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Review Article

Importance of *Listeria monocytogenes* in food safety: a review of its prevalence, detection, and antibiotic resistance

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Abstract

Listeria monocytogenes, as a foodborne pathogenic bacterium, is considered as major causative agent responsible for serious diseases in both humans and animals. Milk and dairy products are among the main sources of energy supply in the human, therefore contamination of these products with *Listeria* spp., especially *L. monocytogenes*, could lead to life threatening infections in a large population of people. Rapid and accurate detection of *L. monocytogenes* in milk and dairy products, vegetables, meat, poultry, and seafood products is needed to prevent its dissemination through the food chain. Upon contamination of food materials with this pathogen, increase in its antibiotic resistance rate can occur after exposure to preservatives, antibiotics, and stress conditions, which has now become another major public health concern emphasizing the need for special attention on its control along the food chain and management of the disease in the patients. This review provides an overview of researches with respect to the prevalence of *Listeria* spp., especially *L. monocytogenes*, in milk and dairy products, methods of their detection and typing, and current status of resistance rates to the antibiotics used for treatment of listeriosis.

Key words: Antibiotic resistance, Foodborne diseases, Laboratory diagnosis, *Listeria monocytogenes*, Prevalence

Introduction

There are more than 200 known diseases that are transmitted through food and food products. According to annual announcement of World Health Organization (WHO) (2007-2015) each year more than one million people are infected with foodborne pathogens (World Health Organization (WHO) (2007-2015) According to a report of The Centers for Disease Control and Prevention (CDC 1999) in the USA, 76 million people get infected and 5000 die each year due to the foodborne pathogens. Despite the improvements in the production of food stuffs, the risk of foodborne diseases has increased during the last 2 decades. It is estimated that almost one-quarter of the world population is at risk of illnesses through contaminated food products, hence mortality due to foodborne diseases is assumed as the main public health concern (Silk *et al*., 2013).

Foodborne diseases refer to any illnesses resulting from the consumption of contaminated food with bacteria, viruses, parasites, and toxins. According to the World Health Organization (WHO) (2007-2015) guidelines, foodborne diseases account for one-third of deaths among children aged under five, and annually 420,000 people die because of foodborne diseases. There are various agents like bacteria, viruses, and parasites causing foodborne diseases, among which, the bacterial agents are more prevalent. *Listeria* is one of the major causative agents that accounts for serious diseases in humans and animals through the consumption of milk and other dairy products as well as meat, poultry, and ready to-eat products.

About 3% of dietary energy supply of Asian and African people is provided from milk, which is lower than those estimated in European and Oceanian countries. This accounts for providing 6-7% of dietary protein of Asian and African people in comparison with 19% for European countries. Milk and milk products are rich in protein, minerals like calcium, magnesium, selenium, riboflavin, vitamin B5 and B12, which while

essential for growth and function of the human body, especially for pregnant women and children, could also provide necessary growth requirements for the contaminating bacteria, such as *Listeria* spp. and other slow growing bacteria. Milk products are defined as "a product obtained by any processing of milk, which may contain food additives and other ingredients that are functionally necessary for their processing" (Park *et al*., 2013). They contain cheese, ice cream, butter, cream, yogurt, etc., which vary in different regions depending on dietary habits and processing of milk. The consumption of milk and milk products is higher in developed countries than in the developing countries, particularly with the improvement of economic status, urbanization, and changes in diet in more populated developing countries. Although industrial milk products provide good supply for this demand, increase in the tendency for usage of freshly prepared products encouraged people toward the use of domestic products. However, this tendency is linked to an increase in infection risk, especially by pathogens such as *Campylobacter jejuni*, Shigatoxigenic *Escherichia coli* (STEC), *Listeria monocytogenes* and *Salmonella* spp. that are more prevalent in milk and dairy products (Murinda *et al*., 2002; Oliver *et al*., 2005; Asgharzadeh *et al.*, 2010; Naseri *et al.*, 2010).

Survey of antimicrobial resistance in foodborne pathogens, e.g. *Listeria* spp., beyond control of food contamination is useful for prevention of the disease occurrence, which should be done to help better management of the infections. Currently, a combination of ampicillin or amoxicillin with gentamicin is the primary therapy for human listeriosis. However, administering excessive antibiotic in animals' feed could contribute to the antimicrobial resistance of this bacterium, which makes the treatment ineffective (Troxler *et al*., 2000).

In this review, we tried to provide a more comprehensive view on the prevalence of *L. monocytogenes* from various sources, their correlation with occurrence of diseases, and status of antibiotic resistance in order to encounter its importance in control and diagnosis.

Epidemiology and characteristics of *Listeria*

Listeria is a Gram-positive, microaerophilic, non-spore forming, catalase positive and rod-shaped bacteria that is an intrinsic pathogen (Larsen *et al*., 2006; Abdollahzadeh *et al*., 2014; Shamloo *et al*., 2015). *Listeria* was classified in the *Corynebacteriaceae* family according to the 7th edition of Bergey's Manual of Systematic Bacteriology (Parte, 2012), but was placed in a new family, named as *Listeriaceae*, in 2004 (Whitman *et al*., 2012). However, the *16S rRNA* sequence of *Listeria* is closely related to *Lactobacillus* and is usually classified along with *Lactobacillus* and *Erysipelothrix* (Vos *et al*., 2011). The optimum temperature for growth of bacteria is 37°C, but could survive at various temperature conditions (Shamloo *et al*., 2018). Like other Gram-positive bacteria, they have teichoic acid. The genus of *Listeria* is comprised of 17 species, among which six species are more frequent: *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri*, and *Listeria grayi* (Gasanov *et al*., 2005). Among these species, only *L. monocytogenes* causes serious disease in both animals and human. *Listeria monocytogenes* is a foodborne bacterium that can be found in different sources, such as water, soil and different kinds of food products as well as in humans and animals (Rabiey *et al*., 2013). *Listeria monocytogenes* produces soluble listeriolysin O (LLO), a unique cytolysin, which could be activated in low pH and produced under heat stress. The disease produced by *L. monocytogenes* is listeriosis, which is mainly caused by eating contaminated food products (Liu *et al*., 2005). *Listeria monocytogenes* is a major causative agent of foodborne illness worldwide, resulting in a high rate of hospitalization and mortality.

Listeria monocytogenes could be sub-classified in 13 serotypes according to somatic O antigen, all of which could cause listeriosis, but serotype 1.2b, 1.2a, 4b, are more prevalent (Abdollahzadeh *et al*., 2017). Serotypes 4c, 4b, 3b, 1.2a, 1.2b, are mostly isolated from food products regardless of the types of food matrixes, their capacity for pathogenicity, or the geographical regions. Serotype 4b is closely related to *listeriosis* outbreaks (Abdi Moghadam *et al*., 2015). The bacterium has a widespread distribution, ranging from soil, water, food products, meat, vegetables, fish, processed foods, ready-to-eat food and dairy products, as well as in the clinical and healthcare facilities. This is due to ability of this pathogen to survive and grow in dry, cold, and high salt environments. The bacterium can easily grow in different food matrixes kept in the refrigerator (Mahdavi *et al*., 2012). *Listeria* can adapt to the conditions of the gastrointestinal tract by overcoming improper conditions of this microenvironment, including the acidity, osmolality, low oxygen, and the antimicrobial effect of bile salts and peptides. It was indicated that *Listeria* could cause the chronic infection and has a unique ability to survive in different microenvironments of the gastrointestinal tract (Gahan *et al*., 2005). *Listeria monocytogenes* infection is accounted for various syndromes in humans, ranging from mild to severe. It causes abortion or immature birth in pregnancy or septicemia and meningitis in immunocompromised patients, such as those with cancer or leukemia, in adults above 65 years old, or patients with acquired immunedeficiency syndrome (AIDS) and Hodgkin. Moreover, *Listeria* was isolated from faecal samples of 5% of healthy adults. In the gastrointestinal diseases, the most common clinical manifestation is diarrhea, mild fever, nausea, and vomiting. It is estimated that more than 1600 people are infected with *L. monocytogenes* annually that cause about 260 deaths in the USA (Thomas, 2016). The infection shows a seasonal trend, with peaks in mortality from July through October (Sauders *et al*., 2003).

Listeria outbreaks

The incidence of *Listeria* is relatively rare and the annual report of infection with *Listeria* has been decreased from 7.7 cases per one million to 3.1 cases during the period 1990 to 2003 in the USA. Moreover, in Europe, the incidence of infection has declined from 4.5 cases per million to 3.4 cases between 1999 and 2003. Annually, 1600 cases of *Listeria* infection are reported from the USA. One study indicated that the annual incidence of *Listeria* infection in USA is about 1795-1860 cases per 100000 persons (Clark, 2015-2017). Also, the mortality rate for this infection is about 30% in some regions of the United States. In European countries, overall 1760 cases of *listeriosis* were reported in human in 2013 by the European Food Safety Authority (EFSA). On average, 99.1% of the cases needed hospitalization (Control and Prevention, 2011).

According to the CDC's National Center for Zoonotic, Vector-Borne, and Enteric Diseases, listeriosis was added to the list of nationally notifiable diseases in 2001. To improve its surveillance study, the Council of State and Territorial Epidemiologists has recommended that all *L. monocytogenes* isolates be forwarded to state public health laboratories for subtyping through the National Molecular Subtyping Network for Foodborne Disease Surveillance. All states have regulations requiring health care providers to report listeriosis cases, and public health officials should try to interview all infected persons promptly using a standard questionnaire about high risk foods. To reach this goal, FoodNet conducts active laboratory-and population-based surveillance (Davidson *et al*., 1989). Based on these surveillance studies in 2006, USA's public health officials announced that among 884 outbreaks of foodborne pathogens, just one outbreak was due to *Listeria*; however, one year later the CDC reported 122 outbreaks of *Listeria* in the world that reached to 158 outbreaks in 2008. However, these data demonstrated that the incidence of infection due to *Listeria* was decreased (42%) in comparison with 1998 (Chen *et al*., 2006). Currently, most of the outbreaks are contributed via the consumption of dairy products, while the number of outbreaks due to consumption of ready to eat foods has been decreased. Consumption of fruits, vegetables, and ice cream are associated with low to moderate numbers of *Listeria* outbreaks. An outbreak of *Listeria* due to contamination of cantaloupe was reported in 2011. In March 2015, an outbreak of *Listeria* with consumption of ice cream was reported. What was unusual about this outbreak was that three serotypes 1/2b, 3b, and 1/2a of *Listeria*, which are basically related to food and the environment, were reported as responsible bacteria in this outbreak. In addition, low level of contamination was observed in the ice-cream samples, representing a lower infectious dose for them. It should also be considered that the health state of patients has a great impact on infection with *L. monocytogenes*, since the rate of infection is higher in immunocompromised patients (Buchanan, 2017).

Most prevalent serotypes of *Listeria* in foods and environments are 1/2a and 1/2b. However, serotype 4b strains account for 50% of human *Listeria* outbreaks, while serotype 1/2a causes 27% of clinical listeriosis (Burall *et al*., 2017). In European countries, overall 1760 cases of *Listeria* in human were reported in 2013 by EFSA. Recent evidence declared that most of *Listeria* outbreaks were linked with contamination of crustaceans, shellfish, mollusks, meat and meat products, cheese, vegetable, and juice in EU regions (Zhu *et al*., 2017). The highest percentage of contamination in Chinese food industries were *L. monocytogenes* according to a 2 year survey conducted by (Wu *et al*., 2015) with about 20% contamination. They found out that the rate of contamination (Serotypes 1/2a and 3a) in North China was higher than southern area, which is as a result of its psychotropic property. North area has a cold climate whereas the south part is mostly warm (Wu *et al*., 2015). Other outbreaks of listeriosis and responsible serotypes which caused the infection through consumption of contaminated milk and dairy products are summarized in Table 1.

Prevalence of *Listeria monocytogenes* in milk

Fleming *et al*. (1988) in 1985 reported the first report of *L. monocytogenes* in 2% of pasteurized milk in Massachusetts, but before that, Weis (1975) confirmed that *L. monocytogenes* is a causative agent of mastitis in dairy cows, which can lead to contamination of excreted milk. From that time, a series of studies were conducted on *L. monocytogenes* in milk and milk products. The contamination rates were reported in raw milk as high as 45% in Spain, and 12% in the USA (Fenlon *et al*., 1989). A Brazilian researcher conducted a survey from October 1989-1990 on the incidence of *Listeria* spp. in raw and pasteurized milk and found *Listeria* spp. in 12.7% of raw milk samples and about 0.9% of pasteurized milk samples (Moura *et al*., 1993). A number of studies were conducted on the prevalence of *L. monocytogenes* and *Listeria* spp. in milk and dairy products. For example, in 2004, a study was conducted in the Czech Republic, which declared an overall rate of 2.6% contamination with *L. monocytogenes* in milk samples, which was due to contamination of milk with soil prior to pasteurization (Navratilova *et al*., 2004). Another study published in the same year in Northern Ireland was based on a year-long survey conducted on milk processing plant in which researcher found the contamination with *Listeria* spp. in 44.4% of raw milk and 5.6% of pasteurized milk samples. *Listeria monocytogenes* constituted 22% of the raw samples; however, it was not detected in pasteurized milk samples (Kells *et al*., 2004). Dairy farming is the leading agricultural sector of various countries. A survey conducted in Latvia on various farms across the country illustrated that *Listeria* spp. was mostly found in raw milk prepared in a conventional way. They demonstrated that *L. monocytogenes* was mostly isolated from bulk milk from an organic dairy farm, but the prevalence of infection was three times higher in milk samples of conventional dairy farms, 33 versus 211 samples, respectively (Konosonoka *et al*., 2012). People use goat

and sheep milk as an alternative to feeding infants that cannot tolerate cow milk due to having an allergy. Furthermore, the milk of goat and sheep has more nutritional value than cow milk. It was demonstrated that *Listeria* spp. are found in 5.6% of goat milk samples and 3.9% of sheep milk samples, in which 33.3 and 25% of them belonged to *L. monocytogenes*, respectively. Accordingly, the hygienic conditions of milking machines and public health consideration should be employed to avoid the infection (Osman *et al*., 2014). In a study that was conducted in Ethiopia, the prevalence of *Listeria* spp. in raw milk and milk products was 28.4%, where 5.6% of them belonged to *L. monocytogenes*. Interestingly, researchers declared that raw milk had the lowest contamination among other milk products (18.9%), while the prevalence of contamination in pasteurized milk was about 40% (Seyoum *et al*., 2015). Although pasteurization could decrease the likelihood of infection, it cannot completely eliminate the threat, as a report from Finland showed that milk could be contaminated through subsequent stages of pasteurization (Lyytikäinen *et al*., 2000). Their results indicated that the prevalence of *L. monocytogenes* in the bottled raw milk was higher than the fresh bulk tank milk (4.8% versus 1.7%). The prevalence of bacteria in milk filter socks was about 39%. They examined the effect of temperature in growth of *Listeria* and found that keeping the milk at refrigerator temperature could decrease the incidence of infection in milk. The most recent report from Iran was from Isfahan in which they found that the prevalence of *Listeria* spp. in raw milk, ice cream, cream, and porridge was 5.49%, 19.04%, 11.11%, and 4%, respectively. However, they did not detect any *Listeria* spp. in yogurt, butter, kashk, and cheese. According to previous reports from Iran, dominant species of *Listeria* was *L. innocua* and *L. monocytogenes* with a prevalence of 5.44% and 1.36%, respectively (Shamloo *et al*., 2015; Sayevand *et al*., 2018). Different studies from Iran reported that the rate of contamination with *L. monocytogenes* ranged from 1-4%. Interestingly, a recent study did not find *Listeria* spp. in cheese samples. Probably, this is due to the correct manufacturing process of cheese. Diversity in the prevalence of *L. monocytogenes* and its serotypes among different studies on milk samples are summarized in Table 2.

Detection of *Listeria* spp.

The contamination of food and dairy products with *L. monocytogenes* is the major cause of foodborne disease in humans. Since researchers have found that *L. monocytogenes* is a foodborne pathogen, there is a continuous challenge about the isolation of the bacterium from food and other samples (Sutherland, 1997). The primary studies indicated that *L. monocytogenes* is able to grow at the low temperature; accordingly, researchers used this phenomenon for its isolation from clinical samples by culturing for a long time at 4°C. However, this method could not differentiate the injured *Listeria*

Table 1: Foodborne outbreaks caused by *Listeria monocyogenes*

Country	Year	Food type	No. of cases	Deaths	Serotype	References
Maryland, USA	1979	Raw milk	20	3	4b	Ho *et al*. (1986)
England	1981	Dairy products	11	5	1.2a	FDA (2003)
Switzerland	1983-87	Soft cheese	122	33	4b	Büla *et al*. (1995)
USA	1983	Milk	49	14	4b	Fleming *et al*. (1985), Jemmi *et al*. (2006)
Switzerland	1983-87	Soft cheese	122	34	4b	Jemmi *et al*. (2006)
USA	1985	Mexican-type cheese	142	48	4b	Linnan *et al*. (1988)
USA	1985	Soft cheese	142	30	4b	Jemmi *et al*. (2006)
Austria	1986	Unpasteurized milk	28	5		Allerberger *et al*. (1988)
Pennsylvania, USA	1986-87	Ice cream	36	44	4b, 1.2b, 1.2a	Schwartz *et al*. (1989)
Denmark	1989-90	Blue Mold cheese	26	7	4b	Jensen *et al*. (1994)
Wisconsin and Michigan, USA	1994	Chocolate milk	45		1.2b	Dalton *et al*. (1997)
USA	1994	Milk	45	0	1.2b	McLauchlin *et al*. (2004)
France	1995	Soft cheese	17	4	4b	McLauchlin *et al*. (2004)
France	1995	Brie de Meaux cheese	36	4	4b	Vaillant *et al*. (1998)
France	1997		14		4b	Jacquet *et al*. (1999)
Finland	1998-99	Butter	25	6	3a	Lyytikäinen *et al*. (2006)
England	1999	cheese	2		4b	Kimball (2016)
North Carolina, USA	2000	Queso Fresco cheese	12	5	4b	MacDonald *et al*. (2005)
North Carolina, USA	2000	Mexican-style cheese	13	5	4b	Swaminathan *et al*. (2007)
Sweden	2001	Soft cheese	120		1.2a	Carrique-Mas *et al*. (2003)
Japan	2001	Washed-type cheese	38		1.2b	Makino *et al*. (2005)
British Columbia	2002	Cheese	47		4b	Pagotto *et al*. (2006)
Quebec, Canada	2002	Cheese	17	0		Swaminathan *et al*. (2007)
British Columbia	2002	Pasteurized cheese	86		4b	Pagotto *et al*. (2006)
Texas, USA	2003	fresh cheese	13	2		Swaminathan *et al*. (2007)
Texas, USA	2003	Mexican-style cheese			4b	Swaminathan *et al*. (2007)
Texas, USA	2003-07	Queso fresco	74	10		Smith (2012)
Switzerland	2005	Soft cheese	10	3	1.2a	Bille *et al*. (2006)
Texas, USA	2005	Raw milk	12			Control *et al*. (2005)
Switzerland	2005	Soft cheese	3	1		Jemmi *et al*. (2006)
Czech Republic	2006	Cheese	75		1.2a	Vit *et al*. (2007)
Germany	2006	Hard cheese	6	1		Helwigh *et al*. (2007)
Germany	2006-07	Cheese	189	26	4b-1.2a-1.2b	Koch *et al*. (2010)
Norway	2007	Raw milk soft cheese	21	5		Helwigh *et al*. (2009)
Massachusetts, USA	2007	Pasteurized milk	5	3		Control *et al*. (2008)
USA	2008	Mexican-style cheese	8		1.2a	Jackson *et al*. (2011)
Quebec	2008	Cheese	38	2	1.2a	Gilmour *et al*. (2010)

Table 2: Prevalence of *Listeria monocytogenes* isolated from dairy products

Year	Country	No. Sample/ positive	% Isolate L.M	Food type	Serovar L.M	Isolation methods	Identification methods	References
2001	Chile	1014/23	2.2	Cheese-ice cream	1.2a-4b-1.2b	FDA	Biochemical-CAMP	Cordano *et al*. (2001)
2001	European	329/21	6.4	Red smear cheese		IDF	Biochemical-CAMP	Rudolf *et al*. (2001)
2001	Mexico	1300/162	13	Raw milk	1-4b	FDA	Phage typing	Carlos *et al*. (2001)
2002	Sweden	295/58	19.6	Silo raw milk	1.2a	ISO	Biochemical-CAMP	Waak *et al*. (2002)
2002	Switzerland	76271/3722	4.9	Cheese ripening	1.2b-1.2a-4b-3b-1.2c	FDA	Hybridization DNA probe	Pak *et al*. (2002)
2004	Italy	96		Gorgonzola cheese	1.2a	USDA	Biochemical-CAMP	Carminati *et al*. (2004)
2004	Spain	340/23	6.8	Cow milk	1.2a-4b-1.2b	FDA		Vitas (2004)
2004	Spain	202/6	3	Sheep milk	4b-1.2c	FDA		Vitas (2004)
2005	Japan	123/15	12.1	Domestic cheese	1.2b	FDA	PFGE	Makino *et al*. (2005)
2005	Portugal	63/29	46	Soft cheese	4b-1.2b-1.2a	USDA	Phage typing	Pintado *et al*. (2005)
2006	Turkey	157/2	1.27	White cheese		FDA	Biochemical-CAMP	Aygun *et al*. (2006)
2007	Iran	500/8	1.6	Raw milk	4b	ISO	Phage typing	Moshtaghi *et al*. (2007)
2007	Turkey	250/12	4.8	Tulum cheese		FDA	Biochemical-CAMP	Colak *et al*. (2007)
2007	Algiers	237/11	4.64	Raw milk	4b-4d-4e	IMMUNO-ENZYMATIC	Biochemical-CAMP	Hamdi *et al*. (2007)
2008	India	2060/105	5.1	Raw milk		USDA	PCR	Kalorey *et al*. (2008)
2008	Turkey	142/13	9.2	White cheese		FDA	Biochemical-CAMP	Arslan *et al*. (2008)
2008	Brazil	10/6	60	cheese	1.2a	FDA	Biochemical-CAMP	Brito *et al*. (2008)
2009	Lebanon	164/24	14.6	Dairy-based food			PCR	Harakeh *et al*. (2009)
2009	Irish	330/21	6	Irish cheese		ISO/FDA	RT-PCR	O'Brien *et al*. (2009)
2010	Croatia	60/2	3	cheese		ISO	Biochemical-CAMP	Frece *et al*. (2010)
2010	Iran	594/18	3	Raw sheep milk		USDA	PCR	Rahimi, Ebrahim *et al*. (2010)
2010	Turkey	280/7	2.5	Cheese		ISO	Biochemical-CAMP	Kahraman *et al*. (2010)
2011	Mexico	200/18	9	Mexican-fresh cheeses		FDA	Biochemical-CAMP	Torres-Vitela *et al*. (2012)
2011	Turkey	120/34	28.3	Semi-hard cheese		FDA	Biochemical-CAMP CAMP	Guner *et al*. (2011)
2012	Iran	91/4	4.3	Raw milk		USDA	PCR	Shamloo *et al*. (2012)
2012	Jordan	350/39	11	White cheese		ISO	PCR	Osaili *et al*. (2012)
2012	Iran	290/5	1.7	Ice-cream		USDA	PCR	Rahimi *et al*. (2012b)
2013	Iran	446/18	4	Raw milk	1.2a-3a-1.2c-3c-4b	USDA	Biochemical-CAMP	Jamali *et al*. (2013)
2013	Morocco	288/17	5.9	Raw milk-traditional dairy products		ISO	Biochemical-CAMP	El Marnissi *et al*. (2013)
2014		20/12	60	Cheese	1.2b-1.2a	ISO	PFGE	Dalmasso *et al*. (2014)
2014	Iran	18/9	50	Lighvan cheese		ISO	Biochemical-CAMP	Moosavy *et al*. (2014)
2014	Pakistan	400/9	2.2	Raw milk		ISO	Biochemical-CAMP	Usman *et al*. (2016a)
2014		200/46	23	Raw milk-cheese		FDA	PCR	Al-Ashmawy *et al*. (2014)
2015	India	307/50	16.2	Raw milk	1.2a-4b	ISO	Multiplex PCR	Karthikeyan *et al*. (2015)
2015	Iran	292/4	1.36	Traditional dairy products		USDA	PCR	Shamloo *et al*. (2015)
2015	Bangladesh	40/0	0	Dairy products		USDA/FDA	Biochemical-CAMP	Sarker *et al*. (2015)
2015	Egypt	133/3	2.2	Dairy products		ISO	Biochemical-CAMP	Meshref *et al*. (2015)
2015	Iran	100/5	5	Raw milk		FDA	PCR	Mansouri-Najand *et al*. (2015)
2015	Ethiopia	443/25	5.6	Dairy products		FDA	Biochemical-CAMP	Seyoum *et al*. (2015)
2016		210/14	6.6	Dairy products	1.2b-4b-1.2a	ISO	PCR	Kevenk *et al*. (2016)
2016	Nigeria	173/14	8.3	Raw milk		FDA	PCR	Usman *et al*. (2016a)
2016	Italy	8716/145	1.66	Raw milk		ISO	RT-PCR	Dalzini *et al*. (2016)
2016	Egypt	122/11	9	Raw milk-soft cheese		COLD ENRICHMENT	PCR	El-Banna *et al*. (2016)
2016	Nigeria	36/9	25	Milk and milk products		FDA	PCR	Usman *et al*. (2016b)
2016	Turkey	279/44	15.7	Pottery cheese		FDA	Biochemical-CAMP	Kaptan (2016)

FDA: Food and Drug Administration, IDF: International Dairy Federation, ISO: International Organization for Standardization, USDA: United States Department of Agriculture, CAMP test: (Christie-Atkinson-Munch-Peterson), PFGE: Pulsed-field gel electrophoresis, PCR: Polymerase chain reaction, RT: Reverse transcription polymerase chain reaction, and L.M: *Listeria monocytogenes*

cells which cannot survive and grow in stress conditions. The approved methods should enrich the bacteria with a detectable level of 10^4-10^5 colony-forming unit (CFU) in one milliliter of a sample and should detect one *Listeria* in each 25 g of the food sample. *Listeria* grows slowly and can be suppressed by competitors. The administration of bacteriostatic agents, e.g., nalidixic acid and acriflavine, into enrichment media was approved in all standard methods of the isolation (Gasanov *et al*., 2005).

The rate of growth in enrichment media depends on the type of food that *Listeria* is isolated from, the production of bacteriophage, and monocins as an inhibitor of growth and existence of competing flora. Hence, based on the source of bacterium in which *Listeria* is isolated, different media have been administered for the enrichment process. The three most popular enrichment mediums are *Listeria* electrical broth, Fraser media, and University of Vermont broth (UVMII) media. *Listeria* electrical broth is suitable for isolation of *Listeria* from seafood and environment and has the highest specificity (Duarte *et al*., 1999). Administering Fraser media has a lower number of false negative results in comparison with UVMII media that has the highest number of false positive (Loncarevic *et al*., 2008).

Although enrichment culture is a standard method in the food industry, the results are usually available after one week. Two of the most important reference methods for detection of *Listeria* in all food samples are Food and Drug Administration (FDA) bacteriological and analytical methods (BAM) and International Organization of Standard (ISO) 11290 methods (Hitchins, 2001). In FDA-BAM method the enrichment step is carried out in media containing selective bacteriostatic agent (nalidixic acid and acriflavine) along with cycloheximide as an antifungal agent. The temperature for enrichment is 30°C for 48 h. The ISO 11290 is a two-step process (Gasanov *et al*., 2005). The first enrichment in half Fraser broth for 24 h, then a full strength Fraser media is used for further enrichment.

The Fraser media contains the same bacteriostatic agents as in FDA-BAM method. It contains esculin for detecting the β-D-galactosidase activity of *Listeria*. However, using bacteriostatic agents can have an adverse effect on the bacterial population. To avoid this effect, in FDA-BAM method, the agent is added after 4 h of incubation allowing the injured cells to recover and grow in media, whereas in ISO method, the half concentration of agents is added in the first step of enrichment. Table 2 summarized the methods used for isolation of *Listeria* in milk and dairy products (Hitchins, 2001).

Researchers also use other reference enrichment methods for particular food products. For meat, eggs, poultry, and environmental samples the two-step the United States Department of Agriculture (USDA) protocol have been used. In the first step, they used University of Vermont Medium containing both bacteriostatic agents (nalidixic acid and acriflavine) and in the next step, Fraser broth and culture in Modified Oxford agar with moxalactam and colistin sulfate as a selective agent were used (Hau *et al*., 2002).

For dairy products, the AOA/IDF method 990.12 is used, which contains the same bacteriostatic agents in enrichment media and is then cultured on Oxford agar. According to the Nordic committee no. 136 (*L. monocytogenes* detection and enumeration in foods), about 94.9-96.4% of *L. monocytogenes* samples are detected using the first step of ISO 11290 with half Fraser media and if the rate of contamination is very low, the second step could not be omitted (Loncarevic *et al*., 2008).

Selective media

In three reference enrichment methods, it is recommended to use polymyxin-Acriflavine-Lithium-Chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) and Oxford agar as a selective media, although they cannot differentiate pathogenic and nonpathogenic *Listeria* spp. (Aurora *et al*., 2008). Recently, the chromogenic media which is based on essential pathogenic virulence factors of *Listeria* has been administered. Chromogenic media is the most popular culture confirmation method because of its easy preparation and explanation. It enables identification of *L. monocytogenes* after 24 h. The detection of *L. monocytogenes* in *Listeria* agar with Ottaviani and Agosti (ALOA) is based on the detection of phosphatidylinositol-specific phospholipase C (PI-PLC) activity, which in *L. monocytogenes* and some strains of *L. inanovii* hydrolyze the l-α-phosphatidylinositol and produce a fatty acid that forms an opaque halo around the colonies (Greenacre *et al*., 2003). The other media that are similar to ALOA plate include BCM *Listeria* (Biosynth, Switzerland), OCLA (Oxoid, UK), CHROM agar_ *Listeria* (BD e Diagnostic Systems, USA) and OAA (bioMérieux, France). Rapid L'mono_ (Bio-Rad Laboratories, USA), could distinguish between hemolytic and nonhemolytic *Listeria* based on fermentation of xylose. *Listeria ivanovii* could ferment the xylose and produce blue colonies with yellow halo; however, *L. monocytogenes* is non-hemolytic and could not ferment the xylose and produce blue colonies without a halo. Other species of *Listeria* could not cleave the 5-bromo-4-chloro-3-indolyl-myo-inositol-1-phosphate (X-IP), a substrate of PI-PLC and grow with white colonies with or without a yellow halo (Jantzen *et al*., 2006). The CHROM agar could be used for the isolation of *Listeria* from meat products. The BBL CHROM agar is used for isolating *Listeria* from different sources, such as food and environmental samples. Comparison of ALOA agar with Oxford and PALCAM showed that there is no difference in the isolation of *L. monocytogenes* between these three media; however, the rate of isolation of *L. innocua* was ten times higher in ALOA media than other media; moreover, the ALOA media gives a better and higher recovery after 24 h (Loncarevic *et al*., 2008).

Confirmation of bacterial species

Two basic phenotypic and genotypic methods have been carried out following enrichment and primary isolation of *Listeria* from food and environmental samples.

1. Phenotypic assays

Immunoassay tests

There are different kinds of immunoassay tests which are based on natural binding of different antibodies (e.g., monoclonal, polyclonal, and recombinant antibodies) to the specific antigen on the surface of bacteria. To increase the rate of detection, pre-enrichment is needed in order to eliminate the background flora noise and low cell count (Gasanov *et al*., 2005). Most of the immunoassay techniques in *Listeria* are used for detecting some structural components, such as flagella, LLO toxin, and protein p60. These techniques include enzyme-linked-immunosorbent assays (ELISA), Sandwich ELISA, competitive ELISA, fluorescently labeled ELISA, and latex agglutination assay, and enzyme-linked immunofluorescent assay (ELFA). All of these techniques need high amounts of samples and are expensive to carry out since they require specialized equipment (Gasanov *et al*., 2005).

Biochemical and culture methods

Biochemical methods are used for the confirmation of *Listeria* colony isolates from selective culture media. These methods could be based on their ability to hemolyse sheep or horse RBC or make the acidic environment via the break down of D-xylose, L-rhamnose, K-methyl-D-mannoside, and D-mannitol. The Christie-Atkins-Munch-Petersen (CAMP) test can be used for differentiation of hemolytic species of *Listeria* in which the suspected bacterium is cultured horizontally between streaks of *Staphylococous aureus* and *Rhodococcus equi* on blood agar (Gasanov *et al*., 2005). For instance, *L. inanovii* enlarge the area of hemolysis produced by *R. equi*, but it was shown that the CAMP test cannot differentiate between *L. monocytogenes* and *L. ivanovii*. So, administrating the commercial β-lysine discs is recommended in USDA method. Also, fermentation of different sugars could be used for identification of non-hemolytic species. Although biochemical methods are useful for identification of bacteria, they have some false and ambiguous results.

2. Molecular assays

Phenotypes, enzymatic activities, and general properties of bacteria that are used by biochemical tests for their detection may be changed by external

conditions, such as growth phase and mutations of responsible genes. Recent advances in genetic and molecular methods, which enable us to target unique genes that are not be affected by natural variations in each species of bacteria, diminished this weakness and increased accuracy of the diagnosis (Liu *et al*., 2003). Identification of *L. monocytogenes* using molecular methods has now become very popular, because these techniques are sorely sensitive, accurate and specific. DNA hybridization and polymerase chain reaction (PCR) are among these used techniques.

Nucleic acid amplification

The *inlB* gene is 100% specific for *L. monocytogenes* that could detect the very low amount of bacteria without prior enrichment process (Aznar *et al*., 2002). In addition, the group of internalins (*inlA*, *B*, *C*) could be amplified using multiplex PCR method that increases the sensitivity (Abdollahzadeh *et al*., 2016b). The most popular virulence factor genes which are amplified using PCR are *hlyA* gene (LLO), *iap* gene (Invasion-Associated Protein), *inl* gene (internalins), *prfA* (regulator protein for activation of virulence cluster), and *16S rRNA*. The detection of two or more virulence factors in *Listeria* could give a precise result. Detection of *Listeria* and rapid discrimination of its isolates at strain level could be done using Multi-Locus Single Strand Conformation Polymorphism (MLSSCP), after amplification of four polymorphic virulence genes (Takahashi *et al*., 2007).

The amplification methods have some limitations; they cannot distinguish between viable and inactivated *Listeria* as well as some background components. The existence of phenol, aldehydes in smoked fish, hemoglobin in blood or protease in dairy products can interfere in PCR process. Furthermore, some factors like the types of culture media that are used for initial enrichment and isolation, the methods of DNA extraction, and sample preparation have effects on PCR results (Simon *et al*., 1996).

DNA hybridization

DNA hybridization is another molecular method that is used for characterization of *Listeria*. In this assay rapid detection of *Listeria* spp. is done using single probe labeled with a radioisotope or immunofluorescent agent which is complemented with target DNA sequences. This technique is currently employed commercially for rapid detection of *Listeria* in food products.

Typing methods for *L. monocytogenes*

Some types of *L. monocytogenes* are linked with human infections, such as serotype 4a, and some others are involved in the food contamination. Several typing methods based on the serological and molecular methods have been approved to differentiate these lineages during an outbreak.

1. Serological methods

Listeria species has been divided into 15 serotypes based on somatic (O) antigen, which is heat-stable, whereas it is divided into 4 serotypes based on the flagellar antigen, which is heat labile. At least 13 serotypes of *Listeria* have been determined by combination of both O and H antigens (i.e. 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) (Seeliger and Jones, 1986). Among these serotypes 1/2a, 1/2 b, and 4b are the most prevalent in human disease. In addition, the 4b serotype is mostly prevalent in epidemic outbreaks of *Listeria*, whereas, 1/2a and 1/2b are mostly sporadic (Jeršek *et al*., 1999). However, recently 1/2a serotype was a major cause of outbreak in countries such as Canada, Germany, and Austria (Chenal-Francisque *et al*., 2011). Despite the ease of application, serotyping methods have less sensitivity and should be followed by other molecular methods.

2. Phage typing

Strains of *Listeria* species could be differentiated based on their sensitivity to defined phages. In this assay, after administration of defined members of phages that target specific antigens on *Listeria* cells, lysis occurs. Phage type of each isolate is then characterized based on its sensitivity. This assay is a reliable test for distinguishing *Listeria* strains (Loessner, 1991).

3. Multi-locus enzyme electrophoresis (MLEE)

Multi-locus enzyme electrophoresis is based on the different patterns of amino acid migration in variable electrostatic charge that reflects the allelic variation of genes coding these sequences of amino acid. Molecular typing using MLEE is a reliable method as most of WHO laboratories used it for detecting *Listeria* serotypes because of the high sensitivity and it is easy to perform (Thomas *et al*., 2012). However, more consideration should be taken for interpretation of the results due to detection of large numbers of electrophoretic bands in the isolates and the variations on test conditions.

4. Esterase typing

This method measures the esterase activity of *L. monocytogenes* cells on a starch membrane using electrophoresis; however, the reproducibility of this method is relatively low (Harvey *et al*., 1996).

5. Pulse field gel electrophoresis (PFGE)

Pulse field gel electrophoresis is an effective and gold standard method for typing of pathogenic *Listeria* strains from contaminated samples (Abdollahzadeh *et al*., 2016a). It could discriminate between different types of *Listeria* from various sources. The total genome of bacteria is cut into several pieces by restriction enzymes. The DNA fragments produce unique band patterns on an agarose gel and then, *L. monocytogenes* is classified into different subtypes (pulsotypes) according to defined PFGE patterns.

6. Ribotyping

This method is based on different ribosomal genes of an organism and their relationship among different subtypes. Mostly, the ribosomal RNA which is constant over the evolution of organism has been used for typing. Briefly, the ribosomal genes are digested with restriction enzymes and hybrid with rRNA gene probes. The pattern of banding indicates the type of *Listeria*. This method is very reliable and reproducible, however the rate of differentiation between *L. monocytogenes* types is less than molecular methods (Bruce *et al*., 1995). This method could also be done based on PCR method (PCR-ribotyping).

7. PCR based methods for typing

There are two prevalent methods for the amplification of a specific part of a gene. One approach is random amplification of polymorphic DNA (RAPD), which is based on using random primers for amplifying the DNA fragments. In this assay, different species are distinguished based on the number and size of the amplified fragments. Advantage of this method is the ability to amplify an unknown microorganism using short-length primers. This method is widely used for epidemiological studies and typing of bacteria isolated from poultry industries, dairy environment, and food products (Zulkifli *et al*., 2009). Another approach is based on DNA fragmentation or conformational variation in PCR products. Two popular methods are SSCP and restriction fragment length polymorphism (RFLP) (Wiedmann, 2002). In RFPL the target gene is ribosomal subunits or virulence factors amplified with PCR that are then cut into different patterns using restriction enzymes. However, this method cannot differentiate serotypes very well. In SSCP, small mutations in the DNA fragment causes conformational changes in DNA strand (Wiedmann, 2002). These changes could be detected using denaturation gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) (Muyzer, 1999). Capillary electrophoresis can increase the sensitivity of detection. DNA sequences used for this approach are mostly *16S rRNA* gene, hemolysin (*hly*) and *iap* genes (Wagner *et al*., 2000).

The recent approach for evaluating variability and relationship among different species of bacteria, especially in outbreak, is the multilocus sequence typing that is high-quality method (Lamon *et al*., 2015). The multi virulence locus sequence typing (MVLST) administered for differentiation of pathogenic and nonpathogenic strains. It evaluates the virulence genes (e.g., *prfA*, *inlB*, *inlC*) (Zhang *et al*., 2004). For accurate identification of different species in epidemiological studies, detection of tandem repeat regions such as variable number of tandem repeats (VNTR) regions is useful. The VNTR locus is located in stable regions of the genome and do not change over the evolution (Sperry *et al*., 2008).

Antimicrobial resistance

1. Antibiotic resistance in food and the environment

Antibiotic resistance of *Listeria* which was isolated from cheese and pork was reported in 1996 (Rota *et al*., 1996). After that, various studies from different countries demonstrated the prevalence of antibiotic resistance in *Listeria* spp. varying from 0.6% up to 59% depending on sources from which they were isolated (Walsh *et al*., 2001; Antunes *et al*., 2002; Srinivasan *et al*., 2005). Resistance to oxacillin has the highest incidence, which was reported from Turkish food industries. The percentage of resistance to antibiotic varies between 59-63% according to the type of sources from which *Listeria* was isolated (Lyon *et al*., 2008). Recently, Byren *et al*. (2016) demonstrated that 50% of *Listeria* spp. was isolated from vegetables, and two *L. monocytogenes* isolates from ready-to-eat vegetables, were resistant to penicillin G (PEG) and tetracycline (TET). In addition, Abdollahzadeh *et al*. (2016a) found high resistant levels of *L. monocytogenes* to ampicillin, cefotaxime, and penicillin among the clinical and seafood isolates, they showed that the percentage of resistance to cefotaxime was 100%. In the case of penicillin the resistance rate was 71.4% for the clinical isolates and 57% for the seafood isolates (Abdollahzadeh *et al*., 2016a). Diversity in the frequency of antibiotic resistance among different studies on *L. monocytogenes* isolates from food and food-producing environments is summarized in Table 3.

2. Therapeutic regimens and mechanisms of antibiotic resistance in *Listeria*

Antimicrobial resistance of *Listeria* spp. is considered as one of the major health problems for management of the outbreaks and the human illnesses. β-lactams (penicillin and ampicillin) alone or combined with an aminoglycoside (e.g. gentamicin), sulfamethoxazole in patients with allergy to penicillin, vancomycin is patients with bacteremia, erythromycin in infected pregnant women, and rifampicin, TET, chloramphenicol, and fluoroquinolones are among general antibiotics that are prescribed for listeriosis. The majority of *Listeria* spp. are sensitive to these antibiotics (Troxler *et al*., 2000). However their exposure to pH, cold and salt stresses, could increase their resistance property. Ability of *Listeria* spp. for biofilm formation, expression of efflux pumps (that could confer resistance to fluroquinolones), carriage of mobile genetic elements (such as *tet* families that originated from *Enterococcus*) are among main known causes of antibiotic resistance (Olaimat *et al*., 2018). Two major efflux pumps, i.e., *MdrL* and *Lde*, exist in almost all *L. monocytogenes* serotypes. The *MdrL* pumps detoxify macrolide, cefotaxime, heavy metals and EtBr. The *Lde* pump detoxifies fluoroquinolone antibiotics and an intercalating dye such as EtBr and acridine orange (Mata *et al*., 2000).

Table 3: Frequency of antibiotic resistance among *Listeria monocytogenes* isolated from food and food-producing environments

Antibiotics	Sources isolated from	References	Year
Amikacin	Dairy-based foods	Harakeh *et al.*	2009
Ampicillin	Retail meats	Walsh *et al.*	2001
	Listeria monocytogenes type strains	Njagi *et al.*	2004
	Dairy farm environment	Srinivasan *et al.*	2005
	Meat products	Yucel *et al.*	2005
	Dairy-based foods	Harakeh *et al.*	2009
	Turkey meat	Aras *et al.*	2015
	Open air-fish market environment	Jamali *et al.*	2015
Bacitracin	Bison	Li *et al.*	2007
Cefotaxime	Cheese, pork sausage	Rota *et al.*	1996
	Bison	Li *et al.*	2007
Cefoxitin	Cheese, pork sausage	Rota *et al.*	1996
Ceftazidime	Open air-fish market environment	Jamali *et al.*	2015
Ceftriaxone	Processing plant	Lyon *et al.*	2008
	Bovine hides, carcasses	Wieczorek *et al.*	2012
Cephalosporin C	Dairy farm environment	Srinivasan *et al.*	2005
Cephalothin	Cabbage, environment	Prazak *et al.*	2002
	Meat products	Yucel *et al.*	2005
	Turkey meat	Aras *et al.*	2015
	Open air-fish market environment	Jamali *et al.*	2015
Chloramphenicol	Pork sausage	Rota *et al.*	1996
	Cheese, pork sausage	Rota *et al.*	1996
	Dairy farm environment	Srinivasan *et al.*	2005
	Meat products	Yücel *et al.*	2005
	Poultry meat	Miranda *et al.*	2008
	Dairy-based food	Harakeh *et al.*	2009
Ciproflaxin	Cabbage	Prazak *et al.*	2002
	Foodstuffs and processing environment	Conter *et al.*	2009
	Poultry meat	Alonso-Hernando *et al.*	2012
Clindamycin	Pork sausage	Rota *et al.*	1996
	Cabbage, water, environment	Prazak *et al.*	2002
	Poultry carcasses	Antunes *et al.*	2002
	Poultry carcasses	Antunes *et al.*	2002
	Cabbage, water	Prazak *et al.*	2002
	Clinical isolates	Safdar and Armstrong	2003
	Poultry meat	Miranda *et al.*	2008
	Poultry meat	Miranda *et al.*	2008
	Dairy-based food	Harakeh *et al.*	2009
	Surface (beef processing plant)	Granier *et al.*	2011
	Sludge, activated sludge	Granier *et al.*	2011
	Bovine hides, carcasses	Wieczorek *et al.*	2012
	Turkey meat	Aras *et al.*	2015
	Open air-fish market environment	Jamali *et al.*	2015
Florfenicol	Dairy farm environment	Srinivasan *et al.*	2005
Fosfomycin	Bison	Li *et al.*	2007
Gentamicin	Environment	Prazak *et al.*	2002
	Dairy-based foods	Harakeh *et al.*	2009
	Poultry meat	Alonso-Hernando	2012
	Bovine hides, carcasses	Wieczorek	2012
	Turkey meat	Aras *et al.*	2015
Kanamycin	Meat products	Yucel *et al.*	2005
Linezolid	Foodstuffs and processing environment	Conter *et al.*	2009
Linomycin	Bison	Li *et al.*	2007
Meticilin	Turkey meat	Aras *et al.*	2015
Nalidixic acid	Meat products	Yucel *et al.*	2005
Nitrofurantoin	Cabbage, water	Prazak *et al.*	2002
Oxacillin	Cabbage, water, environment,	Prazak *et al.*	2002
	Bison	Li *et al.*	2007
	Dairy-based food	Li *et al.*	2007
	Bovine hides, carcasses	Wieczorek *et al.*	2012
Penicillin	Cabbage, water, environment	Prazak *et al.*	2002
	Dairy farm environment	Srinivasan *et al.*	2005
	Dairy-based food	Harakeh *et al.*	2009
	Open air-fish market environment	Jamali *et al.*	2015
	Ready to eat vegetables	Byrne *et al.*	2015
Rifampicin	Dairy farm environment	Srinivasan *et al.*	2005
	Environment	Conter *et al.*	2009
	Foodstuffs and processing	Conter *et al.*	2009
	Poultry meat	Alonso-Hernando	2012
Rifampin	Cabbage, water	Prazak *et al.*	2002
	Dairy farm environment	Srinivasan *et al.*	2005
Streptomycin	Cabbage, water	Prazak *et al.*	2002
	Poultry carcasses	Antunes *et al.*	2002
	Dairy farm environment	Srinivasan *et al.*	2005
	Open air-fish market environment	Jamali *et al.*	2015
Tetracycline	Pork sausage	Rota *et al.*	1996
	Retail foods	Walsh *et al.*	2001
	Cabbage, environment	Prazak *et al.*	2002
	Dairy farm environment	Srinivasan *et al.*	2005
	Human and food origins	Bertrand *et al.*	2005
	Foodstuffs and processing environment	Conter *et al.*	2009
	Dairy-based food	Harakeh *et al.*	2009
	Raw meat and retail foods	Pesavento *et al.*	2010
	Pork cheek, surface (beef processing plants)	Granier *et al.*	2011
	Raw chicken and RTE chicken	Osaili *et al.*	2011
	Bovine hides, carcasses	Wieczorek *et al.*	2012
	Ducks	Adzitey *et al.*	2013
	Open air-fish market environment	Jamali *et al.*	2015
Tobramycin	Pork sausage	Rota *et al.*	1996
	Cabbage, environment	Prazak *et al.*	2002
Trimethoprim-ulfamethoxazole	Cabbage, water	Prazak *et al.*	2002
	Dairy farm environment	Srinivasan *et al.*	2005
	Meat products	Yucel *et al.*	2005
	Dairy-based foods	Harakeh *et al.*	2009
	Poultry meat	Miranda *et al.*	2009
	Open air-fish market environment	Jamali *et al.*	2015
Vancomycin	Dairy-based foods	Harakeh *et al.*	2009
	Foodstuffs and processing	Conter *et al.*	2009
	Raw meat and retail foods	Pesavento *et al.*	2010
	Environment	Pesavento *et al.*	2010
	Open air-fish market environment	Jamali *et al.*	2015

RTE: Ready-to-eat food

Currently, the most common therapeutic strategies for the treatment of listeriosis is administrating penicillin or ampicillin along with aminoglycosides (Swaminathan *et al*., 2007). *Listeria* species are naturally resistant to cephalosporin, fosfomycin, first generation of quinolone and sulfamethoxazole (Troxler *et al*., 2000; Conter *et al*., 2009). Administering other antibiotics like vancomycin, trimethoprim, sulfamethoxazole, rifampicin has been reported in various studies (Srinivasan *et al*., 2005). There are reports showing that *L. monocytogenes* can be resistant to antimicrobial agents such as penicillin, ampicillin, TET, streptomycin, clindamycin, oxacillin, and vancomycin (Troxler *et al*., 2000; Conter *et al*., 2009). Resistance to various antibiotics is the major concern of public health, since the percentage of infection due to *L. monocytogenes* is increasing regardless of improvement in the production process of food and milk (Hansen *et al*., 2005). The antimicrobial resistance is mostly seen in animals rather than humans. The first report of antibiotic-resistant of *L. monocytogenes* was reported from France in 1988 and since then it has been reported frequently (Antunes *et al*., 2002).

3. Role of other bacteria in the emergence of resistance *Listeria* strains

There is evidence about the transmission of plasmid pIP501 through conjugation mechanism from *Streptococcus agalactiae* to *L. monocytogenes*. This plasmid confers resistance to chloramphenicol, macrolides, lincosamides, and streptogramins. The transmission of other plasmids, such as pAMβ1 from *Enterococcus faecalis* that confers resistance to erythromycin, pIP823 from *E. faecalis* and *E. coli* to *L. monocytogenes* was reported. In addition, transfer of *vanA* gene cluster from *E. faecium* and transfer of resistance to erythromycin from lactic acid bacteria by conjugation were demonstrated in various studies. Transmission of a transposon, *tn916* harboring *tetA* gene, from *E. faecalis* to *L. innocua* which confers resistance to TET, was also demonstrated (Flamm *et al*., 1984; Charpentier *et al*., 1995).

Conclusion

Despite many achievements in developed countries, mainly in public health, food safety, administration of health promoting programs, and improvements in the laboratory diagnostics methods, *L. monocytogenes* remain as the major challenge in food industries. This bacterium could survive under an adverse environmental condition and overcome various types of stress like heat inactivation and could persist for a long time in food industry by attaching to food-contact surface, hence, application of good manufacturing practice (GMP) in food industries, such as improvement in food products, methods of storage, shipping and handling along with application of food safety training program, especially for food industry employees and staff in restaurant or distribution centers, should be taken into consideration. Moreover, hazard analysis critical control point (HACCP) system should be applied in each food processing step in order to ensure safe production of food, including the steps for processing of raw materials, storage and transportation. It should be noticed that *L. monocytogenes* has a high rate of mortality and it could be tuned into viable but non-culturable state in the undesirable condition. This form of the bacterium is present in food products, but is chemically inactive and cannot be detected in the culture media. Hence, an improvement in detection methodology and data about routes of its transmission, and resistance mechanisms to antibiotics is an urgent need to prevent its spread and control diseases caused by this bacterium in humans and animals.

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