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Epidemiology and antimicrobial resistance of *Listeria monocytogenes* in food items: A review

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Abstract

Listeria monocytogenes is a ubiquitous organism, and its' reservoirs are soil, forage, water, healthy humans and animals, or infected domestic and wild animals. *L. monocytogenes* is a foodborne microbe that can contaminate food products during or after processing. High-risk food items associated with listeriosis are ready-to-eat foods, including refrigerated ones. Listeriosis is an emerging infection of major public health concern worldwide because of the occurrence of associated foodborne outbreaks and the significant risk of mortality and morbidity. *L. monocytogenes* poses a significant risk to the food industry, particularly producers of ready-to-eat foods, due to its ability to proliferate in a vast range of adverse environmental conditions. The risk factors for this infection are food-storing time, temperature, type of product, infective dose, immunity, and the traditionally accepted consumption of raw foods. The route of *L. monocytogenes* to humans is via the consumption of contaminated food. Itcan be transmitted directly from mother to child through contact with animals or hospital-acquired infections. Antibiotic resistance is a major threat to global public health, food security, and food development because it makes disease harder to treat as antibiotics become ineffective. *L. monocytogenes* may face a broad spectrum of sub-lethal environmental stresses during food production and processing. *L. monocytogenes* is infective for all human population groups; it causes severe problems in pregnant women, neonates, the elderly, and immunosuppressed individuals. Prevention and control of listeriosis are achieved through proper disposal of contaminated materials, bedding, and litter, incineration of infected carcasses, proper hygiene and sanitation at animal farms, and wearing protective clothing while handling infected animals, aborted material, and a retained placenta.

Keywords: Antibiotic resistance, emerging, food, *L. monocytogenes*

1. Introduction

Listeria monocytogenes (*L. monocytogenes*) *is a* ubiquitous organism, widely distributed in the environment. The principal reservoirs are soil, forage, and water, as well as healthy humans and animals or infected domestic and wild animals (EFSA, 2011). *L. monocytogenes* causes listeriosis, which occurs in humans and animals. Since the beginning of the 1980s, *L. monocytogenes* has been recognized as an emerging food-borne pathogen after the occurrence of several sporadic and epidemic cases of listeriosis in Europe and the USA. Recently, the incidence of sporadic cases rose again in Europe (Cairns and Payne, 2009).*L. monocytogenes* is a foodborne microbe that can contaminate food products during or after processing.*L. monocytogenes* poses a significant risk to the food industry, particularly producers of ready-to-eat (RTE) foods, due to its ability to proliferate over a vast range of adverse environmental conditions encompassing low temperature, low pH, and high salt. *L. monocytogenes* causes a major public health concern due to severe human illness with serious consequences (Scallan*et al*., 2011).

Most of the time, infection in animals is subclinical, but severe forms can also occur. Septicaemia, encephalitis, meningitis, meningo-encephalitis, abortion, stillbirth, perinatal infections, and gastroenteritis characterize the disease (Mateus*et al*., 2013; OIE, 2014). The organism has an intracellular life cycle that can pass from cell to cell without release from the cell. This ability presents its potential to cross the placental barrier and the blood–brain barrier, explaining its pathogenesis and clinical signs (Janakiraman, 2008).

Starting in the 1960s, due to the introduction and widespread use of refrigerators, processed foods and foods with extended shelf lives became more associated with *L. monocytogenes*. The prevalence was found to differ from place to place based on hygiene, food content, and environmental contamination rates in the specific areas (Lamon*et al*., 2011). A study conducted in Addis Ababa city showed the prevalence of *Listeria s*pp. to be 32.6% and that of *L. monocytogenes* to be 5.1% in some foods such as meat, cheese, fish, pork, poultry, and ice cream (Molla *et al*., 2004). High-risk food items associated with listeriosis are ready-to-eat foods, including those that are refrigerated, but do not undergo any substantial heat treatment before consumption. The causes of the emerging human case of listeriosis are changes in food production, processing, and distribution; increased use of refrigeration; changes in eating habits toward ready-to-eat foods; and an increase in the number of people at high risk for the disease (Rocourt and Reilly, 2000).

Antimicrobial resistance (AMR) has emerged as a threat to the current effective treatment for an ever-increasing range of microbial infections. It results in reduced efficacy of antibiotics, making treatment complicated, time-consuming, and costly. The discovery of each and every new antibiotic has been followed by reports of emerging resistance against it (WHO, 2014). AMR do not respect geographical boundaries and can traverse among humans and animals across countries, mediated through resistant strains, without any specific information or check (Littman and Viens, 2015). Antibiotic-resistant bacteria may reach humans (i) indirectly along the food chain through consumption of contaminated food or food-derived products and (ii) following direct contact with colonized or infected animals or biological substances such as blood, urine, feces, saliva, and semen, among others (Chang *et al.,* 2015). Therefore, the objective of this paper is to review the available literature on the epidemiology and AMR of *L. monocytogenes* in food items.

2. Literature review

2.1 Etiology

Listeriosis is caused by the genus Listeria, which now has seven species. From these, two species are the most pathogenic for humans and animals. *L. monocytogenes* is pathogenic to human beings and several animal species, whereas *L. ivanovii* is pathogenic to ruminants and occasionally to humans (Mclauchlin and Martin, 2008). *L. monocytogenes* has been divided into 13 serotypes (namely 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) based on somatic and flagellar antigens. Serotypes 1/2a, 1/2b, and 4b are commonly reported in humans, and serotypes 1/2a and 4b in animals. A few cases of human listeriosis have been attributed to *L. seeligeri* and *L. welshimeri* (Weller *et al*., 2015).

2.2 Epidemiology

Listeriosis is an emerging infection with major public health concerns worldwide, and its outbreaks in humans and animals have been reported from different parts of the globe at different times. In Europe, listeriosis surveillance data is available at the national level in 16 countries. 19 recent reports based on these national surveillance systems indicate an increase in the incidence of listeriosis in many European countries including England and Wales, Denmark, Belgium, Germany, the Netherlands, Switzerland and Finland during 2000 to 2006 (Goulet *et al*., 2008). In England and Wales, the incidence of listeriosis has nearly doubled from an annual average of 110 cases in 1990s to 230 per year in 2007(HPA, 2007). Increased risk of listeria infection is predominantly seen in patients aged 60 years or above. Similar trends are also found in other European countries where the incidence of listeriosis is higher among older persons (CDC, 2008).

Epidemiological investigations have revealed that some outbreaks were associated with consumption of contaminated dairy products such as cheese and butter, processed meat, fish products, contaminated salad, and ice cream cake (Devalk *et al*., 2005).Listeriosis in humans is caused by all 13 serotypes, particularly 1/2a, 1/2b, and 4b, and the rate of annual endemic disease varies from 2 to 15 cases per million of population (Munoz, 2012*). L. monocytogenes* isolates of human and food-borne origin, recovered during 20 years (1992–2012) from Argentina, corresponded to serotype 4b mostly (71%), and the rest (29%), to serotype 1/2b (Prieto*et al*., 2016).*Listeria* affects animals, including sheep, goats, cattle, buffalo, horses, pigs, camels, canines, rodents, wild animals, and birds. In animals, sheep are mostly affected (OIE, 2014). *L. monocytogenes* has been isolated from the meat and/or milk of goats, sheep, cattle, pigs, chickens, quail, partridge, ostriches, and buffaloes (Ndahi*et al*., 2014), fish and fishery products (Barbuddhe *et al*., 2008), ice creams, vegetables, other ready-to-eat foods (Lambertz*et al*., 2013), seafood (Ahmed *et al*., 2013), and a mushroom production facility (Viswanath*et al*., 2013).

In Ethiopia, there was a study done in 2004, which showed a prevalence of *Listeria species* of about 31.6% and *L. monocytogenes of* 5.1% in some foods, such as meat, cheese, fish, pork, poultry, and ice cream (Molla *et al.*, 2004). Derra*et al.* (2013) described 4.1% prevalence from raw meat and dairy products like raw milk, cottage cheese, and cream cake collected from the capital and five neighboring towns in Ethiopia. The serotypes of *L. monocytogenes* identified belong to 1/2b, 4b, and 4e.

2.2.1 *Source of infection*

The sources of listeriosis are soil, manure, sewage, farm slurry, sludge, silage, animal feed, water, and the excreta or feces of mammals and birds. Dairy cows usually represent a reservoir for the bacterium, and raw milk is served as a major source of transmission from animals to humans. Contaminated raw foods represent a vehicle for the introduction of L. monocytogenes into food processing plants (Ferreira *et al*., 2014). Persons with compromised immune systems, such as pregnant women and elderly people, are more susceptible to listeriosis than others. The gastrointestinal tract of humans serves as a major reservoir of the pathogen (Hernandez-Milian and Payeras-Cifre, 2014).

2.2.2 *Mode of transmission*

The routes of transmission of *L. monocytogenes* to humans are through the consumption of contaminated food as well as directly from mother to child, contact with animals, and hospital-acquired infections. Healthy individuals can be asymptomatic carriers of *L. monocytogenes*, with 0.6-3.4% of healthy people with unknown exposure to *Listeria* being found to shed *L. monocytogenes* in their feces. However, outbreak investigations have shown that listeriosis patients do not always shed the organism in their feces (Painter and Slutsker, 2007). Intestinal colonization and the presence of L. *monocytogenes* in the feces of poultry play a significant role in the spread of listeriosis in domestic animals and ruminants (Goh*et al*., 2012).

2.2.3 *Risk factors*

The risk factors for infection are food storage time, temperature, type of product, infective dose, immunity, and the traditionally accepted consumption of raw foods. There are other factors that contribute to the occurrence of a high incidence of listeriosis. These are the increased proportions of susceptible people due to age, immunocompromised status, increased use of cold storage to prolong the shelf life of foods and consumption of raw foods (Oliver *et al.*, 2005).

2.3 Antimicrobial resistance

Antimicrobial resistance is the ability of a microorganism to resist an antimicrobial concentration that is used in clinical practice when the organism changes its response to the antimicrobial drug. AMR is considered one of the major threats to global public health, food security, and food development (WHO, 2018). Microorganisms, particularly bacteria, respond differently to antibiotics and other antimicrobial compounds, either due to intrinsic differences or to the development of resistance by adaptation or genetic exchange (Calderon and Sabundayo, 2007).

2.3.1 *Factors influencing the AMR of L. Monocytogenes*

The extensive use of antibiotics has been due to misuse of drugs in humans and animals, which has greatly contributed to the progression and spread of antibiotic resistance among foodborne pathogens, including *L. monocytogenes* (Wilson *et al*., 2018). Antibiotic resistance is believed to develop in bacteria in a number of different ways. Some pathogens develop resistance as a result of their general physiology, whereas others develop resistance as a result of mutation or other types of genetic alteration. In addition, during their adaptation to environmental stresses, pathogens can become more resistant to antibiotics (Munita and Arias, 2016).Antimicrobial drugs are extensively used in animals to prevent, control, and treat illnesses as well as enhance their growth in many countries (Wilson *et al*., 2018). Antibiotic-resistant strains can be transferred between animals and humans; the most probable way is transmission through the food chain. *L. monocytogenes* commonly encounters low levels of antibiotics and other antimicrobials in the food production chain. This may serve as pre-exposure adaptation, which subsequently allows *L. monocytogenes* to resist higher levels of antibiotics or antimicrobial drugs (Castanon, 2007).

L. monocytogenes may face a broad spectrum of sub-lethal environmental stresses during food production and processing. This includes physical stressors and biological stressors, which induce the bacterial cross-protection response that generates cells with increased resistance to the same or other types of stresses. The bacterial response to stress includes changes in cell composition and physiological state, which enable foodborne pathogens to maintain their normal functions and survive in foods during processing (Lungu*et al*., 2011).Al-Nabulsi*et al*. (2015) indicated that exposure of *L. monocytogenes* food isolates to pH, cold, and salt stresses increased their resistance to different antibiotics. The antibiotic resistance of L. monocytogenes was enhanced as the salt concentration increased to 6% or 12%, as the pH was reduced to pH 5, or as the temperature was decreased to 10 °C. Another study reported that exposure of exponential-phase *L. monocytogenes* cells to a concentration of 600 parts per million (ppm) hydrogen peroxide and nonlethal heat (45°C) significantly increased their resistance to antibiotics including penicillin, ampicillin, tetracycline, chloramphenicol, gentamycin, rifampicin, and trimethoprim-sulfamethoxazole (Faezi-Ghasemi and Kazemi, 2015).

Starvation stress may allow *L. monocytogenes* cells to become more resistant to commonly used food preservation techniques such as heat and irradiation. Therefore, the lack of nutrients in areas of processing plants may result in cross-protection against antibiotics as well. Yet, there is not enough evidence available to confirm horizontal antimicrobial resistance gene transfer resulting from food chain stresses (Allen *et al*., 2016).

Some antimicrobials used in food preservation and safety may have an influence on antibiotic resistance. Sodium diacetate, potassium lactate, and nisin are safe antimicrobials that are commonly approved for use in meat and cheese products. Treatment of *L. monocytogenes* inoculated in a model broth system with diacetate, lactate, or nisin was shown to modify the expression of genes involved in regulating membrane permeability and other transport systems. Consequently, it was hypothesized that these compounds could trigger certain efflux pumps to expel certain drugs and toxic substances out of the cell or limit entry into the cell (Stasiewicz*et al*., 2011). Clove oil, cinnamaldehyde, and vanillin were used to inhibit the growth of *L. monocytogenes* in chicken, meat, and other food products. In a study, it was reported that the essential oils (citral and carvacrol) substantiated the antimicrobial effect of bacitracin, colistin, and erythromycin against *L. monocytogenes* and *L. innocua*(Zanini*et al*., 2014).

2.3.2 *Mechanisms of AMR in L. Monocytogenes*

Acquisition of movable genetic elements, including self-transferable plasmids, mobilizable plasmids, and conjugative transposons, is the major mechanism responsible for the development of antibiotic resistance in *L. monocytogenes*. However, efflux pumps were also suggested to be linked with fluoroquinolone, macrolide, and cefotaxime resistance in *L. monocytogenes* (Godreuil*et al*., 2003).

2.3.2.1 Antibiotic resistance mediated by conjugation

It has been reported that *L. monocytogenes* used conjugation as a main strategy to acquire resistance to antibiotics. Conjugation is the process by which genetic materials transfer from a donor cell to a recipient cell. *Enterococci* and *streptococci* represent the main reservoirs of resistance genes for *L. monocytogenes*. The genome of bacteria is composed of the chromosome and accessory movable genetic elements such as transposons and plasmids (Perichon and Courvalin, 2009).

2.3.3.2. *Antibiotic resistance mediated by efflux pumps*

L. monocytogenes has three efflux pumps: one operates to extrude antibiotics, heavy metals, and ethidium bromide, and the second pump is associated with resistance to fluoroquinolones and, partially, the resistance of *L. monocytogenes* to acridine orange and ethidium bromide (Godreuil*et al*., 2003). The third pump is involved in the resistance of L. monocytogenes to fluoroquinolones (Guerin *et al*., 2014).

2.3.3.3. *Antibiotic resistance of L. Monocytogenes isolated from food*

Antibiotic resistance, particularly multidrug resistance, among foodborne bacteria including *L. monocytogenes* has emerged (Pesavento*et al*., 2010). Apparently, the effect of antibiotic resistance is more obvious among vulnerable patients, resulting in prolonged illness and an increased mortality rate (WHO, 2014). It is anticipated that global deaths from infection caused by antibiotic resistant pathogens will increase from 700,000 to 10 million annually, and costs are predicted to reach US $100 trillion by 2050 (ONeill, 2014).

Many *L. monocytogenes* strains resistant to at least one antibiotic have been isolated from different sources, including food, environmental, and human clinical samples. Prazak*et al.* (2002) investigated the antibiotic resistance of 21 *L. monocytogenes* isolates from water, cabbage, and different environmental samples in Texas, USA. In China, 73% of 167 *L. monocytogenes* isolated from retail food products were resistant to sulfonamide, 8.4% were resistant to tetracycline, and 1.8% was resistant to ciprofloxacin. The antibiotic susceptibility of 13 strains of *L. monocytogenes* isolated from homemade white cheeses was examined (Arslan and Ozdemir, 2008).Pesavento*et al*. (2010) reported that 20% of *L. monocytogenes* isolates showed multidrug resistance. However, the percentage of antibiotic resistance among isolates was 20% for ampicillin, 22.5% for methicillin, 27.5% for clindamycin, and 75% for oxacillin. Sakaridis*et al*. (2011) studied the antibiotic resistance of *L. monocytogenes* in chicken slaughterhouses and found that all 55 *L. monocytogenes* isolates displayed resistance to nalidixic acid and oxolinic acid, whereas 83.6% were resistant to clindamycin.

Osaili*et al*. (2012) indicated that out of 39 *L. monocytogenes* strains isolated from different types of cheese, all 39 were resistant to fosfomycin, 92.3% (36/39) were resistant to oxacillin, and 56.4% (22/39) were resistant to clindamycin. However, the isolates showed sensitivity or intermediate susceptibility to gentamycin, imipenem, teicoplanin, rifampicin, linezolid, ciprofloxacin, fusidic acid, vancomycin, trimethoprim-sulfamethoxazole, benzylpenicillin, erythromycin, and tetracycline. Jamal*et al*. (2015) observed that the resistance among *L. monocytogenes* isolates from fish products was 20.9%–27.9% to tetracycline and ampicillin, 14.0%–16.3% to cephalothin, penicillin G, and streptomycin, and 2.3% to rifampicin and chloramphenicol. Garedew*et al*. (2015) from Ethiopia found that 16 (66.7%), 12 (50%), 9 (37.5%), and 4 (16.6%) isolates of 24 *L. monocytogenes* isolates from RTE foods of animal origin exhibited resistance to penicillin, nalidixic acid, tetracycline, and chloramphenicol, respectively. Further, four (16.7%) were multidrug-resistant isolates.

Peter *et al*. (2016) indicated that 16 isolates of *L. monocytogenes* from pork, beef, and chicken were susceptible to gentamycin, cotrimoxazole, erythromycin, and chloramphenicol, but were resistant to amoxicillin, augmentin, cloxacillin, and tetracycline. Abdollahzadeh*et al*. (2016) found that seven *L. monocytogenes* isolates from seafood were resistant to ampicillin and cefotaxime, whereas four isolates were resistant to penicillin. Lee *et al*. (2017) reported that all strains of *L. monocytogenes* that were isolated from RTE seafood and food processing environments were resistant to benzyl penicillin, clindamycin, and oxacillin; 97% (32/33) of isolates were resistant to ampicillin, and 18% (6/33) were resistant to tetracycline. Further, 82% of isolates (27/33) showed resistance to four antibiotics, and 18% (6/33) were resistant to five antibiotics.

Haubert *et al*. (2016) studied the antibiotic resistance of 50 *L. monocytogenes* strains isolated from foods and food environments in Brazil between 2001 and 2010. They found that all isolates were resistant to nalidixic acid and cefoxitin. Further, high prevalences of resistance were observed to clindamycin (68%), streptomycin (10%), meropenem (10%), rifampicin (10%), and trimethoprim–sulfamethoxazole (10%). Wieczorek and Osek (2017) found that 57.9% of *L. monocytogenes* strains isolated from fresh and smoked fish showed resistance to oxacillin, 31.6% and 8.8% were resistant to ceftriaxone or clindamycin, respectively.Kuan*et al*. (2017) tested the antibiotic resistance of 58 *L. monocytogenes* isolates from vegetable farms and retail markets in Malaysia. They found that 100%, 70.7%, and 41.4% of isolates exhibited resistance to penicillin G, meropenem, and rifampicin, respectively. Wilson *et al*. (2018) tested the antibiotic resistance of 100 *L. monocytogenes* isolates from Australian food production chains between 1988 and 2016. They found that all isolates were sensitive to penicillin G and tetracycline. However, only two isolates were resistant to ciprofloxacin, and an isolate was resistant to erythromycin.

In a study comparing the prevalence of antibiotic and multidrug resistance among *L. monocytogenes* isolates from poultry products in north-western Spain, Alonso-Hernando *et al*. (2012) pointed out that, excluding nalidixic acid, to which most isolates were inherently resistant, 37.2% and 96.0% of *L. monocytogenes* isolated in 1993 and 2006, respectively, showed resistance to at least one antibiotic. Multidrug resistance was also more common in 2006 (84.0%) as compared to 1993 (18.6%). Furthermore, the average number of antibiotics to which the *L. monocytogenes* strains were resistant was higher in 2006 (4.2) than in 1993 (1.6). A remarkable increase in the number of resistant strains isolated in 2006 was observed for neomycin, gentamycin, enrofloxacin, streptomycin, furazolidone, and ciprofloxacin.

2.4. Public health importance

Listeria is an opportunistic intracellular pathogen that has become an important cause of human foodborne infections worldwide (Selamawit, 2014). It causes especially severe problems in pregnant women, neonates, the elderly and immune-suppressed individuals. Direct transmission of disease is possible while handling aborted animals. Animals may be diseased or asymptomatic carriers of *L. monocytogenes*, shedding the organism in their feces. Indirect transmission may occur simply by consumption of food products from diseased animals (Osman *et al*., 2014).

Listeriosis occurs as a sporadic, endemic, and foodborne outbreak to induce septicemia, meningoencephalitis, abortion, and infection in other organs. The majority of risks involve contamination of foods during processing and the potential of the organism to grow at refrigeration temperature (Viswanath*et al*., 2013).

2.5. Prevention and control

Control of listeriosis is difficult because of the ubiquitous nature of the causative organism, the lack of a simple method of identifying the presence of *Listeria* contamination in the environment, and a poor understanding of risk factors other than silage (Dhama *et al*., 2015). However, some of the control measures are mentioned as follows: Proper disposal of contaminated materials, beddings and litters, and infected carcasses should be done carefully by incineration or burning methods. Animals should not be fed with rotten vegetables. Proper hygiene and sanitation should be maintained at animal farms. Follow the use of tetracycline in the diet of animals at risk as a preventive measure when there is a disease outbreak. Wear protective cloth while handling infected animals, aborted material, or removing retained placenta. Spoiled silage should not be fed to animals. Routine testing of raw milk or a raw milk testing program should be conducted to reduce the number of cases per year (Latorre *et al*., 2011).

3. Conclusion and recommendations

Listeriosis, caused by *L. monocytogenes*, is a fatal infectious disease of mammals, birds, and humans, characterized by septicemia and encephalitis. It is a major problem in developing countries where there is a scarcity of food as well as unhygienic conditions. The consumption of raw or contaminated food is the most common mode of *L. monocytogenes* infection in humans. The bacteria that survive the acidity of the stomach may enter the small intestine, crossing the epithelial barrier, and then spreading to the liver, spleen, central nervous system, and, in pregnant women, the fetus. *L. monocytogenes* poses a persistent threat to the food industry, particularly operations preparing RTE foods. Antibiotic resistance in *L. monocytogenes* isolated from food products has been developing over the past few decades and represents a serious public health risk worldwide. Generally, most *L. monocytogenes* isolated from different food sources are susceptible to those antibiotics normally effective against gram-positive bacteria. Yet, there is an alarming increase in the prevalence of multidrug-resistant strains of L. monocytogenes from various sources, and therefore monitoring *L. monocytogenes* for changes in its antimicrobial resistance appears prudent. It has been demonstrated that isolates of *L. monocytogenes* from different food sources show resistance to antibiotics commonly used in the treatment of humans and animals.

Based on the above conclusions, the following recommendations are forwarded: (i) avoid the consumption of unpasteurized milk and raw meat and practice good hygiene and adequate heat treatment of food before consuming; (ii) proper disposal of the aborted fetus, retained placenta, and feces of infected animals is necessary to avoid the spread of disease, as is wearing protective clothing when handling infected animals; (iii) provide public health education about the route of transmission and prevention of the disease, especially for people who are at high risk for *L. monocytogenes; and* (iv) alternative use of bacteriophage, egg yolk antibodies, cytokines, herbs, and essential oils to combat *L. monocytogenes is recommended.*

Disclosure

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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