its complex band patterns, however, the interpretation of RFLP results is notably tedious and technically demanding. PFGE uses selected restriction enzymes to yield between 8 and 25 large DNA bands of 40–600 kb in size, and alternating currents to cause DNA fragments to move back and forth, resulting in a higher level of fragment resolution. For this method, *L. monocytogenes* bacteria are first placed in agarose plugs, where they are lysed, and the DNA is then digested with selected restriction enzymes. The plugs containing the digested DNA are transferred into an agarose gel and electrophoresed for 30–50 h with alternating currents. On the basis of distinct DNA band patterns, PFGE classifies *L. monocytogenes* into subtypes (or pulsotypes), providing sensitive subtype discrimination that is considered the reference standard (Brosch *et al*., 1994, 1996; Graves *et al*., 1994). Indeed, after a comparative examination of 35 L. monocytogenes strains by serotyping, esterase typing, ribotyping, RAPD and PFGE, PFGE along with ribotyping produced the most discriminatory outcomes for *L. monocytogenes* (Kerouanton *et al*., 1998). However, due to its time-consuming nature (taking 30 h or longer to perform) and its requirement for special equipment, PFGE is not widely used outside reference laboratories.

3.5 Antimicrobial susceptibility pattern

Antibiotic sensitivity of *L. monocytogenes* isolates to various antibiotics and chemotherapeutic agents was studied by agar disc diffusion method using single antibiotic disc (Bauer *et al*., 1966). The selection of

antibiotic was based on the routinely used antibiotic in field *viz*: Ampicillin, Cephotaxime, Cloxacillin, Erythromycin, Gentamicin, Kanamycin, Nalidixic acid, Norfloxacin, Penicillin–G, Rifampicin, Oxytetracycline and Ceftriaxone. Also some literatures reported that, *L. monocytogenes* is susceptible to wide range of antibiotics like ampicillin, erythromycin, kanamycin, streptomycin, nalidixic acid and others except for cephalosporin and fosomycin/ fluoroquinolones (Abelardo *et al*., 2001; Williams *et al*., 2005: Hansen *et al*., 2005; Aarestrup et al., 2007). However, it has been reported that resistant *L. monocytogenes* strains were found frequently from time to time, even to common conventional drugs like clindamycin, sulfomethoxazole (Shen *et al*., 2006) and also to enrofloxacin (Antunes *et al*., 2002). Plasmid-borne resistance to chloramphenicol, macrolides, and tetracycline has also been identified (FDA/CFSAN, 2003).

Combination (synergy) testing of different antimicrobial drugs against *L. monocytogenes* has also been done, which showed most effective killing power (MacGowan *et al*., 1990). The first choice of therapy is ampicillin combined with different aminoglycosides like gentamicin, TMP/SMX is recommended as the second choice of therapy or as alternative agent. However, other agents are mostly reported as static, and not cidal. On the other hand, the antimicrobial product activity of some aquatic bacteria, were also tested, which showed good inhibitory ability (Molla *et al*., 2004).

4. PREVENTION AND CONTROL

The control of *Listeria* in foods relies largely on a HACCP approach and the establishment of effective critical control points in the process. The careful design and layout of processing equipment in conjunction with the implementation of regular, thorough cleaning regimes of the processing environment can significantly reduce the level of *Listeria* contamination in many processed foods. However, because of its ubiquitous nature it is virtually impossible to totally eliminate the pathogen from many food products. Vulnerable individuals, especially pregnant women, the elders and the immunosuppressed are advised to avoid consuming unpasteurized dairy products to reduce the risk from *listeriosis* (Pal, 2007 and Richard *et al*., 2008).

Preventive measures have to target the organism's nature (conditions for its normal growth) in addition to good sanitation and adequate heat treatment of food before consuming. Moreover, its growth (doubling time) is highly dependent on the temperature, pH, and type of the food sample and background of the microflora present (Morrow *et al.*, 2004). *L. monocytogenes* can survive and multiply within the range of 1°C to 45°C. There are large strain-to-strain variations but some strains seem to be able to grow down to about –1.5°C. The relationship between temperature and *L. monocytogenes* rate of multiplication was studied and reported that the colder the temperature, the slower rate of multiplication was observed (Johan *et al*., 2004).

Several researchers have noted that, different organic acids like acetic acid, lactic acid, citric acid and malic acid play a major role for inhibition of *Listeria monocytogenes* in culture media. For example, acetic acid has more potent anti *listerial* effects than lactic acid/HCl, and causes greater cell destruction when applied in culture media (Doylle, 1999a). Infact it is dependent on the status of the acid whether the acid is total or undissociated. Another good example here is sodium diacetate, which inhibits the growth of *L. monocytogenes* in broth cultures. It was proved that a dip in 5% acetic or lactic acids of some edible foods, not only killed *L. monocytogenes* but prevented its growth during 90 days of storage (Doylle, 1999a) Similarly, it was confirmed that, lactate and diacetate are effective against *L. monocytogenes* contamination in cured ready-to-eat meat products, and also reported about effectiveness of anti-mycotic agents such as benzoate, sorbate, pediocin and propionate in combination with nitrite in inhibiting *listerial* growth in ready-to-eat meat and poultry products (Katla *et al*., 2003). The growth of *L. monocytogenes* can be prevented, or at least reduced, by other means, for example by reducing the pH or by increasing the salt content. (Morrow *et al*., 2004)

5. CONCLUSION AND RECOMMENDATIONS

In general, as tray to review the epidemiology, pathogenesis, means of transmission, diagnosis and control methods, *L. monocytogenes* has gained recognition as a global human pathogen because of its increasing incidence and grow in a wide range of temperature, pH as well as high case fatality rate, emerging, opportunistic, sporadic causes of death in immuno-compromised individuals. Animal origin food products are important vehicles of *L. monocytogenes*, regularly causing outbreaks in different countries of the world. Despite its importance as emerging public health threat world wide, it did not receive due attention and comprehensive studies regarding the prevalence and the current situation of *listeriosis* in cattle, sheep, environment and food of animal origin in Ethiopia.

Based on the above conclusion the following recommendations forwarded:-

- ✓ An extensive survey on the prevalence of *Listeria* species in ready to eat foods in whole of Ethiopia must be undertaken.
- ✓ Consuming foods directly from refrigerator should be avoided unless treating them with sufficient heat (proper cooking).
- ✓ Pregnant woman and immunocompromised individuals should avoid contact with potentially aborted fetuses; they should eat only properly cooked meats and pasteurized dairy products.
- ✓ Serious precautions regarding the food type, storage system and proper cooking should be considered

while handling of food, in order to control *listerial* food contamination.

- ✓ Finally careful design and layout of processing equipment in conjunction with the implementation of regular HACCP approach is nececery.

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