CPS Symposium Session 3 – Executive Summary

The 12th Annual Center for Produce Safety (CPS) Research Symposium reached the half-way point on June 29, 2021, with Session 3 featuring four presentations on *Listeria*. CPS has prioritized *Listeria* produce safety research for nearly a decade assembling a knowledge base stakeholders can use to build risk and science-based strategies for controlling *Listeria montocytogenes* (Lm) contamination. Session 3 added to the knowledgebase with studies on Lm growth and survival on fresh produce including stored apples, an examination of Lm persistence and interventions in production environments, and an evaluation of the potential for Lm to build tolerance to sanitizers. A recording of Session 3 can be found on the CPS website. An executive summary of session 3 is included below.

1. ***Listeria* survival and growth on fresh produce is variable**. Xiangwu Nou from USDA ARS Beltsville presented his project*, "Listeria monocytogenes growth potential, kinetics, and factors affecting its persistence on a broad range of fresh produce"* [Nou 2018]. This project looked at Lm growth kinetics on a variety of whole and fresh-cut produce under retail display and temperature abuse conditions. Highlights include:
    - ***Listeria growth is significantly reduced on whole produce.*** A significant reduction of Lm was observed in all of the 14 whole commodities studied. Significant Lm growth was observed on some fresh-cut products including cantaloupe and mango.
    - ***Elevated temperature increases Lm growth in some fresh cut products.*** Under temperature abuse conditions (60°F), Lm growth on whole produce items was not increased. However, Lm growth was increased on fresh-cut cantaloupe, mango, cauliflower, celery and romaine. It should be noted that these results differ somewhat from those reported by Schaffner [Schaffner 2019] and Strawn [Strawn 2018] at the 2020 CPS Symposium where elevated temperatures (up to 90°F) were observed to support Lm growth more broadly.
    - ***pH is a primary determinate of Listeria growth on fresh produce juices.*** Fresh fruit or vegetable juices with acidic pH did not support Lm growth. Lm-inoculated produce juices with pH adjusted to <4.5 did not support Lm growth while all juices with pH adjusted to 7 did support Lm growth. Accordingly, juices with higher pH like carrot, cauliflower and cantaloupe supported Lm growth. These observations on pH agree with the data and Lm growth models reported in 2020 by Schaffner and Strawn.
    - ***Microbiota can affect Listeria growth on fresh produce***. Recovery of Lm was inconsistent among the commodities studied. One explanation could be related to variations in the microbiome found on different commodities. Lm antagonist strains have been isolated from fresh produce, e.g., Romaine lettuce opening the possibility that these antagonists might be used to control Lm. The potential for manipulating produce microbiomes to control Lm needs further evaluation.

    Working more specifically on Lm persistence and growth on apples, Elliot Ryser from Michigan State University shared the interim results of his project, "*Fate of different Listeria monocytogenes strains on different whole apple varieties during long-term simulated commercial storage"* [Ryser 2018]. Ryser's team employed a collection of Lm outbreak strains to assess Lm survival and growth on Gala, Honey Crisp and Granny Smith apple varieties, both waxed or unwaxed over two months of aerobic or seven months of controlled atmosphere (CA) storage. Some key findings from this work include:
    - **Storage atmosphere does not impact survival of *Listeria*.** Storage atmosphere (aerobic or CA) did not impact *Listeria* survival.
    - ***Surface-grown Listeria cells recovered from biofilms may survive longer on apples***. There is increased persistence of Lm when inoculated with biofilm cultures of Lm versus planktonic or single cell cultures of Lm like might be found in water. This observation is consistent with previous

research that points to the robust nature of bacteria grown under stress conditions like on nutrient depleted surfaces [Harris 2010] or in the presence of non-lethal levels of sanitizers [Allende 2018].

- ***Waxing before CA storage reduces Listeria survival.*** Lm inoculation of apples was conducted to assess survival. If apples were inoculated, waxed, stored and then sampled, Lm survival was increased versus inoculating the fruit after waxing. This result places a premium on avoiding Lm cross-contamination when the apples are waxed.
- ***Harvest year, apple variety, and apple growing source may impact Listeria survival.*** The data are variable from year 1 to year 2 but suggests that Lm survival varies by growing season, location and variety. The data are too limited to draw specific conclusions at this point. This preliminary observation may also be explained by facility-specific practices and growing environments that can impact fruit and Lm metabolism, persistence and growth.

2. **The most effective mitigation for *Listeria* is thorough cleaning and sanitation.** Martin Wiedmann from Cornell University discussed the results of his project, *"Factors affecting persistence of Listeria monocytogenes need to be identified for evaluation and prioritization of interventions*" [Wiedmann 2019]. The mantra in the produce industry for over a decade has been effective cleaning and sanitation can help control *Listeria* persistence or persistent transience. This project supports that mandate. Some important points to consider:
    - ***Listeria can be found in multiple sites in packinghouse operations***. Via an expert literature review of food and fresh produce studies, the top five sites where *Listeria* is found were floors, drains, conveyor belts, slicers and tables. Onsite testing in produce packinghouses revealed these same areas plus floor/wall junctures, forklifts, forklift stops, catch pans, brush beds, waxing areas, decals or stickers on equipment, footings and PVC pipes. Interventions to reduce *Listeria where it was found* generally resulted in reduced detection in packing operations.
    - ***Cleaning and sanitation protocols that involve breaking down equipment to expose hard to access, survival niches is effective in eliminating persistent or persistent transient Listeria.*** The identification of hard to access locations in facilities and on equipment should be an operational priority. It is important not to overlook any piece of equipment as a potential harborage for persistent *Listeria*. For example, forklifts and forklift stops (made with hollow steel tubing) were shown to harbor *Listeria as were brush beds and catch pans*. SOPs for equipment breakdown and SSOPs to guide cleaning and sanitation are critically important to ensure consistency.
    - ***An aggressive and risk-based environmental monitoring program (EMP) is an important tool for Lm mitigation***. Microbial testing post cleaning and sanitation can both verify efficacy and identify areas where additional efforts are needed. EMP data can also guide decisions on how often equipment needs to be broken down for deep cleaning and sanitation. Factors like *Listeria* pressure in a facility, the likelihood of transference to the product from the packing environment or equipment and *Listeria* verification testing results need to be considered to determine if daily, weekly, monthly or seasonal teardown may be required to effectively manage the risk.
    - ***Computer models can be used to test intervention strategies for Listeria***. Computer-based models have been developed and can provide an effective way to test *Listeria* mitigation strategies when experiments are difficult to execute.

3. **Use sanitizers correctly to avoid *Listeria gaining tolerance* and surviving sanitation.** Martin Wiedmann presented a second project in session 3 entitled, "*Listeria develops reduced sanitizer sensitivity but not resistance at recommended sanitizer use levels*" [Wiedmann 2019]. There has been a longstanding industry concern that repeated use of a sanitizer could result in *Listeria* strains developing resistance to the sanitizer rendering sanitation efforts ineffective. This project explored that concern and arrived at these learnings:

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- ***Quaternary ammonia (Quat)) is the most effective sanitizer against Listeria**. Listeria* isolates showed a range of responses to sanitizers at use level concentrations. Experiments were conducted at commonly used concentrations of sanitizers (80 ppm PAA, 300 ppm Quat or 500 ppm NaOCl) and Quat was more likely to achieve target Lm reduction for *Listeria*. It is important to note that additional work is needed to determine optimal concentrations of PAA and NaOCl. While changing or rotating sanitizers has been discussed to prevent sanitizer resistance, this work suggests caution may be warranted before implementing sanitizer rotation or changes that reduce use of Quats, as Quats provided more consistent *Listeria* reduction as compared to other sanitizers. It is important to note that *Listeria* can grow at concentrations of Quat up to 18 ppm when continuously exposed to sublethal concentrations, supporting the importance of ensuring proper Quat concentrations (300-400 ppm), and in compliance with product labels, are always employed at all locations in a facility. It is also important to be diligent in making sure dilution factors like water in drains are considered as existing water may dilute Quat below its effective use concentration.
- ***Presence of sanitizer and Listeria stress resistance genes are not correlated with Listeria survival when sanitizer is applied at use level concentrations***. In other words, use of Quat at the recommended concentration overcomes naturally occurring genetic resistance factors. Using genetic sequence analysis on a large collection of Lm and Listeria spp., it was concluded that there is a limited risk of sanitizer resistance genes spreading through horizontal gene transfer and resulting in Quat resistant *Listeria* strains.

***Acknowledgements**: The Center for Produce Safety would like to thank session 3 presenters and moderators for their work and dedication to produce safety. More detail on the research projects can be found at www.centerforproducesafety.org. This Executive Summary is meant to inform and provoke thought with an eye towards inspiring readers to examine their own produce safety programs and to use the research to make improvements. It is not meant as a directive on what must be done to produce safe food. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli (info@centerforproducesafety.org). Thank you.*