Prevalence and Persistence of *Listeria monocytogenes* in Dairy and Other Ready-to-Eat Food Products in Africa

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Abstract Worldwide, *L. monocytogenes* bacteria are frequently associated with illness in humans, where immunocompromised individuals and pregnant women are at high risk of contracting listeriosis. The pathogen most often spreads through food consumption, so it is a major concern in most food-processing environments. In Africa, however, research on *L. monocytogenes* is scarce, making data on listeriosis limited. This is problematic because in Africa, traditionally produced foods have poor microbial quality. On average, 5.1 % of dairy and ready-to-eat foods are contaminated with *L. monocytogenes*. The pathogen has been isolated from milk, local cheese ("wara"), yogurt, ice cream, "kunu," and ready-to-eat meat products during and after processing. Furthermore, it is resistant to one or more antibiotics and can also form biofilms on various surfaces that contact food. So, while *L. monocytogenes* is persistent in food-processing environments in Africa, the serotypes of its circulating strains are largely unknown. This study therefore expounds the characteristics of *L. monocytogenes* and listeriosis associated with consuming contaminated diary and ready-to-eat foods in Africa. We also present the prevalence and persistence of the pathogen in most food environments as well as the safety measures that can limit its ability to contaminate foods/surfaces and spread.

1 Introduction

L. monocytogenes, a bacterium that causes listeriosis, is continuously viewed as a threat to global public health and is gaining important attention among stakeholders in food safety. Listeriosis affects all species of domestic animals and humans. The human infection is usually severe, resulting in high hospitalization rates and

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mortality, especially for "at-risk" populations, and causes abortions, meningitis, meningoencephalitis, spontaneous peritonitis, septicemia, etc. (Farber and Peterkin 1991; Rocourt 1996; Mead et al. 1999; Acha and Szyfres 2003). At-risk individuals are those with low immunity, such as HIV/AIDS patients, children under 5 years old, pregnant women, and elderly people (Farber and Peterkin 1991; Acha and Szyfres 2003; Borucki and Call 2003; McLauchlin et al. 2004; Liu 2006; Kuhn et al. 2008). *L. monocytogenes* is ubiquitous in nature and can survive harsh environmental conditions such as low pH, water activity (Nolan et al. 1992; Buchanan et al. 2000; Duffy et al. 1994), and a wide temperature range (including refrigeration temperature) (HPA 2009). Food contamination is a major way that *L. monocytogenes* infections are acquired (Taormina and Beauchat 2002). As such, the pathogen has been isolated from various ready-to-eat, dairy, and other minimally processed foods in many countries and has caused various documented foodborne disease outbreaks (Piffaretti et al. 1989; Mahmood et al. 2003; Vitas et al. 2004; Aurora et al. 2009; Rahimi et al. 2010; Rivoal et al. 2010). Its virulence and persistence in the environment is further potentiated by its ability to form fimbriae, cellulose, and biofilms (Hood and Zottola 1995; Abu-lail and Camesano 2003; Gulsun et al. 2005; Adetunji 2010). Furthermore, the pathogen is now more resistant to many commercially available antibiotics and sanitizers (Poyart-Salmeron et al. 1992).

Listeriosis is frequently reported in developed countries, but its representation in Africa is still unclear. Research on *L. monocytogenes* in Africa is scarce, so data on listeriosis is limited. However, the prevalence, virulence, and serotypes of *L. monocytogenes* in ready-to-eat foods have been reported in Ethiopia, Uganda, Morocco, Egypt, Nigeria, Botswana, and Lesotho (Molla et al. 2004; Ennaji et al. 2008; Salihu et al. 2008; Morobe et al. 2009, 2012; Abd El Malek et al. 2010; Mugampoza et al. 2011; Abeer et al. 2012; Moshoeshoe and Olivier 2012; Yakubu et al. 2012), but the serotypes and virulence of the circulating strains remain largely unknown. The objectives of this review are to expound the characteristics of *L. monocytogenes* and listeriosis associated with consumption of contaminated diary and ready-to-eat foods in Africa and to briefly discuss the prevalence and persistence of the pathogen in foods and food environments. We also highlight the safety measures that can be applied to limit its contamination, spread, and persistence.

2 Characteristics of *L. monocytogenes*

Listeria are facultative anaerobes that are gram-positive, non-acid-fast, non-spore-forming, acapsular rod-shaped bacteria, and they measure 0.5–2 μm by 0.4–0.5 μm (Walker 2005). The history of *Listeria* is traced to 1926 when Murray, Web, and Swann demonstrated that it was the causative organism of listeriosis (Parihar 2008). In 1940, Pirie suggested the name *L. monocytogenes*. Other species in the genus *Listeria* include *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria*

welshimeri, and *Listeria grayi*. Of these, only *Listeria ivanovii* and *Listeria monocytogenes* are pathogenic.

L. monocytogenes is a facultative intracellular, anaerobic, psychotropic bacterium with low G+C (36–42 %) content (Vazquez-Boland et al. 2001; Monk et al. 2008; Parihar 2008; Sukhadeo and Trinad 2009), and it moves in a distinctive way called "tumbling motility," propelled by its peritrichous flagellum (Ferreira et al. 2003; Adetunji and Adegoke 2008). *L. monocytogenes* has a high motility (Mead et al. 1999) and grows within a wide temperature range (<0–45 °C) (HPA 2009), with an optimum at 30–37 °C (Swaminathan et al. 1995). However, the pathogen can be destroyed when kept at high temperatures (e.g., pasteurization) for short durations (Walker 2005). This microorganism is oxidase negative and catalase positive, it ferments glucose and produces acid without gas, and it survives in vacuum-packaged products at refrigeration temperatures (Duffy et al. 1994). Furthermore, it has low water activity and a low pH (Nolan et al. 1992; Buchanan et al. 1993) and produces a narrow zone of hemolysis on sheep blood agar (Varnam and Evans 1991). The bacterial particles usually occur singly, in short chains, or in diploforms, producing V-shape arrangements (Parihar 2008). Furthermore, it is genetically heterogeneous (Piffaretti et al. 1989; Wiedmann et al. 1997; Kathariou 2002) and displays great biodiversity and serological cross-reactivity with various bacteria strains in other genera (Parihar 2008). *L. monocytogenes* is ubiquitous and can be isolated anywhere in the environment, making it a very dangerous organism. In 2008, the Office International des Epizooties (OIE) documented many molecular and cellular determinants of virulence for this intracellular pathogen, and although there is evidence that polymorphisms influence virulence in some of the different *L. monocytogenes* strains, this heterogeneity cannot be correlated with the organism's ability or inability to produce disease. Therefore, all *L. monocytogenes* strains are considered to be potentially pathogenic.

3 *L. monocytogenes* Infections

L. monocytogenes causes a severe zoonotic illness known as listeriosis, which is associated with morbidity and mortality in humans and livestock (Borucki and Call 2003). Animals usually affected include both large and small ruminants, pigs, rabbits, mice, birds, and fish (Ireton 2006). Listeriosis commonly affects people with compromised immunity (e.g., HIV/AIDS patients), pregnant women, neonates, and elderly people (Farber and Peterkin 1991; Borucki and Call 2003; McLauchlin et al. 2004; Liu 2006; Kuhn et al. 2008), resulting in meningitis, meningoencephalitis, spontaneous peritonitis, abortion, septicemia, arthritis, pelvic infection, or arthritis (Khelef et al. 2006; Sukhadeo and Trinad 2009; Adetunji and Isola 2011a). Infections often have high risks of hospitalization, and human mortality is also high, ranging between 20 % and 30 % (Farber and Peterkin 1991; Rocourt 1996; Mead et al. 1999) and up to 80–99 % in the vulnerable groups (Farber and Peterkin 1991; Gray and Killinger 1966; Rocourt 1996; Sauders

et al. 2003; Chenal-Francisque et al. 2011). Several listeriosis outbreaks have been reported throughout the world, including many multistate outbreaks in the United States. In 2003, mortality from listeriosis was about 500 people from a reported ~2500 illnesses (Mead et al. 1999). However, listeriosis is underreported in Africa (Boukadidda et al. 1994) because food-processing industries are still evolving. In Nigeria, *L. monocytogenes* was isolated from some patients that showed clinical signs of listeriosis, and the infection produced a mortality rate of 27 % (Onyemelukwe et al. 1983). But in Morocco, human listeriosis is uncommon (Benomar et al. 2000). Chintu and Bathirunathan (1975) reported 85 cases of listeriosis in Zambia, while Hohne et al. (1975) reported that the outbreak serotypes (1/2a and 4b) of *L. monocytogenes* were isolated in slaughtered cattle in Togo.

Contamination of ready-to-eat foods—as well as other minimally processed foods—is widely documented as the main source of *Listeria* outbreaks. Consequently, it is a great public health concern. Outbreaks are often linked with the consumption of several contaminated food products, including ready-to-eat dairy and meat products, such as coleslaw, pasteurized milk, milk from lactating ruminants, soft cheeses, raw and pasteurized eggs, poultry meat, cooked meats, cured meats, and smoked salmon (Piffaretti et al. 1989; Mahmood et al. 2003; Vitas et al. 2004; Aurora et al. 2009; Rahimi et al. 2010; Rivoal et al. 2010).

4 Traditional Foods and *L. monocytogenes*: Isolation, Prevalence, and Persistence

4.1 Isolation and Prevalence of L. monocytogenes in Ready-to-Eat Foods in Africa

In Africa, roughly 5.1 % of dairy and ready-to-eat food sample are contaminated with *L. monocytogenes*, as shown in some of the few available prevalence studies. While Africa comprises over 50 countries, there are reports on *L. monocytogenes* incidence and prevalence from ready-to-eat foods from only a few countries (Fig. 1). In North Africa, Egypt has a prevalence of 5 % (Abd El Malek et al. 2010) and Morocco is at 2.4 % (Ennaji et al. 2008). In these two countries, *L. monocytogenes* isolates were obtained from meats, luncheon, and frozen chicken legs using multiplex polymerase chain reaction (PCR). However, in a study by Abeer et al. (2012), *L. monocytogenes* was not detected in camel milk from Egypt. Camel milk contains lysozyme and lactoferrin (El Agamy et al. 1992; Wernery 2003; Al-Majali et al. 2007; Al-Haj and Al-Kanhal 2010), which prevent the pathogen's growth.

Ethiopia and Uganda represent the only countries in East Africa where studies on the prevalence of *L. monocytogenes* were carried out. Using both conventional and molecular methods, *L. monocytogenes* was isolated from ice cream, pork samples, minced beef, fish, and chicken samples, amounting to a prevalence of 5.1 % in Ethiopia (Molla et al. 2004). In Uganda, *L. monocytogenes* contaminated

Fig. 1 Countries in Africa with known prevalence, serotypes, and virulence of circulating *Listeria monocytogenes* strains. Virulence genes: (**A**) *prfA* (Egypt); (**B**) *actA* (Egypt); (**C**) *hly* (Morocco). Circulating serotypes: (**1**) 1/2b, 3b, 7, 4b, 4d, 4e (Morocco); (**2**) 1/2b, 4b, 4e (Ethiopia); (**3**) 1/2b, 3b, 4a, 4b, 4c, 4d, 4e (Botswana). (*asterisk*) Countries with known prevalence (Botswana, Egypt, Ethiopia, Lesotho, Morocco, Nigeria, Uganda). The outline map of Africa was sourced from http://www.worldatlas.com/webimage/countrys/africa/afoutl.htm

3 % and 13 % of locally processed yogurt and bulk raw milk, respectively (Mugampoza et al. 2011). Currently, there is no clear evidence on its prevalence in central Africa. In Southern Africa, recorded prevalences ranged from 2.5 % in Lesotho (Moshoeshoe and Olivier 2012) to 4.3 % in Botswana (Morobe et al. 2009). In this region, the contaminated ready-to-eat foods were unpasteurized bovine milk, cheese, meat, frozen cabbage, and salads. In South Africa, researchers work more on *Listeria ivanovii* (Nyenje et al. 2012a, 2012b, 2012c) rather than *Listeria monocytogenes* so data on this pathogen are limited. Like other parts of the continent, few reports exist regarding the prevalence of *L. monocytogenes* in West Africa. Using agar-based techniques, some studies carried out in northern Nigeria by Yakubu et al. (2012) and Salihu et al. (2008) obtained prevalences of 5.3 % and 25 % in bovine milk and smoked fish, respectively.

4.2 *Virulence and Serotypes of Circulating* Listeria monocytogenes *Strains*

L. monocytogenes strains are diverse (Kathariou 2002), having dissimilar virulence characteristics. Usually, the pathogen's virulence genes inhabit a 9.6-kb chromosomal region (Gouin et al. 1994), where the *prfA* gene regulates these clustered genes on the chromosome (Chakraborty et al. 1992). In every successful infection of a host, *L. monocytogenes* makes use of several virulence factors (Doyle 2001; Vazquez-Boland et al. 2001; Liu 2006). These include internalins (*inlA* and *inlB*), surface protein p104, listeriolysin O (LLO: encoded by *hly*), ActA protein, phospholipases (phosphatidylinositol-specific phospholipase C (PI-PLC, encoded by *plcA*) and a broad-range or phosphatidylcholine-specific phospholipase C (PCPLC, *plcB*)), zinc-dependent metalloprotease, Clp proteases and ATPases, protein p60, and stress response genes (*opuCA*, *lmo1421*, and *bsh*). Only very few of the virulent genes expressed by *L. monocytogenes* have been determined in the existing circulating strains, and they are mostly from North Africa (Fig. 1). Abd El-Malek et al. (2010) and Abeer et al. (2012) determined the presence of *prfA* and *actA* genes in *L. monocytogenes* in some ready-to-eat foods in Egypt; in camel milk, the prevalence of *actA* in *L. monocytogenes* is 2.16 % (Abd El-Malek et al. 2010). Also, Ennaji et al. (2008) confirmed the presence of virulent *hly* genes in some foods sourced from Morocco. However, there are very few additional reports on other virulence factors responsible for *Listeria* pathogenesis in *L. monocytogenes* isolates from other parts of Africa.

Based on the specific O and H surface antigen of *Listeria* species, 12 or more serotypes of *L. monocytogenes* have been typed using serological detection (Liu 2006; Arun 2008). These serotypes include: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e, 4ab, and 7. Serotypes 1/2a, 1/2b, and 4b are the most virulent causing up to 98 % of human listeriosis. This nomenclature has also been used to define three existing *L. monocytogenes* lineages: Lineage I (highly pathogenic with epidemic clones, 1/2b, 3b, 4b, 4d, 4e), Lineage II (medium pathogenic, 1/2a, 1/2c, 3c, 3a), and Lineage III (not very pathogenic, 4a, 4c). Lineages I, II, and III are responsible for most, rare, and small outbreaks, respectively (Wiedmann et al. 1996, 1997; Jacquet et al. 2002; Arun 2008). In ready-to-eat foods in Africa, circulating *L. monocytogenes* strains have been serotyped into 1/2a, 1/2b, 3b, 4b, 4d, 4e, and 7 (Molla et al. 2004; Ennaji et al. 2008; Morobe et al. 2009, 2012), indicating that more of the highly pathogenic lineages with epidemic clones are circulating in Africa. Also, because these identified serotypes are from only three countries (Fig. 1), studies on serological typing *L. monocytogenes* in African foods are needed.

5 Resistance of *Listeria monocytogenes* to Antimicrobials

Antimicrobial susceptibility test have shown that *L. monocytogenes* isolates in Africa were sensitive to a wide range of antibiotics. However, since the first documented report of multidrug-resistant *L. monocytogenes* in France in 1988 (Poyart-Salmeron et al. 1992), several studies in all parts of the world, including Africa, have isolated pathogens that are resistant to one or more antibiotics.

Table 1 Antibiotics commonly resistant to *L. monocytogenes* in Africa

Resistant antibiotics	LM source	Country	Authors
Ampicillin	Raw milk	Nigeria	Yakubu et al. (2012)
	"Kunu"	Nigeria	Nwachukwu et al. (2009)
Penicillin G	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Sulfamethoxazole/ trimethoprim	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Chloramphenicol	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
Tetracycline	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
	"Wara" (local cheese)	Nigeria	Adetunji and Adegoke (2008)
Nitrofurantoin	"Wara" (local cheese)	Nigeria	Adetunji and Adegoke (2008)
	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Novobiocin	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
Streptomycin	Cheese, raw milk, cabbage, salad	Botswana	Morobe et al. (2009)
	Raw milk	Nigeria	Yakubu et al. (2012)
Gentamicin	Raw milk	Botswana	Morobe et al. (2009)
	Raw milk	Nigeria	Yakubu et al. (2012)
Cloxacillin	Meat tables	Nigeria	Adetunji and Isola (2011a, b)
Nalidixic acid	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)
Colistin	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)
Cephalosporins	Poultry, red meat, and sausages	Morocco	Ennaji et al. (2008)

Antimicrobial resistance is also common in the African food industry. *L. monocytogenes* is confirmed to be resistant to over 13 commonly used antibiotics (Table 1), especially isolates from ready-to-eat foods in Botswana, Morocco, and Nigeria, but *L. monocytogenes* isolates usually have dissimilar resistance patterns. Also, other studies have shown that *L. monocytogenes* strains isolated from various food sources are resistant to tetracycline (Morobe et al. 2009; Adetunji and Adegoke 2008; Adetunji and Isola 2011a, 2011b) more than other commonly used antibiotics, which corroborates findings from other places (Charpentier et al. 1995; Charpentier and Courvalin 1999). One reason for this resistance may be because tetracycline is one of the most commonly used antibiotics in both animal and human therapeutics (Morobe et al. 2009). Of the six classes of tetracycline-resistance genes—*tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(P), and *tet*(S)—that confer the reduced susceptibility of bacterial pathogens to tetracycline (Charpentier et al. 1995), *tet*(L) and *tet*(S) are present in *L. monocytogenes* (Poyart-Salmeron et al. 1992; Charpentier and Courvalin 1999). Other commonly resistant antibiotics include ampicillin, gentamicin, nitrofurantoin, and streptomycin.

6 Biofilm, Cellulose, and Fimbriae Virulence Characteristics in *L. monocytogenes*

Factors such as biofilm, cellulose, and fimbriae formation in *L. monocytogenes* are responsible for its virulence and persistence. The level of production of these characteristics has been described to influence virulence in bacterial isolates (Hood and Zottola 1995; Gulsun et al. 2005; Adetunji 2010) and also has major implications in food-processing environments. Fimbria and cellulose formation help pathogens adhere to food contact surfaces (Abu-lail and Camesano 2003; Adetunji 2010). Furthermore, isolates' level of biofilm production influences the virulence characteristics of such isolates (Hood and Zottola 1995; Gulsun et al. 2005). Biofilms are aggregation of microbial cells organized within a glycocalyx (exopolysaccharide material made up of cellulose). Biofilms facilitate resistance of pathogens to antimicrobials, increase access to nutrients, protect bacteria from external assaults, and promote plasmid and gene transfer through quorum sensing (Jefferson 2004). These characteristics enhance persistence of pathogens in food-processing environments. Furthermore, hydrophobicity, nature and type of incubating surface/medium, pH, surface charge, and temperature all affect biofilm formation by pathogenic *Listeria* spp. (Wong 1998; Sinde and Carballo 2000; Donlan 2002). Some studies have investigated biofilm formation by *L. monocytogenes* strains in Nigeria (Adetunji and Adegoke 2008; Adetunji 2010; Adetunji and Isola 2011b; Adetunji and Odetokun 2012), finding that *L. monocytogenes* can form biofilms on cement, glass, wood, and steel food contact surfaces. These biofilms increase with higher incubation temperature and time, but

the degree of biofilm formation varied across the different surfaces. Reports on biofilms from other countries are scarce. Researchers in developing countries should investigate ways of mitigating biofilms in food-processing facilities since *Listeria* can persist for years (Unnerstand et al. 1996).

7 Controlling the Contamination, Spread, and Persistence of *L. monocytogenes*

Success in controlling *L. monocytogenes* contamination of ready-to-eat foods and dairy products depends largely on the level of sanitation and hygiene present during food processing. Various international food safety authorities have recommended that *L. monocytogenes* should not be detected in 25 g of ready-to-eat foods (HPA 2009). If detected at levels greater than 10^2 cfu/g in foods, this indicates problems in various control points during food handling and processing, and it poses significant hazards for the at-risk populations. *L. monocytogenes* contamination usually arises because of poor-quality raw materials, inadequate cooking of foods, cross-contamination, poor cleaning and sanitation, and inappropriate temperature and time controls (HPA 2009).

Since *L. monocytogenes* is ubiquitous, environmental contamination should be avoided. Processing facilities and equipment should always be kept clean, before and after use. Cleaning must be thorough and strategic. Cleaning and sanitation schedules should be carefully formulated and strictly adhered to in every processing facility to achieve an appreciable level of effectiveness. All areas of the facility should be cleaned, including floors, roofs, walls, drains, pipes, etc. During cleaning, detergents and chemical sanitizers sensitive to *L. monocytogenes* should be used. Suggested sanitizers are formulations of iodoform, quarter ammonium compounds, peracetic/peroctanoic acids, and chlorinated solutions. Hot water should also be used during sanitation because it has been shown to be effective in pathogen removal. Processing facilities should be properly designed and suitable food contact surfaces should be carefully selected. Food contact surfaces that have lesser affinity for *L. monocytogenes* adhesion should be used in processing facilities. This will reduce the rate of biofilm formation and forestall dispersal of organisms that facilitate the spread of disease.

Furthermore, clean and dirty operations should be separated. Wastes generated during processing must be treated and properly disposed of. Local processing of ready-to-eat and dairy foods should also be standardized and employ new technologies. Good manufacturing practices are encouraged. *L. monocytogenes* colonization and infection must be treated as a bacterial hazard, and thus, the principles of hazard analysis critical control points (HACCP) must be applied. A good HACCP plan should be designed for all processing lines and the critical control points carefully identified. The HACCP plan should be well monitored to ensure that the system is working. Also, effective pathogen detection procedures must be utilized.

These steps will reduce *L. monocytogenes* colonization, transmission, and cross-contamination in processing facilities. Personnel and workers handling ready-to-eat and dairy foods should be educated and encouraged to observe maximum cleanliness during processing, packaging, transportation, and display. Protective clothing including neat aprons, hand gloves, head caps, face masks, etc. must be worn at all times. Personnel should maintain regular cleanliness. They should not observe any contacts between raw and finished products. Products must be well packaged and any source of possible *L. monocytogenes* contamination must be avoided. Strict operation measures must also be applied when products are displayed for sale. It is imperative that all African countries establish food safety authorities that will set the required microbiological standards for ready-to-eat and dairy products consumed in these countries. Measures to enforce these set standards must also be instituted.

8 Conclusion

L. monocytogenes is a pathogen that is a public health concern in food-processing environments. Its wide environmental distribution pattern, persistence through biofilm formation, and ability to cause illnesses in humans, especially through consumption of contaminated ready-to-eat foods, are of serious concerns. Mostly, ready-to-eat foods including dairy products are poorly processed, making *L. monocytogenes* infections and outbreaks common in Africa, but they are clearly underreported. To improve the microbial quality and control contamination of ready-to-eat foods, especially by *L. monocytogenes*, the processing lines of these foods should be standardized with modern technologies that would limit pathogen contamination, colonization, spread, and persistence in processing plants. Surveillance of *L. monocytogenes* and its associated infections especially in at-risk populations is appropriate. More studies are required to highlight the current prevalence of listeriosis in all African regions and countries. The virulence and serotypes of circulating *L. monocytogenes* in ready-to-eat foods across the continent need to be unraveled, particularly using current molecular isolation and serotyping techniques. This will allow for an important differentiation of all the circulating *L. monocytogenes* strains. Finally, with the increasing resistance of *L. monocytogenes* to most currently used antibiotics, authorities should also consider shifting to the use of natural medical plants as antimicrobials and biopreservatives.

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