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Antimicrobial-Resistant Listeria Species From Retail Meat In Metro Detroit

Liziane Da Rocha
Wayne State University,

ANTIMICROBIAL-RESISTANT *LISTERIA* SPECIES FROM RETAIL MEAT IN METRO DETROIT

by

LIZIANE SIPPEL DA ROCHA

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DEDICATION

This thesis is dedicated to my husband, Sandro R. P. da Rocha, whose love and support surrounded me at every moment of this journey, my daughters Luiza, Caroline and Giulia for their love and understanding when I was busy with my studies and sometimes there was not time for a playtime; my parents, Jose Walter and Vanete, and parents in law Waldyr (in memorian) and Josefina, for their words of support and encouragement when I needed. To my brother Rafael and sisters in law Andreia and Verginia, and all my friends in a way or the other always encouraged and supported me to follow it through. I can not thank you enough!!

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TABLE OFCONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Listeria are Gram-positive rod-shaped bacteria and ubiquitous in the environment. They are remarkably tolerant to harsh environmental conditions such as temperature changes, detergents, high salt concentrations, extreme pHs, and resistance to freezing and drying [1] [2]. *Listeria* are able to survive in many food manufacturing processes [3] due to their psychrotrophic nature, meaning being able to propagate at the refrigeration temperature. A variety of foods such as smoked seafood, raw and cooked meats, dairy products such as milk, ice cream and soft cheeses, ready to eat (RTE) meats, raw vegetables and fruits have been associated with the disease transmission of foodborne listeriosis [4, 5]. It is also worth to mention that cross contamination can occur at any time during food handling, food processing and post processing.

Listeria are composed of eight species, including *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeria seeligeri*, *Listeria welshimeri*, and two recently-identified species, *Listeria marthii* [6] and *Listeria rocourtiae* [7]. The common *Listeria* isolated from food are *L. innocua*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri* [8-10], among which *L. monocytogenes* is the only human pathogenic species. Listeriosis, the disease caused by *Listeria* infection, is among the leading causes of death from foodborne illness. *L. monocytogenes* causes an estimated of 1,591 cases and 255 deaths in the United States annually, with mortality rates ranging from 20 to 30% in foodborne outbreaks. *L. monocytogenes* invades human intestinal cells and survives and proliferates inside phagocytic vacuoles and nonphagocytic cells, such as epithelial cells, hepatocytes, and endothelial cells [11, 12]. For healthy individuals, *Listeria* may cause mild symptoms or have no effect at all. Gastrointestinal, flu like symptoms, fever, muscle ache, nausea, vomiting and diarrhea are common signs of

infections [13]. At great risk to acquire listeriosis through contaminated foods are individuals with suppressed or immunocompromised health [14]. Those are patients with cancer, transplant, human immunodeficiency virus (HIV), the elderly, infants, and pregnant women [15]. For such individuals, symptoms can be more severe and include septicemia, meningitis, pneumonia, stillbirth, miscarriage, and death [13].

Another relevant public health concern is the emergence of multiresistant strains of *Listeria*. *L. monocytogenes* in particular, and other *Listeria spp*. in general, have been closely inspected for antimicrobial resistance since multiresistant strain was first documented in France in 1988 [16]. Antibiotics are commonly used in human and veterinary medicine to treat and prevent diseases. Antibiotics are also used as growth promoters in food-producing animals [17]. The excessive use of antibiotics can promote the development of resistance and the resistant populations may spread in host-animals [18]. Importantly, antimicrobial resistance in animals can be transferred to humans, and this represents a major threat to human life [19]. It is important, therefore, to promote the judicious use of antibiotics in humans and food-producing animals [20], as well as to promote surveillance studies of the development of resistance in animal products [21].

Listeria strains with resistance to one or more antibiotics have been recovered from food, environment, and from sporadic cases of human listeriosis [9] [22]. Considering the high mortality rate of listeriosis (20 to 30%), it is important to ensure the effectiveness of antimicrobials for listeriosis and monitor the emergence of antimicrobial-resistant *Listeria* strains. The antimicrobials used to treat human listeriosis are ampicillin with gentamicin or penicillin alone [23]. The second choice of treatment is sulfamethoxazole/trimethoprim. Erythromycin, gentamicin, and vancomycin are also alternatives when patients are allergic to the

penicillin antimicrobials or the physical condition of patients does not allow the common antimicrobial treatment [24, 25]. Although *Listeria* is usually susceptible to many antimicrobials of clinical importance, antimicrobial-resistant *Listeria* does occur, both in clinical settings and along the food chain [9, 22]. Additionally, inter-species variation in antimicrobial susceptibilities has been reported in *Listeria*. Antimicrobial resistance was observed in 0.6% of *L. monocytogenes* compared to 19.5% of *L. innocua* from retail foods [8]. Davis and Jackson [22] reported all six *L. welshimeri* and all four *L. innocua* isolates resistant to penicillin, whereas none of 90 *L. monocytogenes* resistant to this antimicrobial. Another study examining *Listeria* of multiple origins found that *L. grayi* had higher resistance prevalence against trimethoprim and sulfamethoxazole/trimethoprim than other *Listeria* species [26]. Although *L. grayi* is distantly related to the other *Listeria* species [10], these findings may still be a reflection of species-dependent antimicrobial susceptibilities in *Listeria*. Since most resistance phenotypes in *Listeria* appear as a result of horizontal transfer of resistance genes [21, 23, 27, 28], the genetic exchange among different species may facilitate the emergence of antimicrobial-resistant strains that have potential to cause human diseases. This is especially of concern when multiple *Listeria* species exist in the same environmental niches.

L. monocytogenes includes 13 serotypes, three of which (1/2a, 1/2b, and 4b) have been associated with the vast majority of foodborne infections. The overrepresentation of *L. monocytogenes* serotype 1/2a in food isolates has been speculated to be associated with their higher resistance prevalence than other serotypes to food sanitizers and phage in food processing environment [29-31]. However, studies comparing antimicrobial resistance among *L. monocytogenes* serotypes are still limited and they may provide more insight into antimicrobial selection on the prevalence of different serotypes in both agricultural and clinical environments.

CHAPTER 2

Objectives of this Study

The present study aimed at understanding the distribution of *Listeria* species and *L. monocytogenes* serotypes in retail meat as it related to their antimicrobial susceptibilities. The data will add to our knowledge of the extent of antimicrobial resistance reservoir in foodborne *Listeria* and also the contribution of antimicrobial resistance to the occurrence of different *Listeria* in the environment.

CHAPTER 3

Materials and Methods

2.1. Sample description and bacterial isolation

A total of 243 raw meat samples, including 133 beef, 65 chicken and 45 turkey samples, collected in local grocery stores in the metro Detroit area were examined for *Listeria* contamination. FDA Bacteriological Analytical Manual protocols [32] were followed with modifications for *Listeria* isolation. Twenty-five grams of each sample was aseptically transferred into stomacher bags containing 225 ml of buffered *Listeria* enrichment broth (BLEB, Difco, Detroit, MI) and homogenized for 2 minutes. Fifty milliliters of the homogenized sample were incubated at 30°C for 4 hours for pre-enrichment, followed by an addition of selective agents, acriflavin (10 mg/L) and nalidixic acid (40 mg/L) and incubation for 48 hours at 30°C. Enriched broth was spread on PALCAM agar (Difco, Detroit, MI) at 24 h and 48 h of selective enrichment. Plates were incubated at 35°C for 24 h. Potential *Listeria* was streaked on Rapid L'mono differentiation agar (Bio-Rad Laboratories, Hercules, CA) for species identification, followed by PCR confirmation of *Listeria* [33].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *Listeria* was performed by the disc diffusion method as recommended by the Clinical Laboratory Standards Institute [34]. The following antimicrobials were tested: ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, penicillin, sulfamethoxazole/trimethoprim, tetracycline, and vancomycin (BD, Franklin Lakes, NJ). Diameters of growth inhibition zones were measured and interpreted according to the breakpoints recommended by CLSI. The only resistance breakpoints available

for *Listeria* are those for ampicillin and penicillin. For antimicrobials that currently have no interpretive criteria, resistance breakpoints for staphylococci and enterococci were applied. *Staphylococcus aureus* ATCC 25923 and *L. monocytogenes* ATCC 19115 were used as quality control microorganisms.

2.3. DNA extraction by boiling method

Bacterial strains of *L. monocytogenes* were recovered from -80ºC and after thawed and streaked on BHI and incubated at 37ºC overnight were used for DNA extraction. Bacterial culture was collected and resuspended in 500 µl of deionized water, vortexed, and placed on thermocycler for 10 min. at 100ºC. The samples were centrifuged at 14,000rpm/min. for 5 min. and the tubes were gently removed from the centrifuge. The supernatant was transferred to microcentrifuge tubes and labeled. Samples were then stored at -20ºC for further use. A 2 µl of supernatant was used for PCR reaction.

2.4. L. monocytogenes species and *serotype identification*

PCR was performed to identify *L. monocytogenes* species, serotypes 1/2a, 1/2b, and 4b, as previously described [33, 35]. Genomic DNA was extracted by the boiling method as discussed above, from *L. monocytogenes*. Primer sequences are: *L. monocytogenes* species, (F) TGTCCAGTTCCATTTTTAACT, (R) TTGTTGTTCTGCTGTACGA; serotype 1/2a, (F) GAGTAATTATGGCGCAACATC, (R) CCAATCGCGTGAATATCGG; serotype 1/2b, (F) AAAGTGAGTTCTTACGAGATTT, (R) AATTAGGAAATCGACCTTCT; and serotype 4b, (F) AGTGGACAATTGATTGGTGAA, (R) CATCCATCCCTTACTTTGGAC. DNA amplicons were separated on a 1.5% agarose gel stained with ethidium bromide and visualized under UV.

2.5. Pulsed-field Gel Electrophoresis (PFGE)

PFGE was carried out following the standardized PulseNet protocol for *L. monocytogenes* [36]. Briefly, genomic DNA was prepared by mixing 240 µl of standardized cell suspension and 60 µl of 10 mg/ml lysozyme solution (Sigma, St. Louis, MO), followed by incubation at 37°C for 10 min. Sample plugs were digested with 25 U of *Asc*I (New England BioLabs, Ipswich, MA) at 37°C for 3 h. Plugs were then loaded on 1.2% Megabase agarose gel (Bio-Rad, Hercules, CA) in 0.5× Tris-Borate EDTA buffer (TBE, EMD Chemicals, Billerica, MA) at 14°C and electrophoresed on a CHEF-DR III apparatus (Bio-Rad Laboratories) using the following parameters: initial switch time, 4s; final switch time, 40 s; run time, 22 h; angle, 120°; gradient, 6 V/cm; temperature, 14 °C; ramping factor: linear. The PFGE patterns were analyzed using the BioNumerics software (version 6.5; Applied Maths, Austin, TX). The TIFF images were normalized by aligning the peaks of the size standard strain (*L. monocytogenes* H2446), which was loaded in at least two lanes on each gel. Clustering was performed by using the Dice similarity coefficient and the unweighed pair group method with arithmetic means (UPGMA), with 1.5% of position tolerance and 1% optimization.

CHAPTER 4

RESULTS

Seventy-four meat samples (30.4%) were contaminated with *Listeria* species, including 45 beef (33.8%), 19 chicken (29.2%), and 10 turkey samples (22.2%) (Table 1). Overall, *L. welshimeri* was the most prevalent species and identified in 35 samples (14.4%), followed by 26 samples contaminated with *L. monocytogens* (10.7%) and 22 carrying *L. innocua* (9%). Nine samples, including two beef, four chicken, and three turkey products, were contaminated with two *Listeria* species, one being *L. monocytogenes* and the other *L. innocua* or *L. welshimeri*. Species ranking differed among meat categories. *L. welshimeri* was recovered the most from chicken, whereas no marked difference in ranking was observed in beef and turkey (Table 1).

A total of 138 *Listeria* isolates, consisted of 58 *L. welshimeri*, 44 *L. monocytogenes*, and 36 *L. innocua,* were examined by antimicrobial susceptibility testing against nine antimicrobials, including those used to treat human listeriosis. Although most isolates were antimicrobial susceptible, resistance phenotypes were detected to eight antimicrobials, except for chloramphenicol, giving an overall resistance prevalence of 23.2% (32 of 138) (Table 2). Species variation in antimicrobial susceptibilities was observed. *L. innocua* demonstrated the highest overall resistance prevalence, being 36.1% (13 of 36), followed by a close second of 34.1% (15 of 44) in *L. monocytogenes*, and 6.9% (4 of 58) in *L. welshimeri*. The same species ranking was recorded in numbers of antimicrobial resistance phenotypes identified. Resistance to five antimicrobials was seen in *L. innocua*, including ampicillin, ciprofloxacin, penicillin, tetracycline, and vancomycin. *L. monocytogenes* were resistant to four antimicrobials, including erythromycin, gentamicin, sulfamethoxazole/trimethoprim, and/or tetracycline. *L. welshimeri* was resistant to ciprofloxacin, gentamicin, and/or vancomycin.

Tetracycline resistance was the most common resistance phenotype and identified in 13 *L. monocytogenes* and nine *L. innocua*. Vancomycin resistance was identified in one *L. innocua* and two *L. welshimeri*. Two each of *L. innocua* and *L. welshimeri* were resistant to ciprofloxacin. Resistance to ampicillin, erythromycin, penicillin, and sulfamethoxazole/trimethoprim was identified in four individual isolates. Of 32 antimicrobial-resistant *Listeria*, 29 were resistant to only one antimicrobial. Three isolates, one each of the three species, demonstrated resistance to two antimicrobials. They were one *L. innocua* resistant to ampicillin and vancomycin, one *L. monocytogenes* resistant to gentamicin and tetracycline, and one *L. welshimeri* resistant to ciprofloxacin and vancomycin. No multidrug-resistant isolates were recovered.

Forty-four *L. monocytogenes* isolates from 26 meat samples were further analyzed by serotype identification by PCR and DNA fingerprinting by PFGE. The isolates consisted of 32 *L. monocytogenes* from beef, seven from turkey, and five from chicken. Serotypes 1/2a, 1/2b, and 4b were identified in 19, 23, and one isolates, respectively (Figure 1). One remaining isolate from turkey was untypeable by the PCR scheme applied. Of 44 *L. monocytogenes*, 15 (34%) demonstrated resistance to at least one antimicrobial and included two of 1/2a, 12 of 1/2b, and one of serotype untypeable. Tetracycline resistance was the most common resistance phenotype in *L. monocytogenes* and identified in 13 isolates (Figure 1), among which 11 were 1/2b and two were 1/2a. The remaining two antimicrobial-resistant *L. monocytogenes* isolates were one of 1/2b resistant to sulfamethoxazole/trimethoprim and one serotype untypeable isolate resistant to erythromycin. In addition to tetracycline resistance, one 1/2b isolate (ID 24-2) was also resistant to gentamicin and intermediately resistant to vancomycin. Intermediate resistance was also observed in the vast majority of *L. monocytogenes* isolates to penicillin and half the isolates to ciprofloxacin. The only 4b isolate was susceptible to all antimicrobials tested.

PFGE identified 40 unique DNA patterns in 44 *L. monocytogenes*. Although most isolates from same meat samples were unique, indistinguishable clones were also recovered, such as isolates 4-2 and 4-4, 24-1 and 24-2, and 26-1 and 26-2 (Figure 1). Isolates 4-2 and 4-4 showed distinct antimicrobial susceptibility profiles in spite of identical PFGE patterns, and so did isolates 24-1 and 24-2. Meanwhile, indistinguishable PFGE patterns were also identified from different meat samples, such as isolates 3-1 and 5-1 from beef samples that were collected two months apart from the same store. Although most 1/2a isolates were clustered on the top of the dendrogram and 1/2b mainly in the middle, some 1/2a and 1/2b isolates did cluster together. Of particular note, there was only one band difference between isolates 14-1 (1/2b) from turkey and 15-1 (1/2a) from beef.

CHAPTER 5

Discussion

The present study demonstrated retail meat as a reservoir of common *Listeria* species, including *L. welshimeri*, *L. monocytogenes*, and *L. innocua*. Recovery of 32 *Listeria* resistant to ampicillin, penicillin, gentamicin, and sulfamethoxazole/trimethoprim suggests resistance to antimicrobials of human clinical importance is not uncommon in *Listeria*. The observation of inter-species variation in antimicrobial susceptibilities and co-existence of more than one *Listeria* species in the same meat samples raises concerns on antimicrobial resistance transfer in *Listeria* as this may contribute to the emergence of new antimicrobial-resistant *L. monocytogenes* clones considering that all nine multi-species-carrying samples had *L. monocytogenes*.

L. monocytogenes and *L. innocua* have been the most prevalent *Listeria* species in food and food processing environments [10, 37]. Although they were also seen in high prevalence in this study, *L. welshimeri* outnumbered both species, which was mainly due to its high recovery in chicken. In a similar study, *L. welshimeri* predominated in ready-to-eat meat and fish in Canada [38], suggesting possible food variations in *Listeria* contamination.

Tetracycline resistance is still a common resistance phenotype in *Listeria* as reported in several studies [8, 21, 39]. This was also evidenced in the current study and may be an indication of extensive tetracycline use in meat production. Identification of ciprofloxacin resistance in *Listeria* was not surprising as nalidixic acid has been commonly used as a selective agent in *Listeria* isolation and may have selected ciprofloxacin resistance in some isolates. Vancomycin is one of the last resources for many Gram-positive bacteria infections and can be used to treat primary *Listeria* bacteremia [40]. Although no clinical infections due to vancomycin-resistant *Listeria* have been reported, resistant strains of *Listeria* have been isolated

from food products. Researchers in Europe have reported vancomycin resistance in *L. monocytogenes* in fish [41] and *L. innocua* in retail food [8]. Together with our findings of vancomycin resistance in two *L. welshimeri* and one *L. innocua*, the data may suggest an important reservoir of vancomycin resistance in *Listeria* of environmental origin. Previous studies also showed that *vanA* was able to transfer via conjugation from *Enterococcus faecium* to multiple *Listeria* species, including *L. monocytogenes* [23, 42]. Failure to identify the resistance genes in our study (data not shown) does not exclude the possibility that the vancomycin resistance phenotype in *L. welshimeri* and *L. innocua* could also transfer to the human pathogenic *L. monocytogenes*. Erythromycin can be used to treat listeriosis during pregnancy [40] and erythromycin resistance has been found to be conjugatively transferable within *Listeria* genus and from *Listeria* to *Enterococcus* [38]. Further characterization of antimicrobial resistance mechanisms in *Listeria* will add more insights into the extent to which food reservoir of antimicrobial resistance contributes to the emergence and dissemination of antimicrobial-resistant *Listeria* with potential to cause human disease.

In spite of the highest contamination rate of *L. welshimeri* in meat, among 58 isolates analyzed by antimicrobial susceptibility testing, only four were antimicrobial resistant, demonstrating much lower resistance prevalence than *L. innocua* and *L. monoctyogenes*. This contrasts with the findings by Davis and Jackson who reported that antimicrobial resistance was more common in *L. welshimeri* than that in the other two species [22]. They also identified higher resistance prevalence in *L. welshimeri* and *L. innocua* to penicillin and clindamycin than *L. monocytogenes*. However, due to the limited number of *L. welshimeri* isolates in that study (six *L. welshimeri*, four *L. innocua*, and 90 *L. monocytogenes*), a species by species comparison of the two studies could not be made, although in both studies *L. innocua* appeared to be a

common reservoir of antimicrobial resistance. Similarly, Walsh et al. [8] reported 19.5% of *L. innocua* versus 0.6% of *L. monocytogenes* were antimicrobial resistant and no resistance phenotypes were observed in *L. seeligeri* or *L. welshimeri*. Higher tetracycline resistance prevalence was also reported in *L. innocua* than other species [39, 43, 44]. Altogether, these data suggest that interspecies variation may exist in antimicrobial susceptibilities in *Listeria*. However, questions remain as to whether *L. innocua* is a larger reservoir of antimicrobial resistance than other *Listeria* species because there was no marked difference between *L. innocua* and *L. monocytogenes* in terms of prevalence of either tetracycline resistance or the overall antimicrobial resistance in our study. Regardless, the fact that all nine samples contaminated with more than one *Listeria* species in this study carried *L. monocytogenes* raises public health concern as co-existence of multiple species on same food samples increases the cell-to-cell contact and thus facilitates resistance gene transfer to the pathogenic species, such as *L. monocytogenes*.

Variation in sensitivity to food sanitizing agents has been reported in *L. monocytogenes* serotypes. Serotypes 1/2a and 1/2b demonstrated higher resistance prevalence to benzalkonium chloride than serotype 4b isolated from the turkey processing plants [31]. The same group also found serotypes 1/2a and 1/2b to be more resistant than serotype 4b to broad-host-range phages isolated from the plants [29]. A French study also identified low sensitivity to quaternary ammonium compounds in seven *L. monocytogenes* serotypes 1/2a and 1/2c from food products and environmental samples in the food industry, in comparison to all 37 serogroup 4 isolates being sensitive [30]. The data suggest a possible variation in the susceptibility of *L. monocytogenes* serotypes to food sanitizers and phages, which may explain the different prevalence of serotypes in the food processing environment. Although there are limited studies

comparing antimicrobial susceptibility profiles among *L. monocytogenes* serotypes, it is not unexpected to see the predominance of serotype 1/2b in the antimicrobial-resistant *L. monocytogenes*, and in fact, 12 of 15 tetracycline-resistant *L. monocytogenes* were serotype 1/2b. However, due to the low number of 4b isolates, the serotype responsible for the most clinical cases, we were unable to attribute the different recovery of these serotypes in food isolates to their antimicrobial susceptibilities. Further research involving larger numbers of isolates of major serotypes of public health importance will be necessary to understand the contribution of antimicrobial selection to the prevalence of different serotypes of *L. monocytogenes* in the environment as well as in human infections.

The PFGE data suggest an overall diverse population of *L. monocytogenes*. Identification of indistinguishable clones in different meat samples from the same store suggests clonal persistence of *L. monocytogenes*. Because serotypes 1/2a and 1/2b belong to different epidemiological lineages [45], it was not surprising to see separate clusters of 1/2a and 1/2b on the dendrogram. However, the one-band difference between 14-1 and 15-1 from each of these two serotypes suggests some relatedness between the serotypes and that they may have evolved from a common ancestor and be able to survive on multiple food vehicles. PFGE patterns may not be a reflection of antimicrobial resistance phenotypes because many antimicrobial resistance genes are carried on plasmids that cannot be typed by PFGE, which is evidenced by indistinguishable PFGE patterns (4-2 and 4-4, 24-1 and 24-2) with different antimicrobial susceptibility profiles. The recovery of these two pairs of isolates from the same beef samples also raises concern of potential gene transfer and emergence of new resistant clones.

CHAPTER 6

Conclusion

It is estimated that approximately 2500 cases of clinical human listeriosis occur every year in the United States, including 500 deaths [45]. The incidence of human listeriosis cases reported range from 0.2 to 0.8 sporadic cases/100,000 people a year in the United States and Europe [11, 12]. The high mortality rate of listeriosis cases had led governments and food safety agencies worldwide to take serious measurements to reduce the occurrence of *L. monocytogenes* in the food production chain. In spite of the fact that the United States, for example, had adopted the so called "Zero-tolerance" for Listeria monocytogenes in RTE foods, [45] outbreaks are still constantly occurring, having on average 2.2 outbreaks per year , from 1998-2008, being reported to CDC [46].

The information obtained in this study shows that *Listeria* species are prevalent (30.5%) in retail meat and resistance to antimicrobials of human clinical importance is common (23.2%). Antimicrobial resistance reservoir in non-*L. monocytogenes* species should not be ignored due to the potential of antimicrobial resistance gene transfer among bacteria, especially those co-existing on the same food products. Standardization of antimicrobial susceptibility testing protocol for *Listeria* and recommendation of interpretive breakpoints by CLSI will be needed to facilitate comparison of studies and monitor antimicrobial-resistant *Listeria* in the environment.

Figure 1

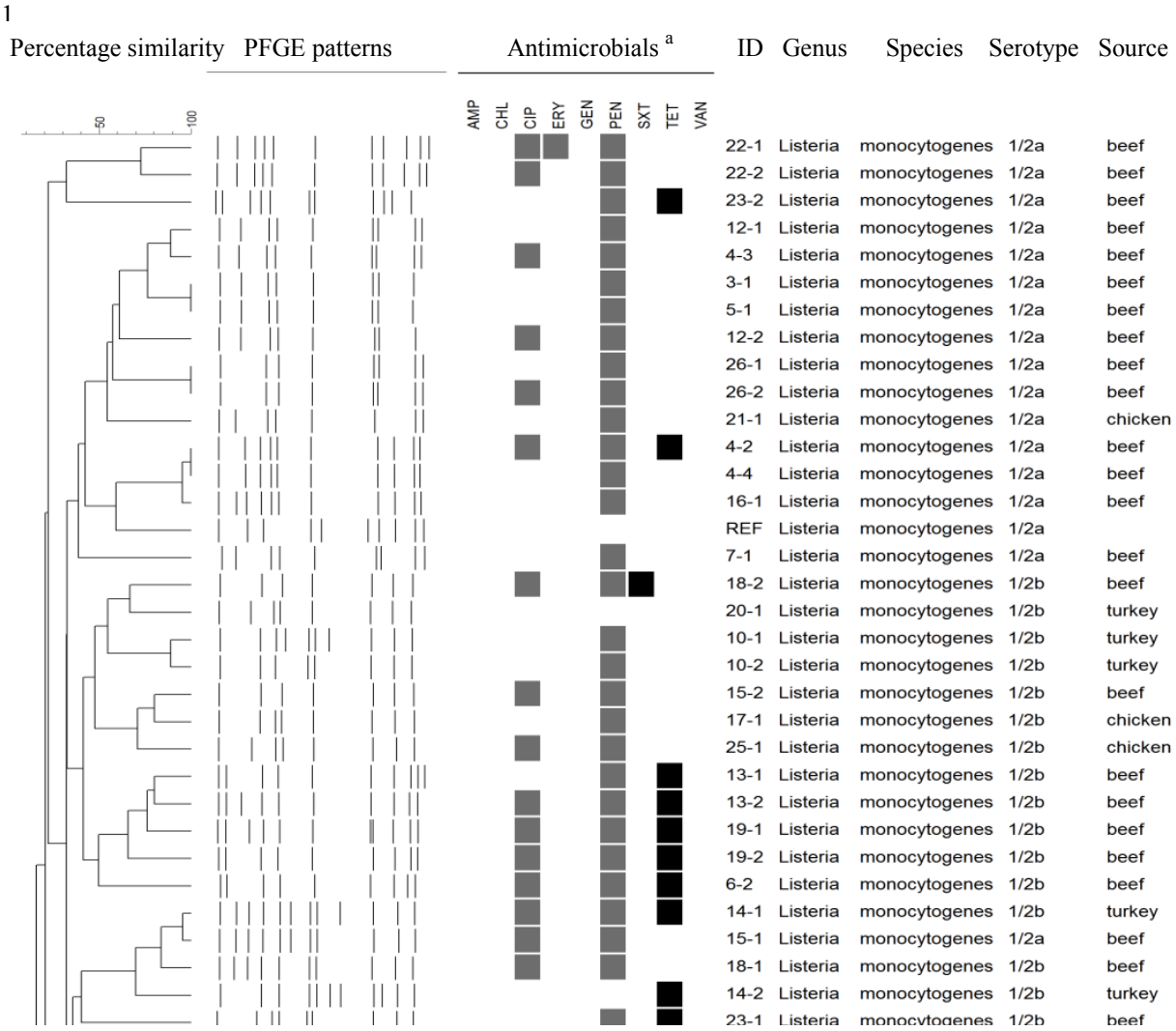

Figure 1 - Dendrogram of PFGE profiles based on *Asc* I digestion of *L. monocytogenes* isolates from retail meat.

Table 1: Contamination of *Listeria* species in retail meat samples

Meat category	No. of samples	No. of *Listeria*+ samples (%)	No. of *Listeria*+ samples		
			L. innocua	*L. monocytogenes*	*L. welshimeri*
Beef	133	45 (33.8)	12	18	17
Chicken	65	19 (29.2)	5	4	14
Turkey	45	10 (22.2)	5	4	4
Total	243	74 (30.4)	22	26	35

Table 2: **Distribution of antimicrobial-resistant *Listeria* species in this study**

Antimicrobials	No. of antimicrobial-resistant *Listeria* (%)			
	L. monocytogenes **(n = 44)**	***L. innocua*** **(n=36)**	***L. welshimeri*** **(n=58)**	**Total (n=138)**
Ampicillin	0 (0)	1 (2.8)	0 (0)	1 (0.7)
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	2 (5.6)	2 (3.4)	4 (2.9)
Erythromycin	1 (2.1)	0 (0)	0 (0)	1 (0.7)
Gentamicin	1 (2.1)	0 (0)	1 (1.7)	2 (1.4)
Penicillin	0 (0)	1 (2.8)	0 (0)	1 (0.7)
Sulfamethoxazole-trimethoprim	1 (2.1)	0 (0)	0 (0)	1 (0.7)
Tetracycline	13 (27.1)	9 (25.0)	0 (0)	22 (15.9)
Vancomycin	0 (0)	1 (2.8)	2 (3.4)	3 (2.2)
Total	15 (34.1)	13 (36.1)	4 (6.9)	32 (23.2)

REFERENCES

1. Indrawattana, N., et al., *Prevelance of Listeria monocytogenes in raw meats marketed in Bangkok and characterization of the isolates by phenotypic and molecular methods.* J. of Health, Population and Nutrition, 2011(1): p. 26-38.
2. Sleator, R.D. and C.G. Gahan, *A postgenomic appraisal of osmotolerance in Listeria monocytogenes.* Appl Environ Microbiology, 2003. **69**(1): p. 1-9.
3. Liu, D., L. Lawrence, and e. al., *A multiplex PCR for species- and virulence-specific determination of LIsteria monocytogenes.* J. Microbiol Methods, 2007. **71**(2): p. 133-140.
4. Bula, C.J., J. Bille, and e. al., *An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults.* . Clin. Infect. Dis, 1995. **20**(1): p. 66-72.
5. Schlech, W.F., *Foodborne listeriosis.* Clin Infec Dis, 2000. **31**(3): p. 770-775.
6. Graves, L.M., et al., *Listeria marthii sp. nov., isolated from the natural environment, Finger Lakes National Forest.* Int J Syst Evol Microbiol, 2010. **60**(Pt 6): p. 1280-8.
7. Leclercq, A., et al., *Listeria rocourtiae sp. nov.* Int J Syst Evol Microbiol, 2010. **60**(Pt 9): p. 2210-4.
8. Walsh, D., Duffy, G., Sheridan, J.J., Blair, I.S. and McDowell, D.A., *Antibiotic resistance among Listeria, including Listeria monocytogenes, in retail foods.* Journal of Applied Microbiology, 2001. **90**: p. 517-522.
9. Arslan, S. and F. Ozdemir, *Prevalence and antimicrobial resistance of Listeria spp. in homemade white cheese.* Food Control 2008. **19**: p. 360-363.
10. Sauders, B.D., et al., . *Diversity of Listeria species in urban and natural environments.* . Applied and Environmental Microbiology 2012. **78**: p. 4420-4433.

11. Vazquez-Boland, J.A., et al., *Listeria pathogenesis and molecular virulence determinants.* Clin Microbiol Rev, 2001. **14**(3): p. 584-640.

12. *Centers for Disease Control and Prevention. Vital signs: listeria illnesses, deaths, and outbreaks - United States, 2009-2011.* MMWR Morbidity and mortality weekly report., 2013. **62**(22): p. 448-52.

13. McLauchlin, J. and R.T.e.a. Mitchell, *Listeria monocytogenes and listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods.* Int J Food Microbiology 2004. **92**(1): p. 15-33.

14. Schlech, W.F., 3rd, *Foodborne listeriosis.* Clin Infect Dis, 2000. **31**(3): p. 770-5.

15. Buffer, J.L., et al., *Health Professionals' Knowledge and Understanding about Listeria monocytogenes Indicates a Need for Improved Professional Training.* Journal of Food Protection, 2012. **75**: p. 1310-1316.

16. Poyart-Salmeron, C., et al., *Transferable plasmid-mediated antibiotic resistance in Listeria monocytogenes.* Lancet, 1990. **335**(8703): p. 1422-6.

17. Phillips, I., Casewell M., et al., *Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data.* Journal of Antimicrobial Chemotherapy, 2004. **53**(28-52).

18. Nilsson, O., *Vancomycin resistant enterococci in farm animals- occurrence and importance.* Vet. PathologInfection, Ecology and Epidemiology, 2012. **2**: p. 16959.

19. http://www.fda.gov/animalveterinary/safetyhealth/antimicrobialresistance/default.htm. *Antimicrobial Resistance. U. S. Food and Drug Administration*. 2015.

20. U. S. Department of Health and Human Services, F.a.D.A., Center for Veterinary Medicine., *The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals.* http://www.regulations.gov 2012.

21. Zhang, Y., et al., *Characterization of Listeria monocytogenes isolated from retail foods.* Int J Food Microbiol, 2007. **113**(1): p. 47-53.

22. Davis, J.A. and C.R. Jackson, *Comparative antimicrobial susceptibility of Listeria monocytogenes, L. innocua, and L. welshimeri.* Microb Drug Resist, 2009. **15**(1): p. 27-32.

23. Charpentier, E. and P. Courvalin, *Antibiotic resistance in Listeria spp.* Antimicrob Agents Chemother, 1999. **43**(9): p. 2103-8.

24. Hansen, J.M., P. Gerner-Smidt, and B. Bruun, *Antibiotic susceptibility of Listeria monocytogenes in Denmark 1958-2001.* APMIS, 2005. **113**(1): p. 31-6.

25. Lorber, B., *Listeriosis.* Clin Infect Dis, 1997. **24**(1): p. 1-9; quiz 10-1.

26. Troxler, R., et al., *Natural antibiotic susceptibility of Listeria species: L. grayi, L. innocua, L. ivanovii, L. monocytogenes, L. seeligeri and L. welshimeri strains.* Clinical Microbiology and Infection, 2000. **6**: p. 525-535.

27. Charpentier, E., et al., *Incidence of antibiotic resistance in Listeria species.* J Infect Dis, 1995. **172**(1): p. 277-81.

28. Roberts, M.C., et al., *Transferable erythromycin resistance in Listeria spp. isolated from food.* Appl Environ Microbiol, 1996. **62**(1): p. 269-70.

29. Kim, J.W., R.M. Siletzky, and S. Kathariou, *Host ranges of Listeria-specific bacteriophages from the turkey processing plant environment in the United States.* Appl Environ Microbiol, 2008. **74**(21): p. 6623-30.

30. Mereghetti, L., et al., *Low sensitivity of Listeria monocytogenes to quaternary ammonium compounds.* Appl Environ Microbiol, 2000. **66**(11): p. 5083-6.

31. Mullapudi, S., R.M. Siletzky, and S. Kathariou, *Heavy-metal and benzalkonium chloride resistance of Listeria monocytogenes isolates from the environment of turkey-processing plants.* Appl Environ Microbiol, 2008. **74**(5): p. 1464-8.

32. Administration., U.S.F.a.D., *Bacteriological Analytical Manual: Detection and Enumeration of Listeria monocytogenes.* . 2011.

33. Chen, Y. and S.J. Knabel, *Multiplex PCR for simultaneous detection of bacteria of the genus Listeria, Listeria monocytogenes, and major serotypes and epidemic clones of L. monocytogenes.* Appl Environ Microbiol, 2007. **73**(19): p. 6299-304.

34. Institute., C.a.L.S., *Performance standards for antimicrobial disk susceptibility tests. Approved standard, 10th ed. CLSI publication M02-A10. Clinical and Laboratory Standards Institute, Wayne, PA.* . 2009.

35. Borucki, M.K. and D.R. Call, *Listeria monocytogenes serotype identification by PCR.* J Clin Microbiol, 2003. **41**(12): p. 5537-40.

36. Graves, L.M. and B. Swaminathan, *PulseNet standardized protocol for subtyping Listeria monocytogenes by macrorestriction and pulsed-field gel electrophoresis.* Int J Food Microbiol, 2001. **65**(1-2): p. 55-62.

37. El-Shenawy, M., J. Manes, and J.M. Soriano, *Listeria spp. in Street-Vended Ready-to-Eat Foods.* Interdiscip Perspect Infect Dis, 2011. **2011**: p. 968031.

38. Kovacevic, J., L.R. Mesak, and K.J. Allen, *Occurrence and characterization of Listeria spp. in ready-to-eat retail foods from Vancouver, British Columbia.* Food Microbiol, 2012. **30**(2): p. 372-8.

39. Chen, B.Y., et al., *Antibiotic resistance in Listeria species isolated from catfish fillets and processing environment.* Lett Appl Microbiol, 2010. **50**(6): p. 626-32.

40. Jones, E.M. and A.P. MacgGowan, *Antimicrobial chemotherapy of human infection due to Listeria monocytogenes.* European Journal of Clinical Microbiology and Infectious Diseases, 1995. **14**: p. 165-175.

41. Conter, M., et al., *Characterization of antimicrobial resistance of foodborne Listeria monocytogenes.* . . International Journal of Food Microbiology 2009. **128**: p. 497-500.

42. de Niederhausern, S., et al., *Glycopeptide-resistance transferability from vancomycin-resistant enterococci of human and animal source to Listeria spp.* Letters in Applied Microbiology 2004. **39**(de Niederhausern, S., Sabia, C., Messi, P., Guerrieri, E., Manicardi, G., Bondi, M. 2004. Glycopeptide-resistance transferability from vancomycin-resistant enterococci of human and animal source to Listeria spp. Letters in Applied Microbiology 39, 483-489.): p. 483-489.

43. Franco Abuin, C.M., et al., *Susceptibilities of Listeria species isolated from food to nine antimicrobial agents.* Antimicrob Agents Chemother, 1994. **38**(7): p. 1655-7.

44. Li, Q., J.S. Sherwood, and C.M. Logue, *Antimicrobial resistance of Listeria spp. recovered from processed bison.* Lett Appl Microbiol, 2007. **44**(1): p. 86-91.

45. Orsi, R.H., H.C. den Bakker, and M. Wiedmann, *Listeria monocytogenes lineages: Genomics, evolution, ecology, and phenotypic characteristics.* Int J Med Microbiol, 2011. **301**(2): p. 79-96.

46. Cartwright, E.J., et al., *Listeriosis outbreaks and associated food vehicles, United States, 1998-2008.* Emerging infectious diseases., 2013. **19**(1): p. 1-9.

ABSTRACT

ANTIMICROBIAL-RESISTANT *LISTERIA* SPECIES FROM RETAIL MEAT IN METRO DETROIT

by

LIZIANE S. DA ROCHA

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A total of 138 *Listeria* isolates, including 58 *Listeria welshimeri,* 44 *Listeria monocytogenes,* and 36 *Listeria innocua,* from retail meat were characterized by antimicrobial susceptibility tests against nine antimicrobials. In addition, the 44 *L. monocytogenes* were analyzed by serotype identification using PCR and genotyping using pulsed-filed gel electrophoresis (PFGE). Resistance to one or two antimicrobials was observed in 32 *Listeria* (23.2%). No multidrug resistance was identified. Tetracycline resistance was the most common resistance phenotype and identified in 22 *Listeria* isolates. Low prevalence of resistance to ciprofloxacin, erythromycin, gentamicin, and vancomycin was also detected. *L. innocua* demonstrated the highest overall prevalence of antimicrobial resistance, being 36.1%, followed by 34.1% in *L. monocytogenes*, and 6.9% in *L. welshimeri*. Serotypes 1/2a, 1/2b, and 4b were identified in 19, 23, and one *L. monocytogenes*, respectively. One isolate was untypeable. Fifteen *L. monocytogenes* were antimicrobial resistant (12 of 1/2b, 2 of 1/2a, and 1 of serotype untypeable). A diverse population of *L. monocytogenes* was identified as evidenced by multiple PFGE patterns in the 44 isolates. The data indicate that *Listeria* contamination is common in

retail meat. Although antimicrobial resistance still occurs at low prevalence, multiple *Listeria* species can serve as reservoir of antimicrobial resistance. Variation of antimicrobial susceptibilities in *L. monocytogenes* serotypes may exist.

AUTOBIOGRAPHICAL STATEMENT

Liziane Sippel da Rocha received a Bachelor of Science Degree in Nutrition and Food Science in 2009 from Wayne State University (WSU), Detroit, Michigan. In 2009, she joined the graduate program at Wayne (WSU) where she has completed her graduate study towards the accomplishment of a Master of Science degree. While completing her graduate degree, Liziane also was an active member of the Phi Tau Sigma Honorary Society Chapter at WSU in which she has been serving as a Treasurer for two consecutive years and also helping with organizing food plant visits for members of the chapter. She worked as a Research Assistant and also as a Graduate Research Assistant in the area of Microbiology and Food Safety at WSU under the guidance of Professor Dr. Zhang. Throughout her course of study, she received several academic and professional awards, mainly Dr. Shelef Scholarship Award (NFS) 2010-2011, the WSU Graduate Professional Scholarship Award for the academic years 2012-2013, and the Safe Quality Food Institute (SQF) – FMI Foundation Scholarship Award 2013-2014. Liziane is an author in an article that was published in the Journal of Food Protection, December 2012: "Antimicrobial-Resistant *Listeria* Species from Retail Meat in Metro Detroit" and co-author in an article that was published in the Emerging Infectious Diseases, 2011: "Methicillin-resistant *Staphylococcus aureus* in Retail meat". Liziane has ANSI accredited Certificate of Accomplishment from NSF International in HACCP Manager Training, also accredited by the International HACCP Alliance, and Implementing SQF Systems by the NSF International. She has the International Food Safety Manager Certification Exam by NRFSP. She also participated in several courses and webinars in the areas of Food Safety and Microbiology and Management Systems to improve compliance and reduce risk in the food chain.