Agricultural Science, Engineering and Technology Research
Vol. 1, No. 4, December 2013, PP: 60-69
Available online at http://asetr.org/

Research article

Characterization of *Listeria monocytogenes* Isolated from Retail Organic Chicken

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Abstract

Listeria monocytogenes is an important foodborne pathogen. It is commonly found in the environment, frequently present in the gut of cattle, poultry, and pigs and can be transmitted to ready-to-eat foods as well as raw meat products. However, no data are available on the prevalence of L. monocytogenes in organic foods. Throughout its history Listeria has been observed, studied and phylogenetically classified by numerous researchers. Because of the uncertainty of its phylogenetic position and its morphological similarity to the group of coryneform bacterium, names such as, Corynebacterium parvulum and Corynebacterium infantisepticum have been used to describe the organism. In addition, serotyping and subtyping isolates of the pathogen L. monocytogenes is not only important for epidemiological reasons but for increasing our knowledge about the ancestry, evolution and virulence of this important foodborne pathogen. **Copyright © ASETR, all rights reserved.**

Key words: Listeria monocytogenes, organic foods, chicken, food borne pathogens

Introduction

Listeria monocytogenes was first reported in 1924 by E.G.D. Murray (Murray *et la.*, 1992). Murray isolated the organism, which caused monocytosis, from rabbits and guinea pigs. In 1929, Nyfeldt described the *Bacterium monocytogenes hominis* [Nyfeldt. *et al.,1992*] as pathogenic to humans as well. It was not until the early 1980's that *L. monocytogenes* was first recognized as a food-borne pathogen (Schlech. et'al.,1983). Although today there is a clearer understanding of the organism and its relationship to other organisms, this has not always been the case. Throughout its history *Listeria* has been observed, studied and phylogenetically classified by numerous researchers.

Because of the uncertainty of its phylogenetic position and its morphological similarity to the group of coryneform bacterium, names such as, *Corynebacterium parvulum* (Schultz. et.al., 1934) and *Corynebacterium infantisepticum* have been used to describe the organism.

It was not until the 1970s that it's phylogenetic relationship, reinforcing its distinctiveness from the coryneform bacteria, was better understood. Its position was better clarified because of the development of numerical taxonomy, chemotaxonomy, DNA/DNA hybridization and rRNA sequencing techniques (Rocourt, 1999). Currently the genus is taxonomically classified with its phylogenetic position being closely related to *Brochothrix* (Collins,1991). However its relationship with other low G+C% Gram-positive bacteria [26], as well as *Bacillus* and *Staphylococcus* still needs to be clarified. Since the organism was first recognized, much information on its ecology, pathogenicity and the epidemiology of listeriosis has been revealed, yet the organism is still not completely understood nor is its presence in food under control.

Organism

Listeria is a gram-positive, non-sporulating, catalase positive, Oxidase negative rod, which measures 0.5 um in diameter and 1 - 2 um in length. Gram stains show that the cells can be found in chains or as single rods. Growth of the organism on bacteriological media is enhanced by the presence of glucose or other fermentable sugars but is also dependent on the atmosphere and temperature in which they are grown. The organism can grow over a wide range of pHs (4.3- 9.6), water activity (~ 0.83) and salt concentrations (up to 10 %) as well (Seeliger, 1986). *Listeria* are aerobic, microaerophilic and facultatively anaerobic and can be cultured over a wide temperature range.

The organism has a growth temperature range of approximately 1°C -45°C, [44], making it a psychrotroph and a mesophile. There are however, growth factors which are temperature dependent. For example, at 20-25°C peritrichous flagella are formed and cause the organism to be motile, whereas at 37°C the organism is weakly or non- motile [29]. Additionally, its ability to not only survive but to grow as a psychrotroph at 4°C makes this pathogen unique from other commonly found food-borne pathogens which are usually inhibited from growth at refrigeration temperatures.

For many years the genus *Listeria* only contained one species, *L. monocytogenes*. Currently however, there are six recognized species including *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii,* and *L. grayi (*Rocourt, 1999). Although there are six distinct species they all have similar genetic homology which helps explain their similar phenotypic traits. Hemolysis as well as acid production are key characteristics in distinguishing among the species. At present only strains of *L. monocytogenes* are pathogenic to humans and animals, while *L. ivanovii* are only pathogenic in animals, particularly ruminants (Van der Elzen,1993).

Virulence

The *L. monocytogenes* genome is approximately 3.0 Mb (Michel, 1992). (Genbank/EMBL accession number AL591824) and information on its sequence can be found at the Institute for Genomic Research (www.tigr.org). Virulence and virulence-like genes on *Listeria's* chromosome code for surface and secreted proteins as well as other regulators which help it to adapt to diverse environments and for expression of virulence traits. Further, species such as *L. innocua* lack genes which are essential for virulence. For example, a virulence gene such as one which codes for a surface protein and plays a role in invasion is present in *L. monocytogenes* but is absent in *L. innocua* and this may help explain the different pathogenic potentials of different species (Kuhn, 1999).*Listeria monocytogenes* and *L. innocua* both contain a virulence gene cluster located on an 8.2 kb pathogenic island on its genome [45] which is regulated by the main positive regulatory factor A regulon (PrfA) [18]. The cluster is located between the *prs* and *ldh* genes on the chromosome (Gouin, 1994).

Listeria's pathogenicity is not only contributed by its existence as an intracellular pathogen but also to its ability to invade and replicate within a wide range of mammalian cells. The rate of internalization is dependent on the cell type and may be mediated by at least one bacterial surface protein. The bacterial surface proteins *inlA* and *inlB* [10] are responsible for promoting the binding and internalization by either E-cadherin or the Met receptor tyrosine kinase and PI3- kinase activation respectively (Cossart, 2001). Once the organism is internalized a key virulence determining protein, LLO, as well as other secretory proteins PI-PLC and PC-PLC, aid the release of the bacteria from the vacuole which it resides in (Gedde, 2000) After cells have multiplied in the cytosol, an actin-based motility protein necessary for cell-to-cell spread, ActA is synthesized. The protein induces the polymerization of host actin filaments and allows the pathogen to propel itself into other cells as a type of pseudo-pod while evading the host's defenses (Portnoy, 2002). This intracellular cycle and cell-to-cell spread is then continued onto the next cells to continue the infection.

Disease

Listeriosis is the disease caused by *L. monocytogenes* infections. *Listeria* is widely distributed in the environment and can also be found in the gastrointestinal tract of individuals who remain as asymptomatic carriers. This non-invasive listeriosis occur s in healthy adults but generally only amounts to gastrointestinal illness, fever, vomiting and diarrhea, where the degree of severity is dependent on the characteristics of the host and the organism's environment. On the other hand, the more severe form of listeriosis is invasive listeriosis. The most common invasive listeriosis infections occur in children, the elderly, pregnant women and their fetuses and the immunocompromised. With the onset of epidemics such as HIV/AIDS, there has been an increase in the size of the population at risk of morbidity and mortality due to this type of listeriosis (Schlech, 2000).

The disease can manifest as septicemia, meningitis, meningoencephalitis or febrile gastroenteritis and can cause still births and abortions (Vazquez, 2001). The infective dose has not yet been definitively determined but it may take less than 1000 cells to cause infection. However, this is dependent on the immunity of the infected individual and the strain of the organism. The incubation period can range from a few days to three weeks and may be preceeded by gastrointestinal symptoms which manifest after approximately 12 hours incubation (Mead, 1999).

The rate of normal healthy adult infection from listeriosis is low. There are approximately 0.7 cases per 100,000 persons. However, the infection is more common in children at a rate of 10 cases per 100,000 person, and the elderly with 1.4 cases per 100,000 person (Gellin, 1991). Pregnant women are seventeen times more likely than healthy adults to acquire the infection (Southwick, 1996). Although listeriosis is considered a food-borne infection and most outbreaks are transmitted by food, there have been reports of large outbreaks attributed to other modes of transmission. For instance, a neonatal outbreak in Costa Rica involved the use of contaminated mineral oil for cleaning infants after delivery (Schuchat. et.al., 1991). In addition the pathogens which cause neonatal bacterial meningitis in North America, *Listeria* is the third most common pathogen followed by group B streptococcus and *E. coli.* Fetuses can acquire the infection through the mother who has either colonized the organism in the gastrointestinal (GI) tract after consumption of contaminated foods or during childbirth if a mother is carrying *Listeria* in the GI or the perianal region, which in turn can contaminate the skin and respiratory tract of the child during birthing. Although cases unrelated to food do occur, foodborne transmission is the most common source of transimission of *Listeria* to humans.

In the U.S there are an estimated 76 million cases of food-borne illnesses each year. The incidences of listeriosis only average 2500 infections yearly but cause 500 fatalities (Mead . et.al,1999.). Although the actual number of infections is low, a mortality rate, which can be as high as 20-30% regardless of antimicrobial treatment, shows the danger that the presence of *Listeria* poses in foods. The mortality rate is considerably higher than the more common

infections from other food-borne pathogens such as *Escherichia coli O157:H7 (E. coli), Campylobacter* spp. and *Salmonella* spp. (Mead . et.al. 1999).

There have been several sporadic and epidemic outbreaks worldwide implicating *Listeria* contaminated foods. Foods which are denoted as ready to eat (RTE) foods (deli meats, salads etc.), unpasteurized dairy foods (cheese and milk), cured and raw meats (hot dogs, undercooked chicken), and items such as prepared seafood salads and even raw and unprocessed meats have been common foods implicated (Schlech, 2000). Although a look back at some initial recordings of the organism and their related outbreaks demonstrate that a food-to- human route of transmission was likely, it was not established until the 1980's (Rocourt, 1999). There is still more to uncover concerning *Listeria's* relationship between environment, human and food. Listeriosis is often treated with antimicrobials. The most favored treatment consists of using a combination of ampicillin and an aminoglycoside (Hof, 1997). However the use of vancomycin in place of ampicillin is acceptable as well. Another treatment is the use of trimethoprim-sulfamethoxazol (TMP-SMZ) and rifampin. Cephalosporins, which are typically used for treatment of meningitis, are not effective for treating listeriosis because of *L. monocytogenes* resistance to this drug. However cephalosporins can be used in combination with ampicillin for listerial meningitis (Schlech, 2000). Overall, antimicrobial treatment against listeriosis can be slow and may even be untreatable or persistant (Slutsker and Schuchat, 1999).

However remaining vigilant about monitoring the pathogen is also important. This can be demonstrated by examining the way outbreaks are analyzed. PFGE patterns show which strains are likely responsible for an outbreak by matching the genomic patterns from clinical samples to suspected foods which may be vehicles of transmission. With the cooperation and standardization of laboratories performing the analysis, this becomes a rapid method for determining the source of infection by sharing of information and perhaps limiting the number of infections and subsequent deaths (Grave. et.al., 1999).

Characterization of the Organism

Phenotyping and genotyping methods to discriminate bacterial strains are valuable tools which have different levels of discriminatory power. These methods are able to provide information on strains which may be responsible for an outbreak, or to identify the relationship between isolates implicated in an outbreak, and also to help determine a source of transmission for an outbreak. Serotyping is based on the antigenic determinations expressed on the cell surface of the organism. These antigens are produced by lipoteichoic acids, membrane proteins, and extracellular organelles such as fimbriae and flagella (Seeliger. et.a.,1979). Different strains of *L. monocytogenes* express different antigenic determinations, thus each strain can be serologically identified. *Listeria* strains are separated based on flagellar (H) and somatic (O) antigens resulting in more than 13 serotypes (Seeliger. et.a.,1979). Genotypic analysis generally group *Listeria* into two main lineages, Lineage I and II while it is believed that there may be a third subgroup, Lineage III, as well. Lineage I includes serotypes 1/2a, 1/2c, 3a, and 3c 1, Lineage II includes serotypes 1/2b, 3b, 4b, 4d and 4e while serotypes 4a and 4c have been grouped with the third less common Lineage III [66, 37, 13]. The three serotypes which most commonly cause disease (> 95%) are 1/2a, 1/2b and 4b [60]. Although serotype 4b is most frequently implicated in foodborne diseases, it is serotype 1/2a which is most frequently isolated from foods (Gilot.et.al. 1996).

Historically, serotyping of *Listeria* by antiserum has shown to be unreliable. A study by (Schlech ,2000) and the World Health Organization (WHO), demonstrated that different laboratories using antiserum serotyping methods, either could not 100% correctly identify or 100% agree on the correct serotype of all the given isolates (Schlech ,2000) . In addition, serotyping with antiserum has been shown to have less discriminating power than other methods. For example, a Mismatch Amplification Mutation Assay (MAMA) using mismatched PCR primers targeting different sites in the *hly* gene was developed to rapidly screen *L. monocytogenes* isolates into their

respective phylogenetic divisions [43]. Other methods such as one using PCR primers which target select sequences in the four major serotypes to adequately differentiate them have also been recently developed. The results obtained with the PCR primers agree significantly enough with the traditional slide agglutination method (Borucki and Call, 2003) that perhaps this can one day be a standardized method. The discriminatory power of phenotypic and genotypic methods has also been investigated. In one study, phage typing was the most discriminatory of the methods tested followed by REA, MEE, and ribotyping when looking at differences based on "O" serotype discrimination. However, REA best discriminates for serotype 1 and phage typing was best for discriminating serotype 4 (Norrung and Gerner, 1993). However, there were studies which noticed that the discriminatory power of methods such as MEE and ribotyping could not adequately differentiate between closely related serotypes such as 1/2b and 4b (Graves, 1994).

Other methods to differentiate *L. monocytogenes* phenotypes, such as antimicrobial susceptibility are of limited use currently. At present there are no standards or standard guidelines which have been established for the organism. *L. monocytogenes* susceptibility to antimicrobials has also been relatively constant for several years; although there has been some evidence found that there are plasmids present which confer resistance to chloramphenicaol, macrolides and tetracyclines (Poyar et.al., 1992). Although phenotypic or conventional methods such as serotyping, bacteriophage typing, bacteriocin typing, and antimicrobial susceptibility have been used, with the development of molecular techniques some of those methods may have limited use in the future.

Some molecular methods that are commonly used include Multilocus Enzyme Electrophoresis (MEE), chromosomal DNA Restriction Endonuclease Analysis (REA) as well as Restriction Fragment Length Polymorphism (RFLP), Ribotyping and PFGE. The advantages to using each of these molecular techniques for genetic analysis vary. Several researchers along with those at the WHO's Multicenter *Listeria monocytogenes* subtyping study have compared the various molecular methods to determine which have the most discriminatory power.

Although MEE is a powerful tool for population genetic, taxonomic and evolutionary studies, its ability to discriminate subtypes for epidemiological investigations is limited [38]. Further, REA is universally applicable and sensitive because an entire genome can be evaluated easily and cost effectively, however its complex profiles, which may consist of hundreds of bands, is not practical to use epidemiologically (Graves, 1999). Ribotyping and RFLP have also been evaluated in their usefulness for epidemiological purposes. Ribotyping has been shown to be not as discriminatory as REA or MEE in subtyping *L. monocytogenes* (Graves, 2001).PFGE, another powerful subtyping technique, is considered the 'gold' standard for subtyping *L. monocytogenes*. PFGE takes advantage of restriction enzymes that cut genomic DNA infrequently which in turn produces simple profiles that can be analyzed by computer based methods. This allows easy, fast and simplified comparison of strains. This is extremely important when investigating food related outbreaks [39]. Strains suspected in outbreaks can be rapidly compared to those stored in a database which contains known epidemiologically significant strains.

Additional researchers demonstrated the usefulness of PFGE in discriminating between closely related 4b strains [12]. PFGE has since been shown to be applicable for use in typing strains involved in outbreaks of listeriosis (Buchrieser et.al.,1993).An additional study done by Brosch (Brosch.et.al. 1996) in collaboration with WHO reinforced the discriminatory and reproducibility of using PFGE in subtyping compared to the other methods which are less discriminatory and not satisfactorily subtyped especially for non-diverse serotypes such as the 4b group. PFGE uses an infrequent restriction endonuclease such as *AscI* to digest the *Listeria* genome into large fragments (> 40 kb). By using countour clamped homogenous electric field (CHEF) PFGE these larger fragments are then subjected to two opposing electrical gradients of a specified angle in order to allow the DNA to 'zig- zag' through the agarose gel. The DNA is then visualized using ultraviolet light after they have been stained and destained with ethidium bromide. The fragments are seen as bands and their patterns can be compared with other patterns, such as

those from other outbreaks or clinical samples, in order to help determine the epidemiology or origin of the strain. PulseNet, the National Molecular Subtyping Network for Foodborne Disease Surveillance (www.cdc.gov/pulsnet), is made up of a national network of public health and food regulatory laboratories in the U.S. These labs use standardized PFGE protocols to subtype bacteria which may be implicated in a food-borne outbreak. This network of laboratories in collaboration with the Centers for Disease Control (CDC) can use computers to rapidly compare PFGE patterns of isolates from patients and implicated samples from across the U.S. in order to determine the source of transmission. The PulseNet network allows for a rapid and standardized medium for public health officials to rapidly determine the epidemiology of an infection and to stop it from spreading. There has been a concerted effort to develop rapid protocols for organisms under the surveillance of PulseNet. Currently, protocols for *E. coli* O157:H7, *Salmonella* spp. and *Shigella* spp. have been developed (Gautom, 1997). In 2001 researchers at the CDC, developed a rapid standardized protocol for subtyping *Listeria* (Gautom, 1997). The result is a 30 hour protocol which has the same discriminatory power as a previously used multi-day protocol.

Listeria and Food

Because *Listeria* is ubiquitous in nature, its route of transmission was not always definitively categorized. Since the 1980's, it has been conclusively determined by epidemiologic and laboratory surveillance of contaminated cheese and raw vegetable outbreaks, that listeriosis is indeed transmitted by consumption of contaminated foods [74]. Although *Listeria's* relationship between the environment, animals, humans, and food is still not absolutely clear, this categorization of *Listeria* as a food-borne pathogen as well as its potential to harm brought the organism to the forefront of food safety and regulatory issues. There have been several sporadic and epidemic outbreaks worldwide implicating *Listeria* contaminated foods. Foods which are denoted as ready to eat (RTE) foods (deli meats, salads etc.), unpasteurized dairy foods (cheese and milk), cured meats (hot dogs, undercooked chicken), and items such as prepared seafood salads and even raw and unprocessed meats have been common foods implicated (Schlech, 2000).Since there is still more to uncover concerning *Listeria's* relationship between environment, human and food, it is necessary to emphasize the importance of prevention. This has prompted the U. S. Food and Drug Administration (FDA), the Food Safety and Inspection Service (FSIS), and the U.S. Department of Agriculture (USDA) to mandate a level of 'zero tolerance' (no detectable level) for *Listeria* in RTE foods.

Other European nations are aware of the risk from listeriosis and have implemented risk-based standards for keeping *Listeria* out of foods. Some noteworthy outbreaks which occurred in the United States include the following. In the fall of 2002, the largest recall in United States history occurred because of *Listeria* contamination. This recent and important outbreak affected multiple states encompassing the northeastern U.S. and involved a recall of approximately 27.4 million pounds of fresh and frozen turkey and chicken deli meats. There were 46 comfirmed cases of infections, with seven deaths and three still births reported across eight northwestern states. The overall mortality rate from this outbreak reached 22%, while 6.5% of those who died were new born infants. In addition, several outbreaks have been linked to soft-style cheeses such as those which occurred in 1985 [4] and another in 2000-01. The former outbreak, which covered a 7.5 month epidemic period, involved 142 listeriosis cases resulting in 48 deaths or a 33.8% mortality rate. Thirty of the deaths were fetal or neonatal deaths. The latter outbreak resulted in 12 infections. All the patients were pregnant women except for one post-partum woman and another immuno-compromised elderly man. The infections resulted in five stillbirths, three premature births and two infected newborns (Cossart, 2001). This also reinforces the need to continue surveillance programs such as PulseNet and CDC's Foodborne Diseases Active Surveillance Network (FoodNet). These programs allow for rapid epidemiological investigations to be performed. By sharing of databases with local, state and federal publich health laboratories, potentially serious outbreaks can be timely identified and foods implicated recalled as quickly as possible.

Food safety programs such as the Hazard Analysis Critical Control Point (HACCP) in addition to consumer education programs such as FIGHT – BACÒ and ThermyÒ (FSIS, USDA) are also in place. The former educate food producers and food handlers while the latter educate consumers about the importance of contamination and food safety at home. Although there is a need for food safety at the production level, the consumers can help lower the risk of foodborne infections through proper education from understanding the need to chill at proper temperatures, separate raw meats and ready to eat foods as well as cooking to proper temperatures. Although on-going efforts and new efforts are continually being made to eradicate food-borne pathogens from the food supply, they still persist and in some instances have adapted to the pressures used to eradicate them. Several case studies done by the CDC (Schwartz et.al.,1988) identified undercooked chicken as a high-risk vehicle of transmission. Although the role that *Listeria* plays in raw and undercooked chicken is still somewhat unclear, because with proper heating and food handling *Listeria* is often killed in raw foods and meats. Food survey studies conducted in other countries and in the U.S. also show prevalence in conventional chickens, although no data are available on organic chickens. For example, in 2001 a study done in Spain showed that *L. monocytogenes* was found on 15% of the chicken carcasses tested (Capita. et.al. 2001). Other studies have indicated that the rate of recovery of *L. monocytogenes* in raw chicken was 8 out of 70 (11.4%) total samples (Wang, 1992) and 34 of 58 (59%) (Lawrenece and Gilmour, 1994).

The pathogen remains problematic for the food industry because contamination often occurs during processing or post processing. With the modernization and centralization of food processing, *Listeria's* ability to survive and multiply has prevailed even with improved food preservation methods. Therefore in order to control the organism, it is necessary to minimize the potential points of entry for the pathogen. Over the years, a pattern has been seen which indicates that the initial point of contamination begins in the food processing environment. This trend is also evident in the slaughter houses of raw meats such as pork and poultry. Prior to slaughter, contamination of live animals with the pathogen is low. However once the animal enters the processing facility and is slaughtered, the prevalence in the environment becomes high. In surveys of a chilling-cutting area of several processing plants, post-slaughtered meats demonstrated a 70-100% increase in prevalence of the *Listeria* organism [92]. Similar trends of post-slaughter contamination in the processing plant are seen with poultry and turkey slaughterhouses and smoked fish plants (Autio et.al.,1999).

It also seems that the packaging of poultry products is important. *Listeria's* survival can be dependent on the ability of a packing method to suppress other organisms. Wimpfheimer (Wimpfheimer et.al., 1990) looked at the behavior of *Listeria* in different packing and storage conditions. There was an increase in the organism when raw chickens were packaged in a micro-aerophilic or an aerobic atmosphere and left in 4°C for an extended storage time. However there was a decrease in the number of spoilage organisms in the same package under microaerophilic conditions and a similar increase to *Listeria* in the aerobic packaging (Wimpfheimer et.al., 1990).

Although there have been data collected on foods both processed and raw, there has been a lack of data on a fast growing segment of the food industry, organic foods. The growth of the organic food industry has increased 20 - 25% in the U.S. over the last seven years. This has amounted to retail sales of $3.5 billion in 1996 up from $1 billion in 1991. There has also been an increase in foreign markets (Dimitri and Greene, 2002). Consumers and farmers have been increasingly interested in organic food products because organic foods are considered natural and healthy, although the health benefits as well as the food safety risks have not been clarified and much work still needs to be done. Organic farming emphazies the use of renewable resources to produce foods. Soil and water are also conserved to improve the quality of the environment. Conventional pesticides, fertilizers or other synthetic ingredients are also not allowed. Livestock animals must be given access to outdoor free range and be fed organic feed. In addition, they are not given any antibiotics for either growth promotion or as treatment for any disease.

Because of the lack in data and microbiological impact with the way organic farming is done, it is interesting to look at the effect of organisms such as *Listeria* in this new type of environment. Organic livestock production may increase the risk of microbial contamination and thus foodborne illness, due to outdoor production and complete prohibition of antibiotic use. However, there is a paucity of data with regards to the microbiological safety of organic food products. **The objective of this study is to review characterization of *Listeria monocytogenes* isolated from retail organic chicken**

Conclusion and recommendation

Despite having made important scientific advances in understanding the genus *Listeria* and its six species, much is still to be discovered of this pathogen especially on new and emerging markets such as in the organic foods market. Therefore an effort should be made to continue to perform surveillance research on the safety of animals and all foods produced under the organic seal. This study is one of the few which demonstrates that there indeed is a prevalence of *L. monocytogenes* on raw foods and one of the first to demonstrate prevalence on organic foods. This in turn can be dangerous if improper food handling occurs either at the processing level and/or at the consumer level. In addition, serotyping and subtyping isolates of the pathogen *L. monocytogenes* is not only important for epidemiological reasons but for increasing our knowledge about the ancestry, evolution and virulence of this important foodborne pathogen.

In order to better understand the relationship between the prevalence of *Listeria* on organic and conventional chickens, the experimental design of this study needs improvement. It would be necessary to continue the isolation from additional conventional chickens in order to get a better idea of how the prevalence rates relate in chickens raised in these two different environments.

Future work can also be done on the remaining isolates which were untested in this study. It would be interesting to understand the virulence potentials among these isolates, especially those included in the 4b serotype because they are most often implicated in outbreaks which cause disease. Since only one isolate from each of the positive *L. monocytogenes* chicken samples were tested, the remaining isolates can also be examined for their clonality as well as their serotype and genetic diversity. Lastly, an antimicrobial susceptibility profile would be of significant value. Because these isolates came from organic chickens, they should exhibit less resistance to antimicrobials commonly associated with *Listeria* antimicrobial resistance.

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