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Duleeka Prasadani Kuruwita Arachchige
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# PERSISTENCE OF *Escherichia coli* O157:H7 AND *Listeria monocytogenes* ON THE EXTERIOR OF COMMON PACKAGING MATERIALS

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A Thesis
Presented to
the Graduate School of
Clemson University

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Food, Nutrition and Culinary Sciences

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by
Duleeka Prasadani Kuruwita Arachchige
December 2017

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Accepted by:
Dr. Angela M. Fraser, Committee Chair
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Dr. Duncan Darby
Dr. Julia L. Sharp

## ABSTRACT

Acute gastroenteritis (AGE), a major cause of illness, results in 179 million AGE cases every year in the United States. AGE bacteria that have low-infectious dose include *E. coli* O157:H7 (<10-100 cells) and *L. monocytogenes* (<1000 cells). Because of their low-infectious dose and high environmental resistance, contaminated surfaces, such as the exterior surface of food packages, could be a source for disease transmission. Our aim was to determine the persistence of *E. coli* O157:H7 and *L. monocytogenes* on three packaging materials – oriented polyethylene terephthalate (OPET), oriented polypropylene (OPP), and nylon-6. Coupons (25 $cm^2$) from each material were sterilized under ultraviolet light for 5 minutes. Spot and spread inoculation was done on treatment coupons with ca. 7 log CFU of a 3-strain-mixture of green fluorescent protein (GFP)-labeled *E. coli* O157:H7. All the coupons were incubated at Technical Association of the Pulp and Paper Industry-TAPPI standards. Surviving *E. coli* O157:H7 cells on duplicate coupons were recovered in saline at selected time intervals (0, 0.25, 0.5, 1, 2, 3, 5, 7, 14, 15 days). Surviving cells were enumerated on tryptic soy broth supplemented with ampicillin using the 3 tubes most probable number-MPN method described in the Bacteriological Analytical Manual. The experiment was performed in triplicate. The same procedure was carried out for 3-strain-mixture of rifampin-resistant (Rif)-*L. monocytogenes* using tryptic soy broth supplemented with rifampin for the MPN method. (GFP)-*E. coli* O157:H7 and (Rif)-*L. monocytogenes* survived on OPET, OPP, and nylon-6 for 15 days. The survival of *E. coli* O157:H7 was significantly different ($p < 0.05$) from the survival of *L. monocytogenes* between days 0.5-1, 1-2, and 3-5. The survival of both

bacteria on all three materials were not significantly different ($p > 0.05$). *E. coli* O157:H7 and *L. monocytogenes* survived for over 2 weeks on OPET, OPP, and nylon 6, suggesting a highly contaminated outer surface of a food package could be a potential fomite for AGE outbreaks.

# DEDICATION

First, I would like to dedicate my thesis work to my parents who gave me a constant encouragement throughout my academic career. Secondly, my husband and my son who always were behind me, supported me, and dedicated for me to finish my work successfully. Finally, I also would like to dedicate my work to my sister and brother who were an encourage to me in my life.

**ACKNOWLEDGMENTS**

My sincerely appreciate goes to my advisor, Dr. Angela Fraser without whom my thesis would not have been completed successfully. Because of her excellent guidance, I had an opportunity to participate in this meaningful project. She always wanted me to do my best, therefore, always thanking for her excellent mentor.

My special thank goes to my committee, Dr. Xiuping Jiang, Dr. Julia Sharp, and Dr. Duncan Darby. I am thankful to Dr. Xiuping Jiang for providing her laboratory resources to conduct my research and guidance to finish the experiment successful. I thank Dr. Julia Sharp for giving her time and energy to teach me statistics, data analysis of my results, and explain the outcomes. I thank Dr. Duncan Darby for his guidance on selecting packaging materials for the study and explaining the chemical properties of these materials and being always supportive throughout my project. I would also like to thank Mrs. Pat Marcondes for sharing her packaging science knowledge and Dr. Kay Cooksey for providing part of the laboratory instruments for my experiment.

I thank the members of Dr. Jiang's laboratory team, Muthu Dharmasena, Hongye Wang. My special thank goes to Muthu Dharmasena who gave her time throughout my experiment and shared her knowledge. I also would like to thank the members of our Clemson University research team Cortney Leone, Kinsey Porter, Morgan G Chao who helped me in my writing process.

Finally, I would like to thank the United States Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative, which provided financial support for this project under the grant No. 2011-68003-30395.

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# CHAPTER ONE

## RATIONALE AND SIGNIFICANCE

Worldwide, acute gastroenteritis (AGE) is the second most common cause of infectious disease (Ahmed et al., 2014). In the United States alone, 179 million cases of AGE are reported each year (Wikswo, 2015). Between 1999 and 2007, the number of U.S. Americans who died from AGE more than doubled, from approximately 7,000 to over 17,000 deaths (CDC, 2012). While most U.S. cases are self-limiting, which ultimately resolve itself without treatment, some result in hospitalization and death. The transmission pathways for AGE are mainly via person-to-person, foodborne, waterborne, infected animal, and contaminated environmental surfaces or fomites (inanimate objects that can transmit pathogenic microorganisms). While environmental contamination is the least frequently reported mode of transmission, some suggest it could be an under-reported contributor to the spread of AGE during outbreak situations (Fankem et al., 2014; Holmes and Simmons, 2008).

The literature describing AGE outbreaks associated with fomites or environmental contamination shows that most AGE outbreaks are associated with viruses, not bacterial pathogens (Repp and Keene, 2012; Fankem et al., 2014; Holmes and Simmons, 2008). Although virus outbreaks are more common, low-infectious dose bacteria that have high environmental resistance particularly to determine their role in fomite or contaminated environmental surfaces. High environmental resistance

is known as lack of sensitivity to environmental conditions especially because of continued exposure or genetic change.

Low-infectious dose bacteria are those that cause an infection at ingestion of <10 to 1000 cells. Bacteria that have a low-infectious dose include *Escherichia coli* 0157:H7 (<10-100 cells), *Salmonella* Typhi (~1000 cells), *Shigella* spp. (<10 cells), and *Listeria monocytogenes* (<1000 cells) (FDA, 2014). *L. monocytogenes* and *E. coli* O157:H7 also have been shown to have high environmental resistance. *L. monocytogenes* causes nearly 1,600 illnesses and 260 deaths annually in the United States, making it the third cause of foodborne AGE death in the United States (CDC, 2013; Scallan et al., 2011). *E. coli* O157:H7 causes 73,000 illnesses and 61 deaths annually in the United States, making it one of the major virulent (Scallan et al., 2011).

Although cross-contamination of food by fomites or environmental surfaces by low-infectious dose bacteria has not been widely reported, the studies on survival of AGE bacteria on environmental surfaces present evidence that contaminated fomites can play a role in the transmission and spread of AGE (Fankem et al., 2014; Wald et al., 2010; CDC, 2008). One potential fomite for which there is scant published literature is food packaging, particularly the exterior surfaces of packaging materials. A large body of research exists that focuses on the interior surface of food packaging materials, but minimal research has examined the exterior surfaces of food packaging materials in relationship to food safety (Pereda et al., 2011; Rivero et al., 2009; Jin and Zhang, 2008; Mecitoglu et al., 2006; Grower, 2001).

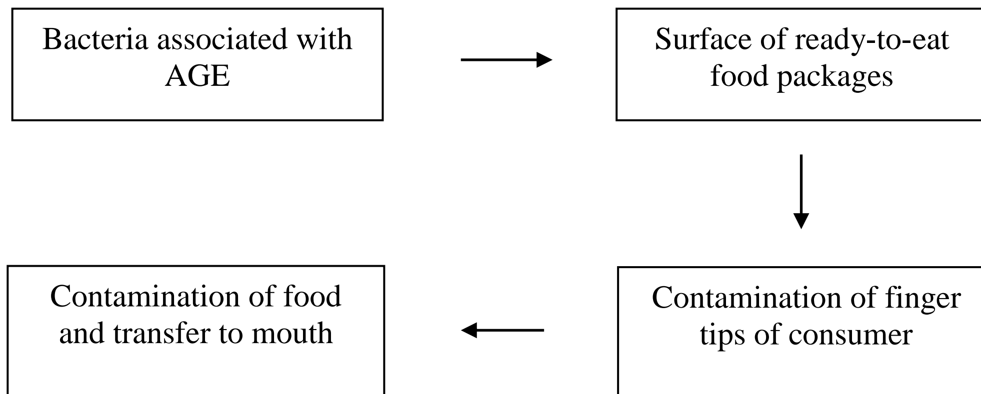


**Figure 1.1 Hypothetical Transfer of Bacteria from Contaminated Hands/Surfaces to Mouth.**

If AGE pathogens survive on exterior surfaces, they could be transferred to the hands of consumers, then transferred to food that is then ingested (Figure 1.1). Therefore, we hypothesize that low-infectious dose AGE bacterial pathogens, such as *E. coli* O157:H7 and *L. monocytogenes*, could survive on the exterior surface of food packaging for several days to weeks depending on the environmental conditions. The study aim was to determine the persistence of *E. coli* O157:H7 and *L. monocytogenes* on three common packaging materials – oriented polypropylene, oriented polyethylene terephthalate, and nylon-6, under Technical Association of the Pulp and Paper Industry (TAPPI) conditions – 23ºC room temperature and 50% relative humidity. The five research hypotheses to meet this aim were as follows:

1. *E. coli* O157:H7 can survive at least two weeks on the surface of three packaging materials (OPET, OPP and nylon-6) under TAPPI conditions.
2. *E. coli* O157:H7 can survive longer on polar packaging materials (OPET and nylon-6) than non-polar packaging materials (OPP) under TAPPI conditions.

3. *L. monocytogenes* can survive at least two weeks on the surface of three packaging materials (OPET, OPP and nylon-6) under TAPPI conditions.
4. *L. monocytogenes* can survive longer on polar packaging materials (OPET and nylon-6) than non-polar packaging materials (OPP) under TAPPI conditions.
5. Gram-positive *L. monocytogenes* can survive better than gram-negative *E. coli* O157:H7.

## LITERATURE CITED


Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *14*(8), 725-730.

Centers for Disease Control and Prevention. (2012). *Deaths from gastroenteritis double*. *CDC newsroom*. Retrieved March 30, 2017, from https://www.cdc.gov/media/releases/2012/p0314_gastroenteritis.html.

Centers for Disease Control and Prevention. (2013). Vital signs: *Listeria* illnesses, deaths, and outbreaks — United States, 2009-2011. *MMWR. Morbidity and Mortality Weekly Report*, *62*(22), 448-52.

Centers for Disease Control and Prevention (CDC). (2008). Norovirus outbreak in an elementary school-district of Columbia, February 2007. *MMWR. Morbidity and Mortality Weekly Report*, *56*(51-52), 1340.

Fankem, S. L., Boone, S. A., Marlene Gaither, M. P. A., and Gerba, C. P. (2014). Outbreak of norovirus illness in a college summer camp: impact of cleaning on occurrence of norovirus on fomites. *Journal of Environmental Health*, *76*(8), 20.

Grower, J. L. 2001. Characteristics of nisin-containing packaging film coating to control *Listeria monocytogenes*. M.S. thesis. Clemson University, Clemson, S.C.

Holmes, J. D., and Simmons, G. C. (2009). Gastrointestinal illness associated with a long-haul flight. *Epidemiology and Infection*, *137*(03), 441-447.

Jin, T., and Zhang, H. (2008). Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging. *Journal of Food Science*, *73*(3).

Mecitoglu, C., Yemenicioglu, A., Arslanoglu, A., Elmac, Z. S., Korel, F., and Çetin, A. E. (2006). Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging. *Food Research International*, *39*(1), 12-21.

Pereda, M., Ponce, A. G., Marcovich, N. E., Ruseckaite, R. A., and Martucci, J. F. (2011). Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids*, *25*(5), 1372-1381.

Repp, K. K., and Keene, W. E. (2012). A point-source norovirus outbreak caused by exposure to fomites. *Journal of Infectious Diseases*, *205*(11), 1639-1641.

Rivero, S., García, M. A., and Pinotti, A. (2009). Composite and bi-layer films based on gelatin and chitosan. *Journal of Food Engineering*, *90*(4), 531-539.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... and Griffin, P. M. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases*, *17*(1), 7.

U.S. Food and drug administration. (2014). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook *Listeria monocytogenes*. Retrieved March 29, 2017, from https://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm070064.htm.

Wald, M., Scherer, K., Nielsen, S., Diedrich, S., Ellerbroek, L., Frank, C., and Koch, J. (2010). Food-borne norovirus-outbreak at a military base, Germany, 2009. *BMC Infectious Diseases*, *10*(1), 30.

Wikswo, M. E., Kambhampati, A., Shioda, K., Walsh, K. A., Bowen, A. B., and Hall, A. J. (2015). Outbreaks of acute gastroenteritis transmitted by person-to-person contact, environmental contamination, and unknown modes of transmission – United States, 2009-2013. *Morbidity and Mortality Weekly Report*, *64(12)*, 1-20.

# CHAPTER TWO

# ACUTE GASTROENTERITIS OUTBREAKS ASSOCIATED WITH FOMITES: A SYSTEMATIC LITERATURE REVIEW

## BACKGROUND AND RATIONALE

Acute gastroenteritis (AGE) is the most common cause of illness worldwide (Ahmed et al., 2014). Pathogens associated with AGE, can be transmitted through direct person to person contact, ingestion of food or water, contact with infected animals, and contaminated environmental surfaces (fomites) (Wikswo et al., 2015). Although the primary transmission of AGE via environmental contamination is reported to be low, two published studies present evidence that contaminated fomites play a role in the transmission and spread of pathogens associated with AGE suggesting the need to conduct a literature review to further explore this relationship (Fankem et al., 2014; Repp and Keene, 2012). The aim of our literature review was to answer two broad research questions: 1) What pathogens are attributed to fomite-associated outbreaks? 2) What AGE-associated outbreaks are attributed to fomites? To our knowledge, no published systematic review of studies examining the role of fomites and AGE outbreaks is available.

## METHODS

### Search Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles guided our literature search (Liberati et al., 2009). The search was performed using four databases – Academic Search Complete, Web of Science, Google Scholar, and Science Direct – using the search string shown in Table 2.1.

**Table 2.1 Search String**

| Terms-Disease | | Term-Outbreak | | Terms-Contamination area |
|---|---|---|---|---|
| "Acute gastroenteritis" OR "acute gastrointestinal illness" OR "foodborne disease" OR "food-borne disease" OR "food borne illness" OR "food-borne illness" | AND | Outbreak | AND | Fomite OR surface |

After compiling articles from all four databases, duplicates were removed and the titles and abstracts were reviewed for inclusion. In addition, the reference lists of all review articles and eligible articles were manually searched to identify additional published articles that might have been missed during database searching. The full texts of included articles were reviewed for final eligibility. To be included, articles had to describe an epidemiological observational study, be peer reviewed, and be published in English between 1970 and May 2015. Studies from all geographic areas were included. Additionally, only studies on the etiology of outbreaks related to AGE or foodborne

disease that discussed fomites or environmental contamination as a potential source of the outbreaks were included. Review articles were excluded from the sample; however, the reference lists of review articles were screened for the eligibility that might have been missed from database search.

## RESULTS

A total of 191 articles were found through the initial database searching (Figure 2.1). After removing duplicate records, 165 records were selected for initial screening of titles and abstracts. A total of 65 eligible articles were identified for full text review and 6 additional records were included through manual searching of the reference lists of the 65 articles. After reviewing the full texts of 71 records, 53 records were excluded for the following reasons: not published in English (n=2), not peer-reviewed articles (n=3), outbreak was not associated with fomites (n=10), outbreak did not match with correct etiology – AGE or foodborne (n=16), or inappropriate experimental design (n=22). A total of 18 studies met the inclusion criteria.

All eligible studies were published between 1997 and 2014 and most were conducted in the United States (6) followed by Australia (3). Nearly all (n=16) reported that the outbreak was caused by human norovirus with the remaining two studies reporting the causative agent as a small round structured virus-SRSV (previously norovirus was referred as SRSV) and virus. None identified bacterial pathogens as the causative agent. In eight studies, fomites were confirmed as the source of the AGE outbreak, the remaining ten studies suggested fomites as the source. Three studies

identified the type of fomites – reusable grocery bag, computer devices, lockers, curtains and commodes – with the remaining fifteen studies reporting that fomites were the mode of transmission without identifying the fomite.

**Figure 2.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses Flow Chart Describing the Literature Search Procedure**

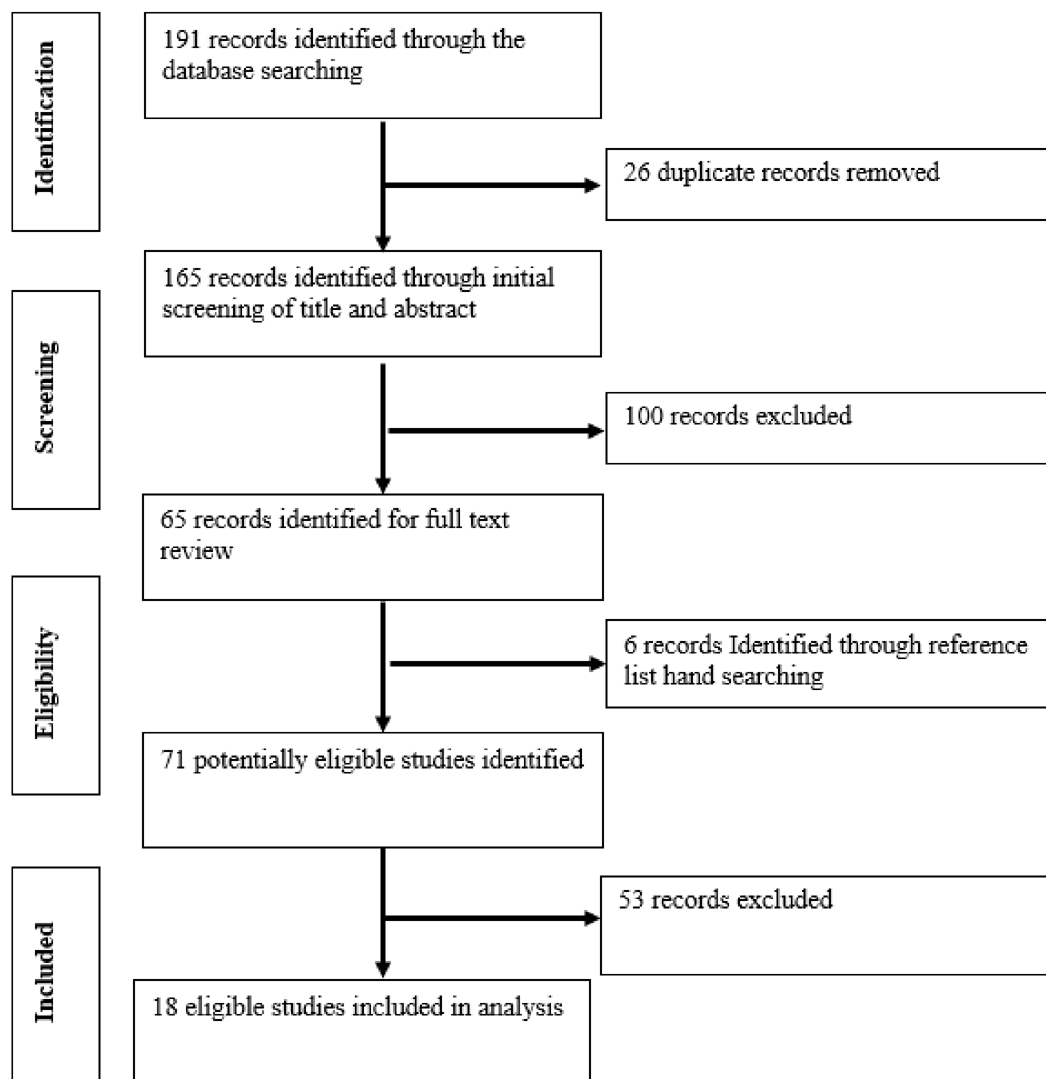

**Table 2.2 Summary of Studies Reviewed for the Initiation/Spread of AGE Outbreaks Associated with Fomites**

| First Author, Year | Location | Setting | Duration of Outbreak | Attack Rate (%)/ Number of People Affected | Causative Agents | Type of Fomites | Key Findings |
|---|---|---|---|---|---|---|---|
| **Cheesbrough et al., 2000** | United Kingdom | Hotel | January-May, 1996 | 2.2%-39.1% guests (19.8%) | Norwalk-like virus (NLV) | **Confirmed:** Re-introduction of outbreak by contaminated environment | • Fecal samples and 61 (42%) of 144 swabs positive for NLV |
| **CDC, 2008** | South America | Elementary school | February 4-8, 2007 | 27 students and 2 staff members | Norovirus GII | **Confirmed:** Non-cleaned computer equipment (key board and mice) | • Stool samples and 1 of 25 swabs (computer mouse and key board) positive for norovirus GII |
| **Evans et al., 2002** | United Kingdom | Metropolitan concert hall | January, 1999 | >300 people | Norwalk-like virus (NLV) | **Suspected:** Direct contact with contaminated fomites | • Two stool samples tested positive for NLV |
| **Fankem et al., 2014** | United States | College summer camp | Summer, 2005 | Beginning- 40%, after initial cleaning- 73%, after proper cleaning and disinfection- 30%. | Norovirus-GII.2 | **Confirmed:** Contaminated fomites in a dorm room, and outbreak spread due to poor cleaning procedure of fomites | • July 21-22, dorm room; 17% positive for norovirus<br>• After cleaning with soap 22% and after disinfection <35% norovirus positive<br>• 45% fomites-norovirus positive |
| **Green et al., 1997** | United Kingdom | Hospital | May, 1994 (25 days) | 28 patients | Small round structured virus (SRSV) particles | **Confirmed:** positive swabs from lockers, curtains and commodes | • 30 fecal (40%) and 7 vomitus (14%) and 28 throat swabs (9.5%) samples-SRSV positive<br>• 36 environmental swabs (30%) positive for SRSV |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Holmes and Simmons, 2008** | New Zealand and Australia | Trans-pacific aircraft flight | January 20, 2007 | Reported at Auckland, after 60 hours of flight. – 41 of 122 (33.6%) passenger | Viral Infection | **Suspected:** Surface contamination in a closed environment, and enhanced spread by cross-contamination in toilet cubicle | • Routine GI illness surveillance data collected by medical staff<br>• Did not test stool samples<br>• Projectile vomiting and diarrhea >50% of cases<br>• Incubation of 10-50 hours |
| **Isakbaeva et al., 2005** | United States | Cruise ship | November 20, 2002 | Cruise ship 1 – 84 (4%) of 2,318 passengers following cruise 2, Cruise 3 – 192 (8%) of 2,456 passengers and 23 (2.3%) of 999 crew | Norovirus (GII) | **Suspected:** Environmental contamination-Persistence of virus despite sanitization onboard | • 25/55 tested stool samples positive for norovirus (45%) |
| **Kuusi et al., 2002** | Europe | Rehabilitation center | December, 1999-February, 2000 | > 300 guests and staff members | Norwalk-like calcivirus (NLV GII) | **Suspected:** Environmental contamination-spread of pathogen | • Stool and environmental samples positive for NLV |
| **Liu et al., 2003** | Australia | Aged-care residential hostel | September, 2002 | 28 residents and 5 staff members (42%) | Norwalk-like virus (NLV) | **Suspected:** Direct contact with index case and fomites | • Stool samples (5) positive for NLV |
| **Love et al., 2002** | United States | Hotel | November, 2000 (2 weeks) | At least 76 of guests and 40 hotel employees | Norwalk-like virus | **Suspected:** Environmental contamination | • Stool samples positive for NLV |
| **Menezes et al., 2010** | South America | Long-term care facility | July 8-29, 2005 | Inpatients 41.3% and employees 16.25% | Norovirus GII | **Suspected:** Fomite transmission | • Norovirus positive for 4 stools (44.4%) and water positive for *P. aeruginosa* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ohwaki et al., 2009** | Japan | Hospital and attached long-term care facility | February 22, 2007 | 47 (16%) of 285 staff members and 55 (13%) of 413 patients | Norovirus GII/4 | **Suspected:** Contact with various contaminated environmental surfaces | • 23/ 32 stool samples positive for norovirus GII/4<br>• *S. aureus* detected in 3 foods samples and 2 environmental samples |
| **Repp and Keene, 2012** | United States | Hotel | October, 2010 | 9 members of a soccer team | Norovirus GII | **Confirmed:** Touching a Reusable grocery bag or consuming its packaged food contents | • Reusable grocery bag positive for G II Norovirus |
| **Schmid et al., 2005** | Australia | Nursing home and hospital | November 9-17, 2004 at nursing home; November 11-28, 2004 at hospital | 17 of 23 (73.9%) residents and 7 of 18 (38.9%) staff members at nursing home,<br><br>10 of 46 (21.7%) hospital patients, 18 of 60 (30%) hospital staff | Norovirus GGII.4 (Jamboree-like) | **Suspected:** Environmental contamination | • 8 of 10 samples positive for Norovirus genotype GGII.4 (Jamboree-like) in both settings |
| **Thornley et al., 2011** | New Zealand | Airplane | October, 2009 | Flight attendants 27 of 77 (43%) | Norovirus GI.6 | **Suspected:** Exposure (contaminated surfaces) on airplane during successive flight sectors | • Stool samples positive for norovirus GI.6<br>• Swab samples negative for norovirus |
| **Wadl et al., 2010** | Germany | Military base | December 24, 2008-February 3, 2009 | 36 persons at a military base, total 101/815 (12.4%) persons | Norovirus (GII.4) | **Confirmed:** Military base canteen-environmental surfaces | • Norovirus detected only in stool samples and environmental samples |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Wu et al., 2005** | United States | Long-term care facility | Fall and winter, 2002 | 127 (52%) of 246 residents, 84 (46%) of 181 employees | Norovirus GII | **Confirmed:** environmental contamination | • Stool samples, 1 of 3-vomit sample, 5 of 10 environmental samples positive for norovirus GII |
| **Xue et al., 2014** | China | Boarding school | December, 2012 | >200 students and teachers, Attack rate 13.9%. | Norovirus GII | **Confirmed:** Spread contaminated environmental surfaces-kitchen surfaces | • 20 swab samples positive for norovirus<br>• Environmental samples positive for norovirus |

**DISCUSSION**

Human noroviruses were the only AGE pathogen identified in these studies, which was not surprising given that norovirus is the most common cause of AGE worldwide and the environmental contamination has been shown to play a role in norovirus outbreaks (Fankem et al., 2014; CDC, 2012; Menezes et al., 2010; CDC, 2008). However, it was surprising that no other pathogens were reported to be associated with fomites. There might be several reasons for this – absence of surveillance systems in some countries, lack of resources to track every outbreak, inconsistences of outbreak investigations.

Many countries do not have good surveillance systems. Even in developed countries those that do, such as the United States, there might be a lack of resources to investigate every outbreak and inconsistences of outbreak investigations. Many developing countries lack financial support for the investigation of outbreaks. Although the physicians in developing countries identify the causative agents to treat the patients, the transmission pathways for AGE pathogens remain under-recognized in the absence of a good outbreak investigation system.

Lack of resources, such as funding to support outbreak investigations, enough laboratories and laboratory facilities with technology, trained and qualified human resources can prevent a proper outbreak investigation. This is a significant issue especially in developing countries. Even in the United States, there might be insufficient resources to investigate each outbreak. Therefore, there may be many unreported cases of AGE outbreaks associated environmental fomites.

Inconsistences in how outbreak investigations are conducted could be another reason for the lack of bacterial AGE outbreaks associated with fomites. Some of the developed countries have multiple outbreak surveillance systems while few developing countries even have a simple tracking system. Therefore, each outbreak in the world is probably not monitored or investigated properly. In addition, there are many uncertainties of the current surveillance systems that do not investigate each AGE outbreak. This could be due to failure in the identification and reporting cases like fomites-associated AGE outbreaks, which remain under-attention. Literature provides evidences on these identified flaws listed in a current surveillance system. Some of them are failure to identify and report cases, failure to inform other countries, inadequate preparedness planning, and inadequate funding arrangements. Further, these flaws confirm the reasons for unavailable epidemiological data in related to fomites-associated AGE outbreaks in the world (Maclehose et al., 2001).

## LIMITATIONS

We only reviewed articles published in the English language. Thus, relevant articles published in other languages might have been excluded. We only searched for foodborne bacteria or AGE bacteria in the key terms. List of all the names for the foodborne or AGE bacteria were not included for the search. This might have caused exclusion of some relevant articles.

## CONCLUSIONS

The only pathogen identified in fomites-associated AGE outbreaks is human norovirus. Fomites identified in AGE outbreaks were a contaminated reusable grocery bag, computer mouse devices and keyboards, lockers, curtains and commodes. Considering these findings, investigators who study fomites can use the results to inform their research. In addition, our findings can be useful to develop appropriate strategies to prevent and control AGE outbreaks using how outbreak investigations were conducted. In future, researchers can study the survival of pathogenic microorganisms on fomites to cause AGE outbreaks under laboratory conditions. As well as, swabbing of fomites/environmental surfaces can be performed to determine the level of contamination to determine ability to cause AGE outbreaks in the place of retail food services.

## LITERATURE CITED


Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *14*(8), 725-730.

Atmar, R. L., and Estes, M. K. (2006). The epidemiologic and clinical importance of norovirus infection. *Gastroenterology Clinics*, *35*(2), 275-290.

Centers for Disease Control and Prevention. (2012). *Deaths from gastroenteritis double*. *CDC newsroom*. Retrieved March 30, 2017, from https://www.cdc.gov/media/releases/2012/p0314_gastroenteritis.html.

Centers for Disease Control and Prevention (CDC). (2008). Norovirus outbreak in an elementary school-District of Columbia, February 2007. *MMWR. Morbidity and Mortality Weekly Report*, *56*(51-52), 1340.

Cheesbrough, J. S., Green, J., Gallimore, C. I., Wright, P. A., and Brown, D. W. G. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiology and Infection*, *125*(01), 93-98.

Buckley, D., Fraser, A., Huang, G., and Jiang, X. (2017). Recovery optimization and survival of human norovirus surrogates, feline calicivirus and murine norovirus on carpet. *Applied and Environmental Microbiology*, AEM-01336.

Evans, M. R., Meldrum, R., Lane, W., Gardner, D., Ribeiro, C. D., Gallimore, C. I., and Westmoreland, D. (2002). An outbreak of viral gastroenteritis following environmental contamination at a concert hall. *Epidemiology and Infection*, *129*(02), 355-360.

Fankem, S. L., Boone, S. A., Marlene Gaither, M. P. A., and ME, R. (2014). Outbreak of norovirus illness in a college summer camp: impact of cleaning on occurrence of norovirus on fomites. *Journal of Environmental Health*, *76*(8), 20.

Green, J., Wright, P. A., Gallimore, C. I., Mitchell, O., Morgan-Capner, P., and Brown, D. W. G. (1998). The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. *Journal of Hospital Infection*, *39*(1), 39-45.

Hall, A.J., Lopman, B.A., Payne, D.C., Patel, M.M., Gastanaduy, P.A., Vinje, J., and Parashar, U.D. (2013). Norovirus Disease in the United States. *Emerging Infectious Disease, 19*, 1198–1205.

Harris, J. P., Lopman, B. A., and O'Brien, S. J. (2010). Infection control measures for norovirus: a systematic review of outbreaks in semi-enclosed settings. *Journal of Hospital Infection*, *74*(1), 1-9.

Holmes, J. D., and Simmons, G. C. (2009). Gastrointestinal illness associated with a long-haul flight. *Epidemiology and Infection*, *137*(03), 441-447.

Isakbaeva, E. T., Widdowson, M. A., Beard, R. S., Bulens, S. N., Mullins, J., Monroe, S. S., and Glass, R. I. (2005). Norovirus transmission on cruise ship. *Emerging Infectious Diseases*, *11*(1), 154-8.

Kaplan, J. E., Gary, G. W., Baron, R. C., Singh, N., Schonberger, L. B., Feldman, R., and Greenberg, H. B. (1982). Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. *Ann Intern Med*, *96*(6 Pt 1), 756-761.

Kim, A. N., Park, S. Y., Bae, S. C., Oh, M. H., and Ha, S. D. (2014). Survival of norovirus surrogate on various food-contact surfaces. *Food and Environmental Virology*, *6*(3), 182-188.

Kuusi, M., Nuorti, J. P., Maunula, L., Minh, N. T., Ratia, M., Karlsson, J., and Von Bonsdorff, C. H. (2002). A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation center due to environmental contamination. *Epidemiology and Infection*, *129*(01), 133-138.

Liu, B., Maywood, P., Gupta, L., and Campbell, B. (2003). An outbreak of Norwalk-like virus gastroenteritis in an aged-care residential hostel. *New South Wales Public Health Bulletin*, *14*(6), 105-109.

Love, S. S., Jiang, X., Barrett, E., Farkas, T., and Kelly, S. (2002). A large hotel outbreak of Norwalk-like virus gastroenteritis among three groups of guests and hotel employees in Virginia. *Epidemiology and Infection*, *129*(01), 127-132.

MacLehose, L., Brand, H., Camaroni, I., Fulop, N., Gill, O. N., Reintjes, R., ... and Weinberg, J. (2001). Communicable disease outbreaks involving more than one country: systems approach to evaluating the response. *Bmj*, *323*(7317), 861-863.

Menezes, F. G. D., Correa, V. M. D. S. D., Franco, F. G. D. M., Ribeiro, M. I., Cardoso, M. F. D. S., Morillo, S. G., ... and Pasternak, J. (2010). An outbreak of norovirus infection in a long-term care facility in Brazil. *Einstein (Sao Paulo)*, *8*(4), 410-413.

Ohwaki, K., Nagashima, H., Aoki, M., Aoki, H., and Yano, E. (2009). A foodborne norovirus outbreak at a hospital and an attached long-term care facility. *Japanese Journal of Infectious Diseases*, *62*(6), 450-454.

Repp, K. K., and Keene, W. E. (2012). A point-source norovirus outbreak caused by exposure to fomites. *Journal of Infectious Diseases*, *205*(11), 1639-1641.

Rusin, P., Maxwell, S., and Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*, *93*(4), 585-592.

Schmid, D., Lederer, I., Pichler, A. M., Berghold, C., Schreier, E., and Allerberger, F. (2005). An outbreak of norovirus infection affecting an Austrian nursing home and a hospital. *Wiener Klinische Wochenschrift*, *117*(23-24), 802-808.

Thornley, C. N., Emslie, N. A., Sprott, T. W., Greening, G. E., and Rapana, J. P. (2011). Recurring norovirus transmission on an airplane. *Clinical Infectious Diseases*, cir465.

Vega, E., Barclay, L., Gregoricus, N., Williams, K., Lee, D., and Vinje, J. (2011). Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerging Infectious Diseases*, *17*(8), 1389.

Wald, M., Scherer, K., Nielsen, S., Diedrich, S., Ellerbroek, L., Frank, C., and Koch, J. (2010). Food-borne norovirus-outbreak at a military base, Germany, 2009. *BMC Infectious Diseases*, *10*(1), 1.

Wikswo, M. E., Kambhampati, A., Shioda, K., Walsh, K. A., Bowen, A. B., and Hall, A. J. (2015). Outbreaks of acute gastroenteritis transmitted by person-to-person contact, environmental contamination, and unknown modes of transmission – United States, 2009-2013.

Wu, H. M., Fornek, M., Schwab, K. J., Chapin, A. R., Gibson, K., Schwab, E., and Henning, K. (2005). A norovirus outbreak at a long-term-care facility: the role of environmental surface contamination. *Infection Control and Hospital Epidemiology*, *26*(10), 802-810.

Xue, C., Fu, Y., Zhu, W., Fei, Y., Zhu, L., Zhang, H., and Sun, Q. (2014). An outbreak of acute norovirus gastroenteritis in a boarding school in Shanghai: a retrospective cohort study. *BMC Public eHealth*, *14*(1), 1092.

# CHAPTER THREE

# PERSISTENCE OF BACTERIA THAT CAUSE ACUTE GASTROENTERITIS ON PACKAGING MATERIALS: A SYSTEMATIC LITERATURE REVIEW

## BACKGROUND AND RATIONALE

The exterior surfaces of food packaging can act as fomites on which AGE bacteria can survive. The published literature presents evidence that AGE associated bacteria can survive on the surfaces of materials, which are commonly used in packaging (Brozkova et al., 2014; Wilks et al., 2006; Ak et al., 1994). If the exterior surface of a food package becomes contaminated with AGE causing bacteria via hands or contaminated surfaces, AGE bacteria could survive on the surfaces until the environmental conditions get favorable. Under favorable conditions, these AGE pathogens could multiply and survive for several days, weeks or months on material surfaces possibly resulting in AGE outbreaks.

According to the results of the literature review presented in Chapter 2, published studies on bacterial AGE outbreaks associated with packaging materials are not available. This might be due to several reasons, such as absence of good surveillance systems, lack of resources to track every outbreak, and inconsistences of outbreak investigations. However, we hypothesize that low-infectious dose AGE bacteria could survive on packaging material surfaces for extended periods, and could possibly cause AGE illnesses. Epidemiological studies present evidence that, these bacteria can be directly transferred from fomites to the human hands and then finger pad to the lip (Rusin et al., 2002; Scott and Bloomfield, 1990). The aim of our review was to answer the research

question “Can AGE associated bacteria survive on common packaging materials?” To our knowledge, this is the first systematic review that summarizes the survival of AGE associated bacteria on materials used in food packaging.

## METHODS

### Search Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was used as the guidance for the systematic literature review (Liberati et al., 2009). The selection criteria were: published in the English language, peer reviewed, and published between 1970 and August 2015 in all geographic areas. A combination of key terms outlined in table 3.1 were used to search four databases – Search Complete, Web of Science, Google Scholar, and Science Direct.

**Table 3.1 Search String**

| **Terms – Survival** | | **Term – Pathogen** | | **Terms – Contamination area** |
|---|---|---|---|---|
| Persistence OR Survival | AND | Bacteria | AND | Surfaces OR fomites OR “Packaging materials” OR “Food packages” |

Duplicates were removed, and the title and abstract were reviewed for the relevance of our search terms. The full text of the included articles was reviewed to determine final eligibility. To be included, each article had to meet following eligibility criteria – published in English, peer reviewed, published between 1970 and end of August 2015. In addition, articles were excluded if they addressed bacteria other than AGE bacteria and surface materials that cannot be found as food packaging material.

Although review articles were excluded, the reference lists of review articles were manually searched to collect additional articles that might have been missed during the electronic search and the reference lists of all eligible articles were manually screened to locate articles that might have been missed during database searching.

**RESULTS**

The initial electronic database search yielded 2760 articles (Figure 3.1). After removing duplicates and screening titles and abstracts, 149 records were identified for full text review. After manual searching the reference lists of the articles included for full text review, 22 additional records were included. After reviewing the full text of 171 potentially eligible articles, 125 records were excluded for the following reasons: not published in English (n=3), not peer reviewed articles (n=5), other bacteria do not cause AGE (n=8), surface materials that cannot be found as food packaging materials (n=25), and had an inappropriate study design (n=84). A total of 24 studies were included in the review.

The 24 eligible articles are listed in Table 3.2. All studies were published between 1973 and 2014. The studies mainly focused on common AGE bacteria – *Escherichia coli* (n=8), *Salmonella* spp. (n=8), *Staphylococcus aureus* (n=5), *L. monocytogenes* (n=5), and *P. aeruginosa* (n=4). AGE bacteria were shown to survive on various packaging materials – plastic polymers (n=16), glass (n=8), aluminum (n=2), and steel (n=1).
In studies (n=5 of 7), which compared the survival of gram-positive bacteria with the survival of gram-negative bacteria, gram-positive bacteria survived longer than the longest surviving gram-negative bacteria on packaging materials. AGE bacteria survived

over a period of incubation from zero time to several minutes, hours, days, weeks, or months depending on the sampling period of each study (Table 3.2).

A range of environmental conditions was also studied – temperature (4°C, 10°C, 21°C, 30°C), humidity (<25%-85%), wet and dry (moist or desiccated) surfaces, and light (dark, UV, fluorescent). Most survived better at colder temperatures (e.g. 4°C) compared to warmer temperatures (e.g. 30°C) (n=5). In two studies, bacterial survival was reduced under dry/desiccation conditions (n=2). Three studies reported that bacteria survived best at high relative humidity (e.g. ~75%) (n=3). In three studies, bacteria survived longer at both very low and very high relative humidity (n=3). One study showed a lower survival at high relative humidity (53%-85%) than at low relative humidity (11%-33%).

**Figure 3.1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses Flow Chart Describing the Literature Search Procedure**

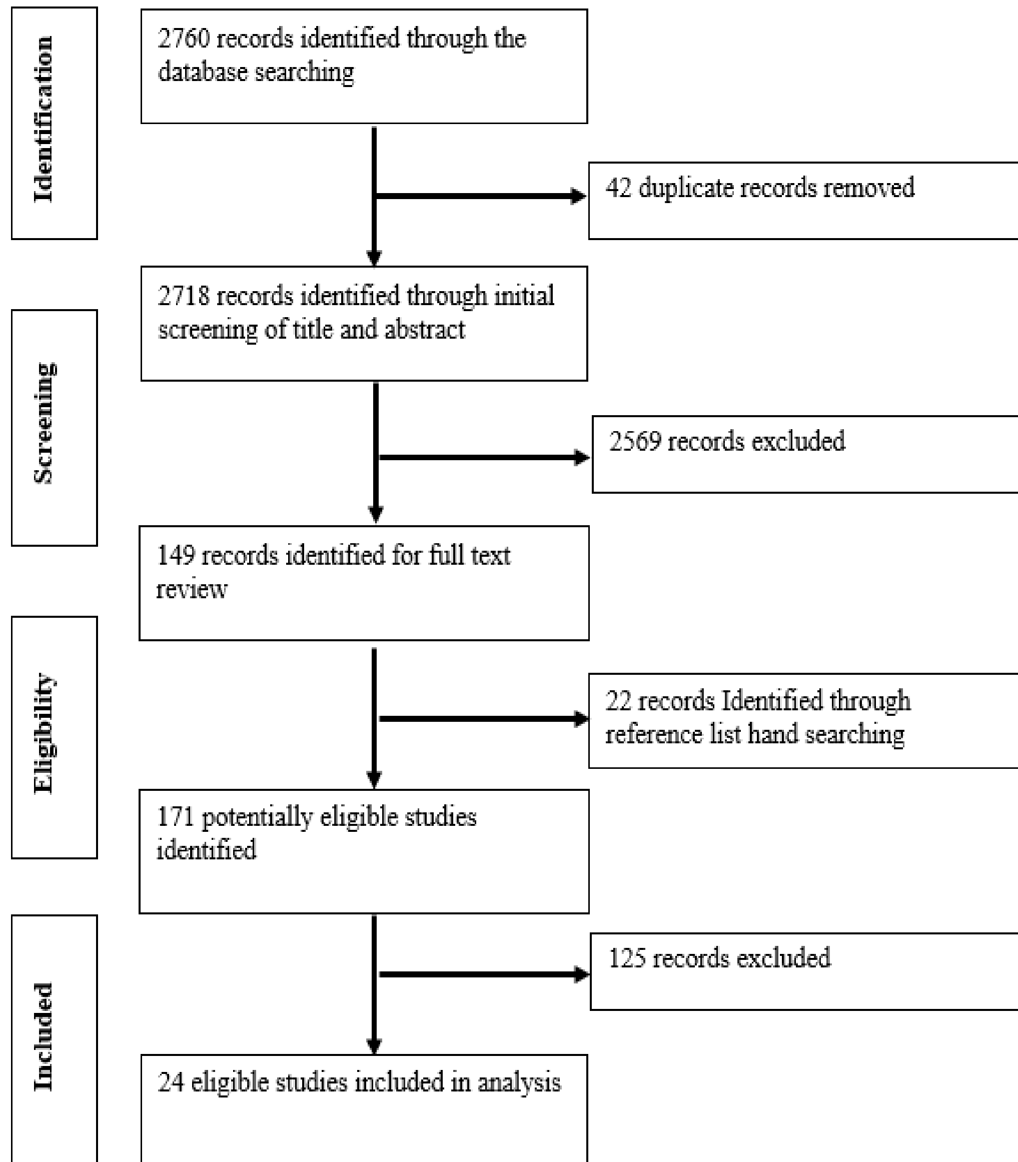

**Table 3.2 Summary of Studies Reviewed for the Persistence of Bacteria that cause AGE on Materials used in Packaging**

| First Author, Year | Foodborne Bacteria | Material | Survival Period Tested | Environmental Conditions | Key Findings |
|---|---|---|---|---|---|
| **Ak et al., 1994** | **GRAM (+)**<br>*L. innocua*<br>*L. monocytogenes*<br><br>**GRAM (-)**<br>*E. coli* spp.<br>*E. coli* O157:H7<br>*S. typhimurium* | Polyacrylic<br>Polyethylene<br>Polypropylene<br>Polystyrene | 0, 3, and 10 minutes, and 12 hours | Tem: 4 ℃, RT: 18°C-28 °C<br>Saturated humidity | *E. coli* O157:H7<br>• No significant difference of recovery among plastics, between tem. or RH<br>• Survived at cold tem. and multiplied at RT<br><br>*L. monocytogenes*<br>• Significantly reduced (90% in 3 hours) when opened to air drying<br>• No significant changes (increased slightly) when surfaces were covered, at RT |
| **Bale et al., 1993** | **GRAM (-)**<br>*P. aeruginosa*<br>*S. enterica*<br>*E. coli* spp.<br><br>**GRAM (+)**<br>*Enterococcus* spp. | Polypropylene<br>Polystyrene<br>Glass<br>Corrugated cardboard<br>Corrugated paper | *Enterococcus* spp. – 11 weeks, other bacteria over 2 weeks | RT: 18°C-28°C<br>RH: 40%-95 % | • All the species survived at least 14 days with *Enterococcus* spp. survived 11 weeks on glass<br>• All species showed 0.5-1.5 log reduction in numbers on polypropylene and glass<br>• *S. enterica* survived significantly better than *E. coli* on plastic surfaces<br>• *E. coli* survived well at very low and very high RH |
| **Brozkova et al., 2014** | **GRAM (-)**<br>*Arcobacter* spp. | Aluminum | 0-180 minutes and 0-24 hours | Tem: 5 ℃, RT: 18°C-8°C | • At 5℃, significantly decreased and completely inactivated after 8 hours<br>• After 2 hours, the survival of *Arcobacter* spp. were 9.0%-27.0% of the original cells<br>• At 25℃, bacteria inactivated significantly faster and survived up to 120 minutes |

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| **Cervenka et al., 2008** | **GRAM (-)**<br>*A. butzleri* | Glass<br>Polypropylene | 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.5, 6.5 hours | RH: 32%-64%<br>Tem: 30℃ | • No colony counts after 1.5 hours at 32% RH and 2.0 hours at 64% RH<br>• Survived after 3.5 hours on polypropylene and after 2.5 hours on glass at 32% RH and survived after 3.5 hours at 64% RH for all the surfaces<br>• Decreased the survival significantly after 1.5 hours at 32% RH, and at 64% RH on polypropylene and after 1.5 hours at 64% RH on glass |
| **Cools et al., 2005** | **GRAM (-)**<br>*C. jejuni* | Polypropylene | Immediately after incubation, 30, 60, 90, and 120 minutes | RT: 18°C-28°C | • Reduced by 3 log CFU per 25 $cm^2$ in first 30 minutes<br>• After 30 minutes, the recovery remained constant |
| **Gough and Dodd, 1998** | **GRAM (-)**<br>*S. typhimurium* | Polyethylene | After rinsing for 10 minutes<br>After drying for 30, 60, 90, and 120 minutes | RT: 18°C-28°C | • After 10 minutes of absorption with bacteria was significantly greater from plastic than other surface types<br>• Significantly higher recovery at 30 minutes than at 60, 90, and 120 minutes |
| **Hirai, 1991** | **GRAM (+)**<br>*S. aureus*<br>*S. epidermidis*<br>*Enterococcus faecalis*<br><br>**GRAM (-)**<br>*S. Enteritidis*<br>*S. typhimurium*<br>*E. coli*<br>*K. pneumonia*<br>*P. aeruginosa*<br>*P. cepacia* | Glass plate | 1, 2, 3, 5, 7 hours | Tem: 21 ± 1ºC<br>RH: 50 ± 5%<br>Dry conditions in a chamber | • Survival rate of gram (-) bacteria decreased with time<br>• *S. epidermidis* was resistant to dry conditions and > 90% survived after 7 hours<br>• Survival of gram (+) bacteria was significantly higher than gram (-) bacteria |

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| **Iibuchi et al., 2010** | **GRAM (-)**<br>*Salmonella* | Polypropylene | Every 2 days to 30 days | Two days in a chamber with water, after 2 days with silica gel<br>RH: < 25%<br>RT: 18°C -28°C | • Biofilm forming strains >$10^4$ CFU/plate on day 175<br>• Biofilm deficient strains <$10^2$ CFU/plate on day 20<br>• Decreased the bacteria more slowly in biofilm forming strains than in biofilm deficient strains until day 30<br>• Significant difference in average population of biofilm forming strains and biofilm deficient strains on day 5, 10, 20 |
| **Joseph et al., 2001** | **GRAM (-)**<br>*Salmonella* spp. | High density polyethylene (HDPE) | 10 days | RT: 18°C -28°C | • *S. weltevreden* cell density of biofilm of 3.4 x $10^7$ CFU/$cm^2$<br>• *Salmonella* cell density of biofilm 1.2 x $10^7$ CFU/$cm^2$ |
| **McEldowney and Fletcher, 1988** | **GRAM (-)**<br>*Pseudomonas* sp.<br><br>**GRAM (+)**<br>*Staphylococcus* sp.<br>*S. aureus* | Glass | 5 days-10 days | RH: 0%, 34%,75%<br>Tem: 4°C, 15°C,25℃<br>Desiccation | • No distinction between gram (+) and gram (-) species in desiccation survival<br>• The longest survival except *S. aureus* showed at 4℃ and the shortest survival for all species was at 25℃<br>• *S. aureus* showed highest survival at 15℃ only under 0% and 75% RH |
| **Moore et al., 2007** | **GRAM (-)**<br>*S. typhimurium* | Polypropylene | 0, 1, 2, 3, 4, 5, 6 hours | RT: 18℃ -28℃ | • Bacteria decreased rapid within first hour inoculation with 3.02 log reduction<br>• Recovered number declined slowly after 1 hour up to 6 hours with 0.87 log reduction |

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| **Morino et al., 2011** | **GRAM (-)**<br>*E. coli*<br><br>**GRAM (+)**<br>*S. aureus* | Glass dishes | 1, 2, 3, 4, 5 hours | RT: 18ºC -28ºC<br>RH: 54 ± 2%<br>Fluorescent light<br>$ClO_2$ gas (mean 0.05 ppmv, 0.14 $mgm^{-3}$)<br>Wet surface | • *E. coli* >2 $\log_{10}$ reduction after 3 hours, >5 $\log_{10}$ after 5 hours<br>• *S. aureus* >2 $\log_{10}$ reduction after 5 hours<br>• *S. aureus* survived significantly higher than *E. coli* under low concentration $ClO_2$ gas, wet surface, at RT, 54% RH and under fluorescent lights |
| **Neely, 2000** | **GRAM (-)**<br>*P. aeruginosa*<br>*E. coli,*<br>*K. pneumonia,*<br>*Enterobacter* spp. | Polyester nylon-polyvinyl | Immediately after inoculation, every hour up to 24 hours and every day after the first day up to 2 months | Tem: 22.5ºC - 26.2ºC<br>RH: 20-49% | Survival of bacteria<br>• 1 hour to 8 days ($10^2$ bacteria per swatch)<br>• 2 hours to >60 days ($10^4$ to $10^5$ bacteria per swatch) |
| **Neely and Maley, 2000** | **GRAM (+)**<br>*S. aureus*<br>*Staphylococci,*<br>Vancomycin resistant enterococci (VRE) | Polyester<br>Polyethylene | Immediately after inoculation and hourly up to 8 hours, daily after first day up to no turbidity<br>(total 3 months) | Tem: 22.9ºC-24.5ºC<br>RH: 30%-49% | • *S. aureus* survived ~ >90 days on polyethylene and >20 days on polyester<br>• *Enterococci* spp. Survived ~ >90 days on polyethylene and >90 days on polyester, the shortest survival time was 11 days<br>• Longest *Staphylococcal* viability was on polyester (1 to 56 days) and on polyethylene (22 to >90 days) |
| **Rodrigues et al., 2013** | **GRAM (+)**<br>*L. monocytogenes* | N – $TiO_2$ coated on glass | After 30 minutes | RT: 18°C-28°C<br>Visible light – fluorescent and incandescent, ultraviolet (UV)<br>Dark room | • *L. monocytogenes* did not show an effective reduction after 30 minutes exposure to each light source<br>• Significantly reduced the number of viable bacteria under all conditions except in dark condition and fluorescent light |

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| **Rossi et al., 2013** | **GRAM (-)** *S. enterica*<br><br>**GRAM (+)** *Staphylococcus* | Polyethylene | 0, 1, 2, 3, 4, 24 hours | RT: 18°C-28°C<br>Air conditioning | • Reduced the number of bacteria with time<br>• Reduction was high in first 4 hours<br>• Bacteria survived after 24 hours |
| **Somers and Wong, 2004** | **GRAM (+)** *L. monocytogenes* | Polyester<br>Silicone rubber | Sampled after 2 to 5 days of incubation | Tem: 10ºC, 4ºC<br>RH: 78% -65% | • Developed the biofilms after 2 days on all materials<br>• Silicone rubber was more resistance to biofilm development<br>• Did not decrease the bacterial numbers significantly at 4ºC<br>• Decreased bacterial cell number significantly (>50 to >95%) at 10ºC |
| **Tolba et al., 2007** | **GRAM (+)** *S. aureus* | Glass | Day 0, daily interval up to day 7, and hourly for 12 hours | Tem: 20ºC<br>RH: 60%<br>Incubated in dark | • After 24 hours, no colonies were detected<br>• Bacterium was detected qualitatively and quantitatively up to 4 hours |
| **Turner and Salmonsen., 1973** | **GRAM (-)** *Klebsiella* | Glass | 2, 4, 8, 24, 48,72 hours | Tem: 25ºC<br>RH: 85%, 53%, 33%, 11% | • Survival has reduced with high RH (significantly at 53% and 85% RH)<br>• Bacteria remained viable at least 3 days at 11% and 33% RH |
| **Wendt et al., 1998** | **GRAM (+)** *E. faecium* strains | Polyvinyl chloride | 0, after 4 hours, 1 day, and 1, 2, 4, 8, 16 weeks | Tem: 22 ± 2ºC<br>RH: 50% | • Recoverable proportion of the strains varied greatly with material 8 to 98%, no association identified<br>• All strains survived at least 1 week under dry condition and two strains (*E. faecium* 26 and *E. faecium* 547) survived 4 months under dry condition |

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| **Wilks et al., 2006** | **GRAM (+)**<br>*L. monocytogenes* | Aluminum | 100 minutes having 4-6 sample intervals | RT: 18°C -28°C | • Survival is greatly reduced compared to stainless steel<br>• No viable bacteria after 60 minutes of incubation (5 log reduction) |
| **Williams et al., 2005** | **GRAM (-)**<br>*E. coli* O157 | Steel | 0, 3, 7, 14, 28 days | Tem: 5ºC, 20ºC<br>Moisture (moist or dry) | • Persistence greatest on moist and at 5ºC than dry and at 20ºC<br>• At 5ºC and dry, *E. coli* survived up to 28 days but at 5ºC moist, survival was > 28 days<br>• At 20ºC and dry, *E. coli* survived 3-7 days and at 20ºC and moist, survival was 7 days |
| **Yazgi et al., 2009** | **GRAM (-)**<br>*E. coli*<br>*P. aeruginosa*<br><br>**GRAM (+)**<br>*S. aureus*<br>*Vancomycin – resistant (VR)*<br>*E. faecalis* | Vinyl | Day 3 and every other day up to no growth in 3 times consequently | RT: 18°C -28°C<br>RH: 70% humidity<br>AP: ~ 823.0 mb | • Survival for all the bacteria, significantly short<br>• *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli* survived respectively 63 days, 48 days, 11 days, and 5 days<br>• Gram (+) bacteria survived longer than gram (-) bacteria<br>• *S. aureus* survived longest, and *E. coli* survived shortest period |

**NOTE:** Room temperature – used this term for ambient temperature too. Mostly it is 21 ± 1°C, but it is a range 18-28°C.
RT, room temperature; Tem, temperature; RH, relative humidity; AP, atmospheric pressure; Gram (+), gram positive; Gram (-), gram negative.

**DISCUSSION**

AGE bacteria (*Escherichia coli* spp., *Salmonella* spp., *S. aureus*, and *L. monocytogens*) were shown to survive on a wide range of material surfaces (plastics, glass, aluminum, and steel). Survival of bacteria were different on various materials, such as wood, paper, plastic, glass or metal surfaces (Williams et al., 2005; Bale et al., 1993). Two studies highlighted a significant difference ($p < 0.05$) between the recovery of bacteria from plastic and steel surfaces. However, a significant difference ($p > 0.05$) was not observed among types of plastic (e.g. polyacrylic, polyethylene, polypropylene, and polystyrene) (Williams et al., 2005; Ak et al., 1994). The surface chemistry of materials, such as polar or non-polar, which describes surface energy and hydrophilicity, influences the survival of bacteria (Cervenka et al., 2008). However, according to the studies included in our review, the relationship of surface type and the survival of AGE bacteria is inconclusive. Most studies examined a single material.

The survival of AGE bacteria on material surfaces is dependent upon a range of interacting environmental factors, such as temperature and relative humidity. Many showed that bacteria can survive longer time at low temperatures (e.g. 4°C), (Brozkova et al., 2014; Somers and Wong, 2004; McEldowney and Fletcher, 1988). How bacteria survival was favorable under low temperature could not be explained well from the literature. However, most of the bacteria studied are psychrotrophs so they are more likely to survive under cold temperatures. Furthermore, bacteria were shown to survive at high relative humidity compared to the low relative humidity, but this finding was contradicted in other studies. Therefore, the effect of relative humidity on survival of bacteria is inconclusive.

In addition to the environmental conditions and the type of material surfaces, the chemistry of the bacterial cell wall also was shown to have an impact on the survival pattern of bacteria. For example, most studies showed that gram-positive bacteria survived longer than the longest surviving gram-negative bacteria (Yazgi et al., 2009; Ak et al., 1994; Bale et al., 1993; Hirai, 1991). However, two studies presented similar survival in both gram-positive and gram-negative bacteria (Rossi et al., 2013; Ak et al., 1994). Therefore, more research is needed to explain the role of chemical composition and functional groups of bacterial cell wall on the survival of bacteria on the surface of materials.

Finally, the survival period of each bacterium cannot be interpreted correctly because the protocols for many studies was not completed until death (zero survival) (Table 3.2). For example, one study was conducted until no bacteria survived, which was 63 days at room temperature and high humidity, while a few studies presented the survival patterns of bacteria for several hours (e.g. 0-72 hours) (Yazgi et al., 2009; Wilks et al., 2006). Therefore, bacteria may survive longer than the selected sampling period used in studies.

## LIMITATIONS

We only reviewed articles published in the English language. Therefore, relevant articles published in other languages might have been excluded. Bacteria that cause AGE or foodborne diseases and materials that can be used as common food packaging were carefully selected as two main limiting factors in this review. The studies that used

nutrition to the bacterial surviving environments have been excluded. Therefore, we could limit nutrition, which is as one of the bacterial survival-enhancing factors.

**CONCLUSION**

AGE bacteria survived on range of packaging materials from several days to months depending on the environmental conditions, properties of the material surface and the chemistry of bacterial cell wall. However, total survival period of bacteria cannot be interpreted correctly. In future, investigators who study the survival of AGE bacteria can use our findings to determine the survival of AGE bacteria on materials that can be used in food packaging. More research is needed to make a conclusion about the survival of AGE causing bacteria on packaging materials and its' relation to the AGE outbreaks. Therefore, studies should be conducted on the survival of AGE pathogens on common food packaging materials to investigate the potential of the exterior surface of food packaging to act as fomites.

**LITERATURE CITED**

Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *14*(8), 725-730.

Ak, N. O., Cliver, D. O., and Kaspari, C. W. (1994). Decontamination of plastic and wooden cutting boards for kitchen use. *Journal of Food Protection*, *57*(1), 23-30.

Bale, M. J., Bennett, P. M., Beringer, J. E., and Hinton, M. (1993). The survival of bacteria exposed to desiccation on surfaces associated with farm buildings. *Journal of Applied Bacteriology*, *75*(6), 519-528.

Brozkova, D. S. L. H. I., and Vytrasova, P. M. J. (2014). Survival of selected bacteria from the genus *Arcobacter* on various metallic surfaces. *Journal of Food and Nutrition Research (ISSN 1336-8672)*, *53*(3), 217-223.

Cervenka, L., Kristlova, J., Peskova, I., Vytrasova, J., Pejchalova, M., and Brozkova, I. (2008). Persistence of *Arcobacter butzleri* CCUG 30484 on plastic, stainless steel and glass surfaces. *Brazilian Journal of Microbiology*, *39*(3), 517-520.

Chow, C. M., Leung, A. K., and Hon, K. L. (2010). Acute gastroenteritis: from guidelines to real life. *Clinical and Experimental Gastroenterology*, *3*, 97.

Cools, I., Uyttendaele, M., Cerpentier, J., D'haese, E., Nelis, H. J., and Debevere, J. (2005). Persistence of *Campylobacter jejuni* on surfaces in a processing environment and on cutting boards. *Letters in Applied Microbiology*, *40*(6), 418-423.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, *293*(5538), 2248-2251.

Gough, N. L., and Dodd, C. E. R. (1998). The survival and disinfection of *Salmonella typhimurium* on chopping board surfaces of wood and plastic. *Food Control*, *9*(6), 363-368.

Hirai, Y. (1991). Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. *Journal of Hospital Infection*, *19*(3), 191-200.

Iibuchi, R., Hara-Kudo, Y., Hasegawa, A., and Kumagai, S. (2010). Survival of *Salmonella* on a polypropylene surface under dry conditions in relation to biofilm-formation capability. *Journal of Food Protection*, *73*(8), 1506-1510.

Joseph, B., Otta, S. K., Karunasagar, I., and Karunasagar, I. (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology*, *64*(3), 367-372.

McEldowney, S., and Fletcher, M. (1988). The effect of temperature and relative humidity on the survival of bacteria attached to dry solid surfaces. *Letters in Applied Microbiology*, *7*(4), 83-86.

Moore, G., Blair, I. S., and McDowell, D. A. (2007). Recovery and transfer of *Salmonella Typhimurium* from four different domestic food contact surfaces. *Journal of Food Protection*, *70*(10), 2273-2280.

Morino, H., Fukuda, T., Miura, T., and Shibata, T. (2011). Effect of low concentration chlorine dioxide gas against bacteria and viruses on a glass surface in wet environments. *Letters in Applied Microbiology*, *53*(6), 628-634.

Neely, A. N. (2000). A survey of gram-negative bacteria survival on hospital fabrics and plastics. *Journal of Burn Care and Research*, *21*(6), 523.

Neely, A. N., and Maley, M. P. (2000). Survival of *enterococci* and *staphylococci* on hospital fabrics and plastic. *Journal of Clinical Microbiology*, *38*(2), 724-726.

Price, P. B., and Sowers, T. (2004). Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(13), 4631-4636.

Rodrigues, D., Teixeira, P., Tavares, C. J., and Azeredo, J. (2013). Food contact surfaces coated with nitrogen-doped titanium dioxide: effect on *Listeria monocytogenes* survival under different light sources. *Applied Surface Science*, *270*, 1-5.

Rossi, E. M., Scapin, D., and Tondo, E. C. (2013). Survival and transfer of microorganisms from kitchen sponges to surfaces of stainless steel and polyethylene. *The Journal of Infection in Developing Countries*, *7*(03), 229-234.

Rusin, P., Maxwell, S., and Gerba, C. (2002). Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*, *93*(4), 585-592.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... and Griffin, P. M. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases*, *17*(1), 7-15.

Scott, E., and Bloomfield, S. F. (1990). The survival and transfer of microbial contamination via cloths, hands and utensils. *Journal of Applied Microbiology*, *68*(3), 271-278.

Somers, E. B., and Wong, A. C. (2004). Efficacy of two cleaning and sanitizing combinations on *Listeria monocytogenes* biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat meat residue. *Journal of Food Protection*, *67*(10), 2218-2229.

Thornley, C. N., Emslie, N. A., Sprott, T. W., Greening, G. E., and Rapana, J. P. (2011). Recurring norovirus transmission on an airplane. *Clinical Infectious Diseases*, cir465.

Tolba, O., Loughrey, A., Goldsmith, C. E., Millar, B. C., Rooney, P. J., and Moore, J. E. (2007). Survival of epidemic strains of nosocomial and community-acquired methicillin-resistant *Staphylococcus aureus* on coins. *American Journal of Infection Control*, *35*(5), 342-346.

Turner, A. G., and Salmonsen, P. A. (1973). The effect of relative humidity on the survival of three serotypes of *Klebsiella*. *Journal of Applied Bacteriology*, *36*(3), 497-499.

Wendt, C., Wiesenthal, B., Dietz, E., and Ruden, H. (1998). Survival of vancomycin-resistant and vancomycin-susceptible *enterococci* on dry surfaces. *Journal of Clinical Microbiology*, *36*(12), 3734-3736.

Wilks, S. A., Michels, H. T., and Keevil, C. W. (2006). Survival of *Listeria monocytogenes* Scott A on metal surfaces: implications for cross-contamination. *International Journal of Food Microbiology*, *111*(2), 93-98.

Williams, A. P., Avery, L. M., Killham, K., and Jones, D. L. (2005). Persistence of *Escherichia coli* O157:H7 on farm surfaces under different environmental conditions. *Journal of Applied Microbiology*, *98*(5), 1075-1083.

Wu, H. M., Fornek, M., Schwab, K. J., Chapin, A. R., Gibson, K., Schwab, E., …..and Henning, K. (2005). A norovirus outbreak at a long-term-care facility: the role of environmental surface contamination. *Infection Control and Hospital Epidemiology*, *26*(10), 802-810.

Yazgi, H., Uyanik, M. H., Ertek, M., Aktas, A. E., Igan, H., and Ayyildiz, A. (2009). Survival of certain nosocomial infectious agents on the surfaces of various covering materials. *Turkish Journal of Medical Sciences*, *39*(4), 619-622.

# CHAPTER FOUR

# PERSISTENCE OF *ESCHERICHIA COLI* O157:H7 AND *LISTERIA MONOCYTOGENES* ON THE EXTERIOR OF COMMON PACKAGING MATERIALS

## INTRODUCTION

Acute gastroenteritis (AGE) is a major cause of illness in the United States, resulting in 179 million episodes every year (Wikswo, 2015). While environmental transmission is estimated to be low, several studies suggest that environmental surfaces may play an under-recognized role in the transmission of AGE-associated pathogens (Xue et al., 2014; Thornley et al., 2011; Menezes et al., 2010; Holmes and Simmons, 2008). To date, most of the literature examining the role of environmental surfaces (or fomites) and transmission of AGE pathogens has centered on viruses and not bacterial pathogens (Repp and Keene, 2012; Fankem et al., 2014; Holmes and Simmons, 2008). This is not surprising, as norovirus is the leading cause of AGE outbreaks in the United States and contaminated environmental surfaces is one documented mode of transmission (Hall et al., 2013). Even so, we believe it is important to examine survival of bacterial pathogens as some bacteria have a very low-infectious dose and high environmental resistance – *E. coli* O157:H7 (less than 10 to 100 cells) and *L. monocytogenes* (<1000 CFU/g) (FDA, 2014).

One rarely studied fomite is the exterior of food packages. Determining bacterial survival on the exterior of a food package is important because these bacteria could be transferred to the hands of consumers after which they could be transferred to food and

then one's mouth. We believe that the exterior surfaces of packages for ready-to-eat snack foods, which are often consumed using fingers, could be a potential, not well-documented source for AGE-associated bacteria.

Three commonly used packaging materials are oriented polyethylene terephthalate (OPET), oriented polypropylene (OPP), and Nylon-6. OPET and OPP are commonly used to package ready-to-eat snack foods, such as potato chips, candy pieces, fruit snacks, crackers or cookies, almonds, or cashews. Nylon-6 is used as an outer layer co-extrusion of food packages for baked goods, meats, and ready-to-eat cheese sticks.

The aim of our research was to determine whether *E. coli* O157:H7 and *L. monocytogenes* could survive at least two weeks on three packaging materials – OPET, OPP, and nylon-6 at retail storage conditions defined by the Technical Association of the Pulp and Paper Industry (TAPPI) as 23℃ room temperature and 50% relative humidity-RH. The following five hypotheses guided our research:

1. *E. coli* O157:H7 can survive at least two weeks on the surface of three packaging materials (OPET, OPP and nylon-6) under TAPPI conditions.
2. *E. coli* O157:H7 can survive better on polar packaging materials (OPET and nylon 6) than on non-polar packaging material (OPP) under TAPPI conditions.
3. *L. monocytogenes* can survive at least two weeks on the surface of three packaging materials (OPET, OPP and nylon-6) under TAPPI conditions.
4. *L. monocytogenes* can survive better on polar packaging materials (OPET and nylon 6) than on non-polar packaging material (OPP) under TAPPI conditions.
5. Gram-positive *L. monocytogenes* can survive longer than gram-negative *E. coli* O157:H7.

**METHODS**

The experimental procedure is summarized as in Figure 4.1.

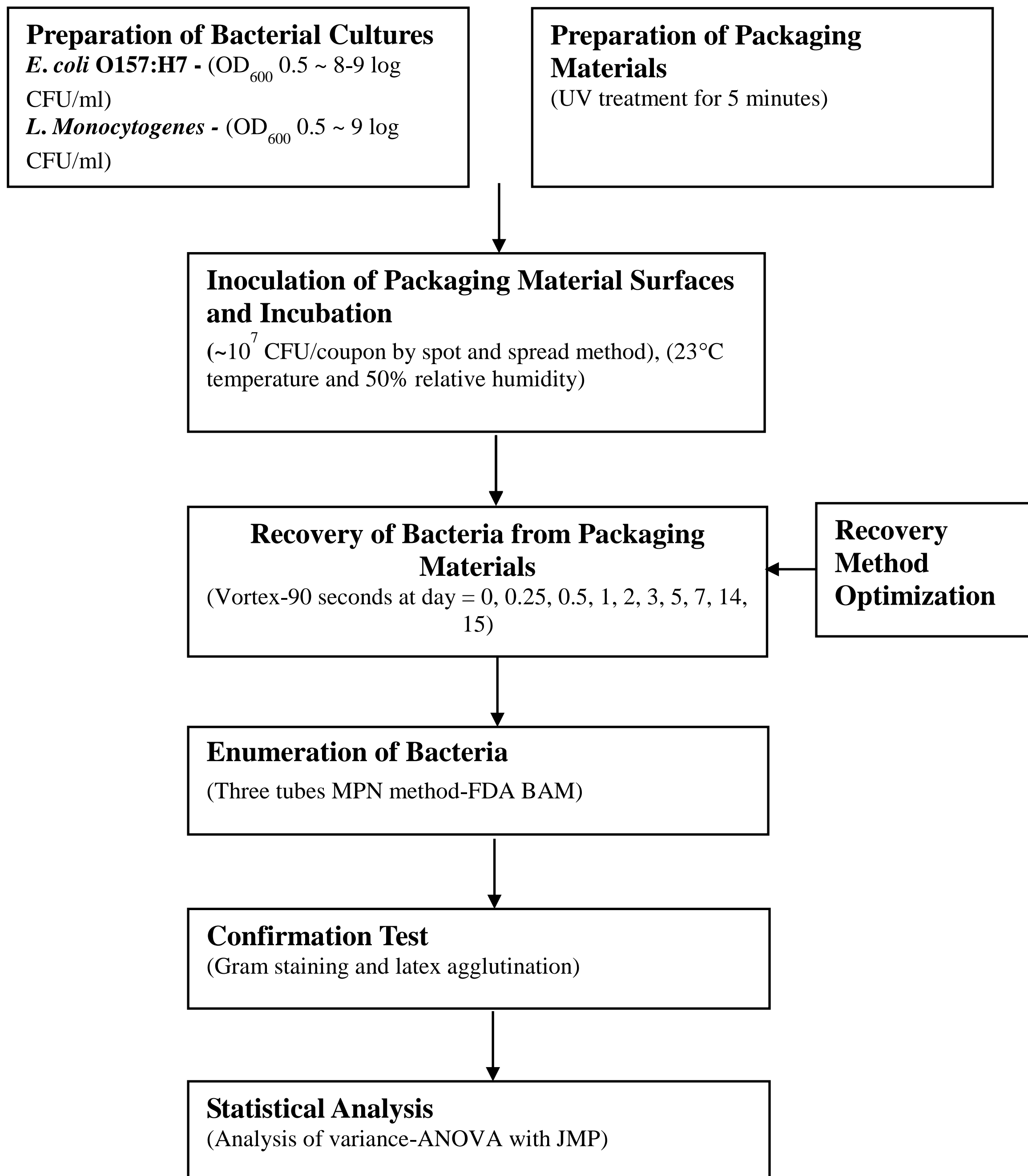



**Figure 4.1. Experimental Protocol**

**Preparation of Bacterial Cultures**

A three-strain mixture of green fluorescent protein (GFP)-labeled *E. coli* O157:H7 (strain #286, C7927, and EØ 654, kindly provided by Dr. Xiuping Jiang, Clemson University, Clemson, SC) and a three-strain mixture of rifampin-resistant (Rif) *L. monocytogenes* (strains 101M, 109, and 201, kindly provided by Dr. Michael P. Doyle, The University of Georgia, Center for Food Safety, Griffin, GA) were used for gram-negative and gram-positive bacteria. A stock culture (at -80ºC) of each (GFP)-labeled *E. coli* O157:H7 strain was streaked on tryptic soy agar (TSA) (Becton Dickinson, Sparks, MD) supplemented with 100 µg/ml ampicillin (Sigma-Aldrich Co., St. Louis, MO) (TSA-A). Each strain of (Rif) *L. monocytogenes* was then streaked on TSA supplemented with 100 µg/ml rifampin (Sigma Chemical Co., St. Louis, MO) (TSA-R). All plates were incubated lid down at 37ºC for 12-24 hours. Subsequently, two sub-culturings were done from the stock culture for each strain. (GFP)-labeled *E. coli* O157:H7 strains were grown separately overnight in 25 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) containing 100 µg/ml ampicillin (TSB-A). (Rif) *L. monocytogenes* strains were grown overnight in 25 ml of TSB containing 100 µg/ml rifampin (TSB-R) at 37ºC in a rotary incubator. Then, the bacterial cultures were sedimented by centrifugation at 5,000 rpm for 5 minutes and washed twice with 25 ml of sterile 0.85% saline solution. The bacterial pellets for each strain were dissolved separately in 25 ml of 0.85% saline to measure the optical density at 600 nm ($OD_{600}$) and adjusted to ca. 0.50 to ensure the bacterial culture was ca. $10^8$ to $10^9$ CFU/ml for (GFP)-labeled *E. coli* O157:H7 and ca. $10^9$ CFU/ml for (Rif) *L. monocytogenes*. *E. coli* O157:H7 and *L. monocytogenes* strains were diluted separately to make the concentration of ca. $10^7$ CFU/ml. Equal volumes from the three

strains of (GFP)-labeled *E. coli* O157:H7 culture were mixed to make a cocktail inoculum with the concentration of ca. $10^7$ CFU/ml. Equal volumes from the three strains of (Rif) *L. monocytogenes* culture were mixed to make a cocktail inoculum with the concentration ca. $10^7$ CFU/ml (Similarly, for the three strains of (Rif) *L. monocytogenes* culture).

A ten-fold dilution series ($10^{-1}$-$10^{-6}$) was prepared by using 0.85% saline for each initial bacterial mixture. One hundred microliters of each bacterial cell suspension from the $10^{-6}$ dilution were plated on duplicate TSA-A plates for (GFP)-labeled *E. coli* O157:H7 and TSA-R plates for (Rif) *L. monocytogenes*. The plates were spread evenly over the surface by a sterile hockey stick shaped spreader, and incubated at 37ºC for 24-48 hours. Fluorescent colonies of (GFP)-labeled *E. coli* O157:H7 were enumerated under UV light using Bio-Rad Universal Hood UV Transilluminator Light Table Molecular Imaging System to determine the initial inocula of *E. coli* O157:H7. Colony counts were reported as CFU/coupon. Initial inocula of *L. monocytogenes* colonies were enumerated using a colony counter and colony counts were reported as CFU/coupon.

**Preparation of Packaging Material Surfaces**

Packaging materials: oriented polyethylene terephthalate (OPET) (Hostaphan 2600, kindly provided by Mitsubishi Polyester Film, Greer, SC) and nylon-6 (Capran 1500RT, kindly provided by Honeywell Films, Pottsville, PA) as polar materials, and oriented polypropylene (OPP) (HSCT1, kindly provided by Transilwrap Company Inc, Franklin Park, IL) as a non-polar hydrophobic material were used for the study. Exterior surfaces of commonly used food packaging materials – OPET, OPP, and nylon-6 were cut into $5 \times 5$ $cm^2$ coupons. Exterior surfaces of each material were identified by non-

print side and labeled for the identification of the surface. Before each experiment, the coupon surfaces from each material were sealed in separate plastic bags and were sterilized under UV light (Zeta 7400, Loctite Corporation, Newington, CT) for 5 minutes.

**Inoculation of Packaging Material Surfaces**

Sterilized coupons of each material were aseptically transferred to labeled petri plates. Fifty microliters of a three-strain-mixture of (GFP)-labeled *E. coli* O157:H7 bacterial cell suspension (ca. $10^7$ CFU/ml of initial inoculum) was transferred by spot and spread inoculation method on the surfaces. Inoculum was evenly spread over the surfaces of 22 coupons from each three packaging material using a sterile loop (12000-810, VWR International, LLC, Radnor, USA) within 15-20 minutes to be used as treatment samples. Fifty microliters of 0.85% sterilized saline was transferred to each sterilized coupon (n=22 for each material) in labeled petri plates and spread evenly over the surfaces with a sterile loop to be used as control samples. All coupons were incubated in a humidity-controlled chamber at TAPPI conditions (23°C and 50 ± 0.2 % relative humidity). Relative humidity was maintained using a saturated solution of Ca $(NO_3)_2.4H_2O$ (Alfa Aesar, Ward Hill, MA) kept in a corner of the chamber. Temperature and relative humidity inside the chamber were monitored daily and at each sampling time. The same procedure was also performed for the inoculation and incubation of the (Rif) *L. monocytogenes* cocktail.

**Recovery Method Optimization**

Four recovery methods – vortex for 90 seconds as described previously (Bale et al., 1993), stomacher for 90 seconds, sonication for 5 minutes, and combination of 90 seconds of stomacher with 5 minutes of sonication, were chosen to identify the best

recovery method. Green fluorescent protein (GFP)-labeled *E. coli* O157:H7 (strain #286) was used in the recovery method optimization experiment.

Sterilized coupons were aseptically (under sterilization conditions) transferred to labeled petri plates under the biosafety cabinet. Fifty microliters *E. coli* O157:H7 bacterial cell suspension (ca. $10^7$ CFU/ml of initial inoculum) was transferred to surfaces by spot and spread inoculation and evenly spread over the surfaces of each material coupons (24 coupons for one material) using a sterile loop (12000-810, VWR International, LLC, Radnor, USA). For control samples, fifty microliters of 0.85% sterilized saline was transferred to sterilized coupons (n=12) in labeled petri plates and spread evenly over the surfaces with a sterile loop. All coupons were incubated in a humidity-controlled chamber at TAPPI conditions (23°C and 50 ± 0.2% relative humidity). Relative humidity was maintained using a saturated solution of $Ca(NO_3)_2.4H_2O$ (Alfa Aesar, Ward Hill, MA) inside the chamber. Temperature and relative humidity inside the chamber were monitored at each sampling time.

After the required incubation time, two test coupons for each recovery method were collected from the humidity chamber at 0 hour (T-0), 2 hours (T-2), and 4 hours (T-4). For the vortex method, duplicate treatment coupons were aseptically transferred into 10 ml of sterile 0.85% saline in a labeled centrifuge tube and vortexed for 90 seconds. For the stomacher and sonication methods, two sets of duplicate coupons for each method were transferred to four labeled stomacher bags containing 10 ml of sterile 0.85% saline. One set was stomached for 90 seconds and second set was sonicated for 5 minutes. The same procedure was repeated for control coupons for each recovery method. Dilution series ($10^{-1}$-$10^{-4}$) for each test sample were prepared for each recovery method by adding

1 ml of sample to 9 ml of 0.85% saline in test tubes. One hundred microliters from the last two dilutions at 0-hour and all four dilutions at 2-hours and 4-hours were plated on duplicate TSA plates. The colonies were counted and surviving *E. coli* O157:H7 cells were reported as CFU/coupon. The same procedure was applied to control samples. The experiment was performed in duplicate with two experimental trials conducted. All procedures were carried out under aseptic conditions in a biosafety cabinet to minimize cross-contamination.

**Recovery of Bacteria from Packaging Material Surfaces**

The surviving (GFP)-labeled *E. coli* O157:H7 cells were recovered by 90 seconds vortex with maceration, which means that the sample is soaked in the solution as, described previously (Bale et al., 1993). After the required incubation time, two test coupons and one control coupon for each material were collected from the humidity chamber at the appropriate sampling time (0, 0.25, 0.5, 1, 2, 3, 5, 7, 14, 15 days) and aseptically transferred to 10 ml of sterile 0.85% saline in labeled centrifuge tubes. All tubes were vortexed for 60 seconds then kept aside for 60 seconds to soak the coupon in the suspension. Then, vortexed another 30 seconds to release the attached cells from the surface of the coupon to the suspension. Dilution series for test samples and control samples were prepared for each sampling coupon by adding 1 ml of test or control sample to 9 ml of 0.85% saline in centrifuge tubes. Dilution series for each sample and control at each sampling time were prepared (e.g., for day 0 test sample $10^{-1}$-$10^{-8}$ and for control sample $10^{-1}$-$10^{-3}$). The surviving (GFP)-labeled *E. coli* O157:H7 bacterial cells were enumerated in TSB-A using the 3 tubes most probable number (MPN) method described in the Bacteriological Analytical Manual (Food and Drug Administration-

FDA). One milliliter of the last 3 dilutions was transferred to three tubes containing TSB-A for each dilution, which were labeled properly to identify the dilution and the material. The same procedure was applied to control samples. Survival was observed qualitatively on day 30 to verify the possibility of *E. coli* O157:H7 survival until day 30. The experiment was performed in triplicate and each trial was duplicated. All the microbiological methods were carried out in aseptic conditions in a biosafety cabinet to avoid any cross-contamination. This experimental procedure was followed for (Rif) *L. monocytogenes* using TSB-R for the 3 tubes most probable number (MPN) method and TSB-R enrichment tubes which means the action of enhancing the bacterial growth in the solution

**Confirmation Tests**

One sample of positive MPN tubes for each packaging material from sampling days 14 and 15 in all three trials were randomly selected and streaked onto TSA-A plates for (GFP)-labeled *E. coli* O157:H7, and TSA-R plates for (Rif) *L. monocytogenes*. The plates were incubated at 37ºC for 12-24 hours. A total of 18 *E. coli* O157:H7 isolates and 18 *L. monocytogenes* isolates were confirmed by gram staining (Becton Dickinson and Company, Sparks, MD) for the colony morphology of each bacteria. Latex agglutination tests for *E. coli* O157:H7 isolates (Remel Europe Ltd, Dartford, Kent, United Kingdom) and for *L. monocytogenes* isolates (Microgen Bioproducts Ltd, Camberley, Surrey, United Kingdom) were performed to confirm the bacteria by serological identification.

**Statistical Analysis**

The MPN count for the survival of (GFP)-labeled *E. coli* O157:H7 and *L. monocytogenes* were converted to log MPN per coupon for each sampling day. Log

values of bacterial counts were analyzed (full factorial analysis of variance) using an analysis of variance (ANOVA) with JMP (pro 12; SAS Institute, Inc., Cary, NC, USA). *P*-values below 0.05 were considered significant. Survival of each bacteria at each sampling time, on each material as well as the comparison of the survival between *E. coli* O157:H7 and *L. monocytogenes* on each material were determined.

## RESULTS

### Recovery Method Optimization

The initial inoculum of *E. coli* O157:H7 was 2 X $10^7$ CFU/coupon in trial 1 and 8 X $10^7$ CFU/coupon in trial 2. Surviving *E. coli* O157:H7 colonies at each sampling time were recovered using all recovery methods (Table 4.1). Recovery of *E. coli* O157:H7 by each method showed a similar recovery immediately after inoculation (T-0). Recovery of *E. coli* O157:H7 from OPET using the vortex, stomacher, sonication, and combination of stomacher and sonication were not significantly different ($p > 0.05$). The vortex method showed highest detachment at 2 and 4 hours of recovery (T-2 and T-4) without any significant difference ($p > 0.05$). Therefore, the vortex method was selected as the best recovery method.

**Table 4.1. Recovery of GFP-labeled *E. coli* O157:H7 (Log MPN/coupon) from the Exterior Surface of OPET[a] by each Recovery Method**

| Recovery Time (Hour) | Recovery – Mean Log CFU/coupon by Recovery Method | | | |
|---|---|---|---|---|
| | Vortex (1) | Stomacher (2) | Sonication (3) | Stomacher + Sonication (4) |
| T-0 | 7.04±0.08 | 7.08±0.03 | 7.04±0.08 | 7.00±0.13 |
| T-2 | 6.60±0.23 | 6.32±0.21 | 6.36±0.18 | 5.90±0.35 |
| T-4 | 4.15±0.51 | 3.68±0.17 | 3.88±0.44 | 4.08±0.46 |

[a] oriented polyethylene terephthalate.

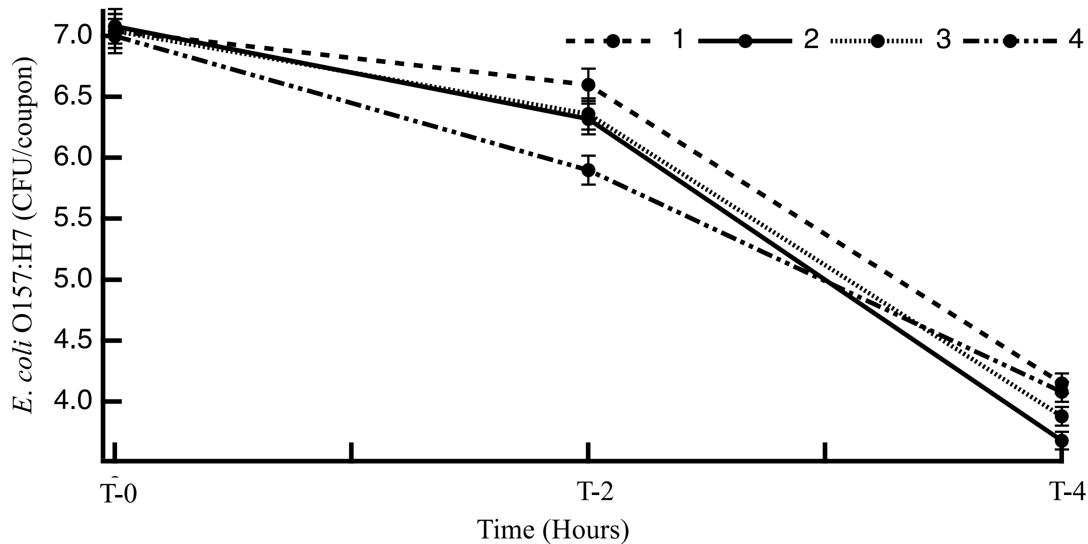



**Figure 4.2. Recovery of GFP-labeled *E. coli* O157:H7 (Mean Log CFU/coupon) from the Surface of Oriented Polyethylene Terephthalate (OPET) by Time (Hour) under each Recovery Method – 1. Vortex, 2. Stomacher, 3. Sonication, 4. Combination of Stomacher and Sonication.**

**Survival of (GFP)-labeled *E. coli* O157:H7**

The initial inoculum level of GFP-labeled *E. coli* O157:H7 was between 0.8 and 4.8 X $10^7$ CFU/coupon across the three trials. *E. coli* O157:H7 survived on all three materials (OPET, OPP and nylon-6) under TAPPI conditions until day 15 of the incubation period (Table 4.2). The *E. coli* O157:H7 population decreased on OPET, OPP, and nylon-6 by 5.74 log MPN, 5.25 log MPN, and 5.12 log MPN, respectively, from day 0 to day 15 (Table 4.2). A significant difference ($p > 0.05$) was not observed between the survival of *E. coli* O157:H7 on polar packaging materials (OPET and nylon 6) and on non-polar packaging material (OPP) (Table 4.2). The survival of *E. coli* O157:H7 was significantly different ($p < 0.05$) at each sampling time and on each of the three packaging materials. A significant difference ($p < 0.05$) was also observed for the survival of *E. coli* O157:H7 over time between individual packaging material (within a packaging material).

**Table 4.2. Recovery of GFP-labeled *E. coli* O157:H7 (Log MPN/coupon) on the Exterior Surface of OPET[a], OPP[b], and Nylon-6 under TAPPI Conditions**

| **Sampling Frequencies (Day)** | **Survival Count on Materials – Mean Log MPN/coupon** | | |
|---|---|---|---|
| | **OPET** | **OPP** | **Nylon-6** |
| 0 | 7.38± 0.33 | 7.50± 0.41 | 7.34± 0.52 |
| 0.25 | 7.17±0.25 | 7.03±0.46 | 6.94±0.15 |
| 0.5 | 6.36±0.71 | 6.55±0.53 | 6.60±0.46 |
| 1 | 5.06±0.76 | 5.34±0.64 | 5.42±0.53 |
| 2 | 4.40±0.31 | 3.83±0.64 | 4.12±0.37 |
| 3 | 3.60±0.34 | 3.73±0.08 | 3.85±0.13 |
| 5 | 3.28±0.53 | 3.05±0.47 | 3.30±0.14 |
| 7 | 2.42±0.57 | 2.99±1.89 | 2.93±0.44 |
| 14 | 1.93±1.37 | 2.18±0.42 | 2.34±0.31 |
| 15 | 1.64±1.11 | 2.25±0.37 | 2.22±1.45 |

[a] oriented polyethylene terephthalate.
[b] oriented polypropylene.

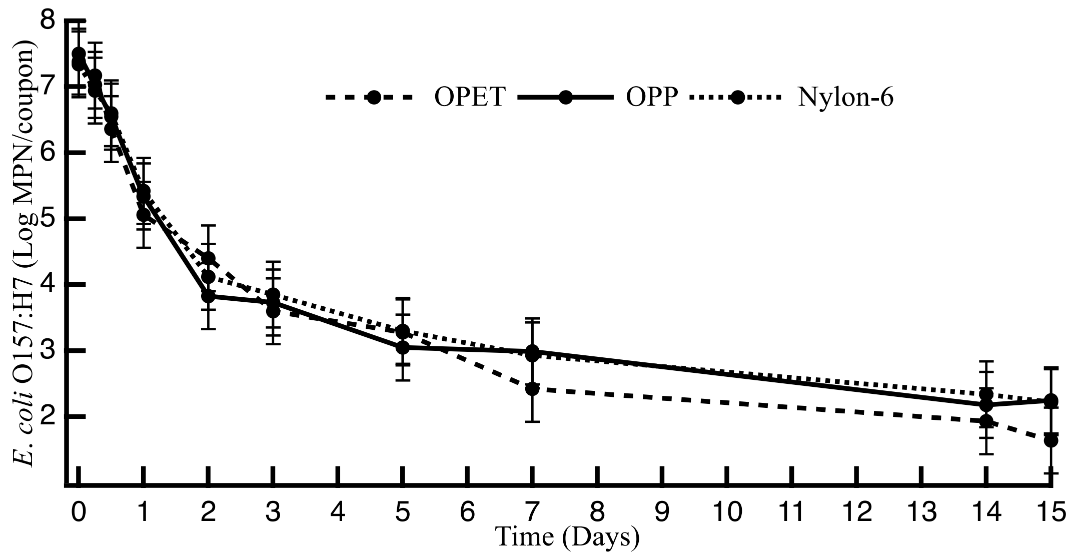



**Figure 4.3. Survival of *E. coli* O157:H7 (Mean Log MPN/coupon) on Oriented Polyethylene Terephthalate (OPET), Oriented Polypropylene (OPP), and Nylon-6 by Time (Day) under TAPPI Conditions.**

**Survival of (Rif)-*L. monocytogenes***

Initial inoculum levels of *L. monocytogenes* across the three trials were between 1.4 X $10^7$ CFU/coupon and 2 X $10^7$ CFU/coupon. *L. monocytogenes* survived on the surface of all three materials under TAPPI conditions for 15 days with survival decreasing over time (Table 4.3). The population reduction of *L. monocytogenes* on each material over 15 days was 6.54 log MPN on OPET, 6.28 log MPN on OPP, and 5.12 log MPN on nylon-6. *L. monocytogenes* survived on each of the three materials (both polar materials and non polar material), during 15 days of survival period was not significantly different ($p > 0.05$) (Table 4.3). The survival of *L. monocytogenes* was significantly different ($p < 0.05$) at each sampling time on individual packaging material (within a packaging material). A significant difference ($p < 0.05$) was observed for the survival of *L. monocytogenes* over time between each packaging material.

**Table 4.3. Recovery of (Rif)-*L. monocytogenes* (Log MPN/coupon) on the Exterior Surface of OPET[a], OPP[b], and Nylon-6 under TAPPI Conditions**

| Sampling Frequencies (Day) | Survival Count on Materials – Mean Log MPN/coupon | | |
|---|---|---|---|
| | OPET | OPP | Nylon-6 |
| 0 | 6.90±0.28 | 7.06±0.32 | 7.21±0.37 |
| 0.25 | 6.80±0.13 | 7.30±0.21 | 6.83±0.19 |
| 0.5 | 7.01±0.49 | 7.01±0.49 | 7.08±0.25 |
| 1 | 3.41±0.05 | 5.10±0.83 | 5.24±1.63 |
| 2 | 1.37±0.02 | 2.15±0.79 | 2.14±1.13 |
| 3 | 0.96±0.85 | 1.13±1.45 | 2.03±0.52 |
| 5 | 1.34±0.67 | 1.81±1.09 | 2.93±0.31 |
| 7 | 1.04±0.91 | 0.44±0.50 | 2.76±0.30 |
| 14 | 0.77±0.49 | 0.78±0.68 | 2.68±0.88 |
| 15 | 0.36±0.62 | 0.78±1.35 | 2.39±0.96 |

[a] oriented polyethylene terephthalate.
[b] oriented polypropylene.

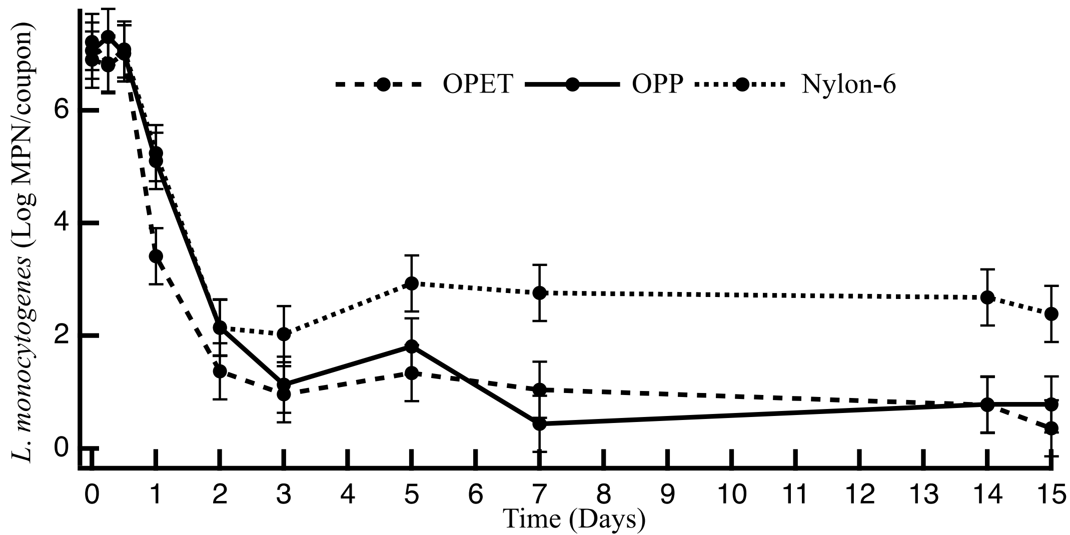



**Figure 4.4. Survival of *L. monocytogenes* (Mean Log MPN/coupon) on Oriented Polyethylene Terephthalate (OPET), Oriented Polypropylene (OPP), and Nylon-6 by Time (Day) under TAPPI Conditions.**

Our findings illustrate that the survival of *L. monocytogenes* was significantly higher ($p < 0.05$) than the survival of *E. coli* O157:H7 between days (0.5-1), (1-2), and (3-5). Qualitative analysis of enriched samples on day 30 presented a survival of *L. monocytogenes* on OPET, OPP, and nylon-6 in most of the samples 5/6, 4/6, and 6/6, respectively. *E. coli* O157:H7 survived on OPET, OPP, and nylon-6 in all the samples 6/6, 6/6, and 6/6, respectively.

**Table 4.4. Recovery of GFP-labeled *E. coli* O157:H7 (Log MPN/coupon) (Rif)-*L. monocytogenes* (Log MPN/coupon) on the Exterior Surface of OPET[a], OPP[b], and Nylon-6 under TAPPI Conditions**

| Sampling Frequencies (Day) | Survival Count on Materials – Mean Log MPN/coupon | | | | | |
|---|---|---|---|---|---|---|
| | OPET | | OPP | | Nylon-6 | |
| | E | L | E | L | E | L |
| 0 | 7.38 | 6.90 | 7.50 | 7.06 | 7.34 | 7.21 |
| 0.25 | 7.17 | 6.80 | 7.03 | 7.30 | 6.94 | 6.83 |
| 0.5 | 6.36 | 7.01 | 6.55 | 7.01 | 6.60 | 7.08 |
| 1 | 5.06 | 3.41 | 5.34 | 5.10 | 5.42 | 5.24 |
| 2 | 4.40 | 1.37 | 3.83 | 2.15 | 4.12 | 2.14 |
| 3 | 3.60 | 0.96 | 3.73 | 1.13 | 3.85 | 2.03 |
| 5 | 3.28 | 1.34 | 3.05 | 1.81 | 3.30 | 2.93 |
| 7 | 2.42 | 1.04 | 2.99 | 0.44 | 2.93 | 2.76 |
| 14 | 1.93 | 0.77 | 2.18 | 0.78 | 2.34 | 2.68 |
| 15 | 1.64 | 0.36 | 2.25 | 0.78 | 2.22 | 2.39 |

[a] oriented polyethylene terephthalate.
[b] oriented polypropylene.
E, *E. coli* O157:H7; L, *L. monocytogenes*

**DISCUSSION**

When studying the pattern of the two survival curves, the population of *E. coli* O157:H7 and *L. monocytogenes* exponentially decreased at the beginning of the survival curves (day 0 through day 2), slowly decreased in the middle part of the survival curves (day 2 through day 5), and stabilized from day 5 through day 15. This reduction of the bacterial population from day 0 through day 2 could be due to the initial exposure to the environment and a lower resistance of bacterial cells to survive on the material surfaces under TAPPI conditions. However, some resistance of bacterial cells could build up over time hence resisting the dry surface and TAPPI condition. This resistance could be due to

continuous exposure to stressful environmental conditions (Holmes et al., 2009). The lower survival counts of *E. coli* O157:H7 and *L. monocytogenes* between days 7 and 15 could be attributed to the unfavorable environment, presence of many dead bacterial cells and lack of nutrients necessary for survival, such as nutrition and water. The survival of *E. coli* O157:H7 was significantly different from the survival of *L. monocytogenes* between days (0.5-1), (1-2), and (3-5), which suggests the initial survival patterns between these two bacteria are not the same. This suggests different behaviors of survival of the two bacteria in the same environment.

We hypothesized a higher bacterial survival on polar packaging materials (OPET and nylon 6) than on non-polar packaging material (OPP). However, both *E. coli* O157:H7 and *L. monocytogenes* showed similar survival patterns on all three materials without any significant difference. Ak and colleagues (1994) reported no significant difference ($p > 0.05$) among plastic types (polyacrylic, polyethylene, polypropylene, and polystyrene), or among wood types, under cold temperature, room temperature or saturated humidity, which supports our findings but disproves our hypothesis. The survival of *E. coli* O157:H7 or *L. monocytogenes* on OPET, OPP and nylon-6 presumably is not a function of individual material characteristics, e.g. polarity of the material, chemical structure or hydrophilic/hydrophobic property.

We also hypothesized that the gram-positive bacteria, *L. monocytogenes,* would have a better survival rate compared to the gram-negative bacteria, *E. coli* O157:H7 based on the findings of three published studies (Ak et al., 1994; Bale et al., 1993; Hirai, 1991). However, our findings showed a higher survival of *L. monocytogenes* compared to the survival of *E. coli* O157:H7 only on nylon-6 after 14 days but this was not

significantly different. The survival of *L. monocytogenes* on surfaces can vary based on the serotypes, however, that would need to be confirmed by additional experiments. Other investigators result support our findings that gram-positive *L. monocytogenes* and gram-negative *E. coli* O157:H7 have similar survival patterns with no significant difference ($p > 0.05$) among plastic types (polar-polyacrylic, non-polar-polyethylene, polypropylene, and polystyrene) (Wilks et al., 2006; Wilks et al., 2005; Ak et al., 1994). The survival of these two bacteria may not have been affected by individual bacterial properties, such as the polarity of the cell wall, or the chemical structure of the cell wall. We believe our findings can be used to illustrate that the exterior of food packages could be a source of AGE-associated bacteria.

## LIMITATIONS

During inoculation and incubation, a dry surface throughout the incubation period was not maintained. An air-drying method was not used to dry the surfaces as it affects the required TAPPI conditions and takes time to initiate day 0 sampling. At the beginning of the incubation period (day 0), all coupon surfaces were wet until day 0.5-day 1, and thereafter, the surfaces became dry, exposing them to the chamber environmental conditions (23°C and 50% relative humidity). Another limiting factor was the humidity inside the incubation chamber, which varied due to opening the chamber at sampling times and exposure to the temperature and humidity in the laboratory. In this case, the humidity was re-equilibrated, allowing the atmosphere to saturate within a few minutes (~15-30 minutes) to eliminate the variations of humidity. Difficulty in equal spreading of the bacterial suspension over the coupon surface was challenging. The entire coupon

surface was covered with small droplets using a pipette and spreading with an inoculation needle. This approach allowed us to maintain the same inoculum (~$10^7$ CFU/coupon) on each coupon.

## CONCLUSIONS

Our results confirm that both E. *coli* O157:H7 and *L. monocytogenes* survived on OPET, OPP, and nylon-6 for 15 days under TAPPI conditions suggesting the exterior of food packages could be a source for AGE-associated bacteria. *E. coli* O157:H7 and *L. monocytogenes* had similar survival curves across all three materials. In addition, there was no significant difference between the complete survival of gram-positive and gram-negative bacteria for 15 days. These findings suggest that material properties or characteristics of the bacterial cell wall do not affect the whole survival pattern of each bacterium. While our results illustrate the survival of *E. coli* 0157:H7 and *L. monocytogenes* at high inoculum levels (~ $10^7$ CFU), this might not represent contamination under real-world conditions, where inoculum levels might be much lower. Additional research under real-world conditions is needed to explain the role of contaminated food packages and AGE.

## ACKNOWLEDGEMENTS

This research was funded by a grant from the U.S. Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative, “Building Capacity to Control Viral Foodborne Disease: A Translational, Multidisciplinary Approach,” Grant No. 2011-68003-30395.

## LITERATURE CITED

Ak, N. O., Cliver, D. O., and Kaspari, C. W. (1994). Decontamination of plastic and wooden cutting boards for kitchen use. *Journal of Food Protection*, *57*(1), 23-30.

Bale, M. J., Bennett, P. M., Beringer, J. E., and Hinton, M. (1993). The survival of bacteria exposed to desiccation on surfaces associated with farm buildings. *Journal of Applied Bacteriology*, 75(6), 519-528.

Fankem, S. L., Boone, S. A., Marlene Gaither, M. P. A., and ME, R. (2014). Outbreak of norovirus illness in a college summer camp: impact of cleaning on occurrence of norovirus on fomites. *Journal of Environmental Health*, *76*(8), 20.

Hall, A.J., Lopman, B.A., Payne, D.C., Patel, M.M., Gastanaduy, P.A., Vinje, J., and Parashar, U.D. (2013). Norovirus Disease in the United States. *Emerging Infectious Disease, 19*, 1198–1205.

Hirai, Y. (1991). Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. *Journal of Hospital Infection*, 19(3), 191-200.

Hoffmann, A. A., & Hercus, M. J. (2000). Environmental stress as an evolutionary force. *AIBS Bulletin*, *50*(3), 217-226.

Holmes, J. D., and Simmons, G. C. (2009). Gastrointestinal illness associated with a long-haul flight. *Epidemiology and Infection*, *137*(03), 441-447.

Menezes, F. G. D., Correa, V. M. D. S. D., Franco, F. G. D. M., Ribeiro, M. I., Cardoso, M. F. D. S., Morillo, S. G., ... and Pasternak, J. (2010). An outbreak of norovirus infection in a long-term care facility in Brazil. *Einstein (Sao Paulo)*, *8*(4), 410-413.

Repp, K. K., and Keene, W. E. (2012). A point-source norovirus outbreak caused by exposure to fomites. *Journal of Infectious Diseases*, *205*(11), 1639-1641.

Thornley, C. N., Emslie, N. A., Sprott, T. W., Greening, G. E., and Rapana, J. P. (2011). Recurring norovirus transmission on an airplane. *Clinical Infectious Diseases*, cir465.

U.S. Food and drug administration. (2014). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook *Listeria monocytogenes*. Retrieved May 30, 2017, from https://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm070064.htm.

Wikswo, M. E., Kambhampati, A., Shioda, K., Walsh, K. A., Bowen, A. B., and Hall, A. J. (2015). Outbreaks of acute gastroenteritis transmitted by person-to-person

contact, environmental contamination, and unknown modes of transmission – United States, 2009-2013. *Morbidity and Mortality Weekly Report*, *64(12)*, 1-20.

Wilks, S. A., Michels, H. T., and Keevil, C. W. (2006). Survival of *Listeria monocytogenes* Scott A on metal surfaces: implications for cross-contamination. *International journal of food microbiology*, *111*(2), 93-98.

Wilks, S. A., Michels, H., and Keevil, C. W. (2005). The survival of *Escherichia coli* O157:H7 on a range of metal surfaces. *International journal of food microbiology*, *105*(3), 445-454.

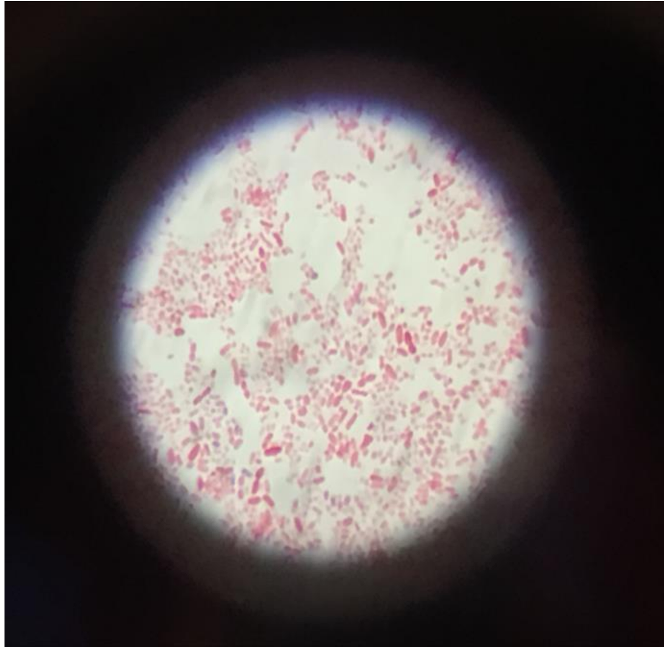
Williams, A. P., Avery, L. M., Killham, K., and Jones, D. L. (2005). Persistence of *Escherichia coli* O157 on farm surfaces under different environmental conditions. *Journal of Applied Microbiology*, *98*(5), 1075-1083.

Xue, C., Fu, Y., Zhu, W., Fei, Y., Zhu, L., Zhang, H., and Sun, Q. (2014). An outbreak of acute norovirus gastroenteritis in a boarding school in Shanghai: a retrospective cohort study. *BMC Public eHealth*, *14*(1), 1092.

# APPENDICES

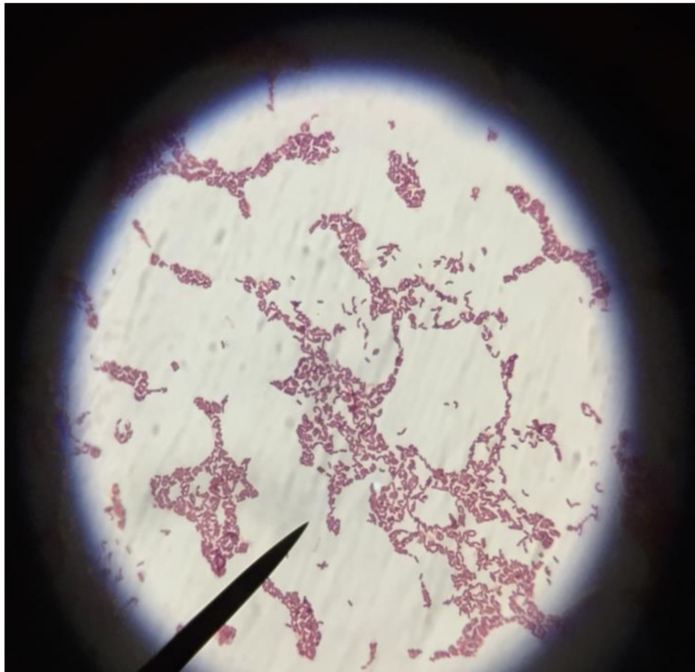
## Appendix A

### Gram Staining of *E. coli* O157:H7 (Gram Negative) under the Light Microscope



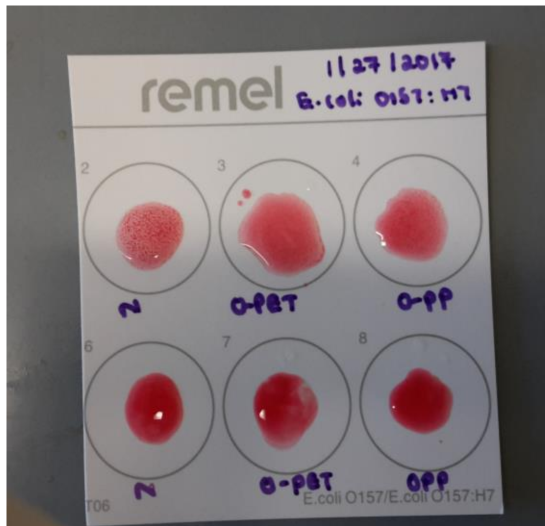
## Appendix B

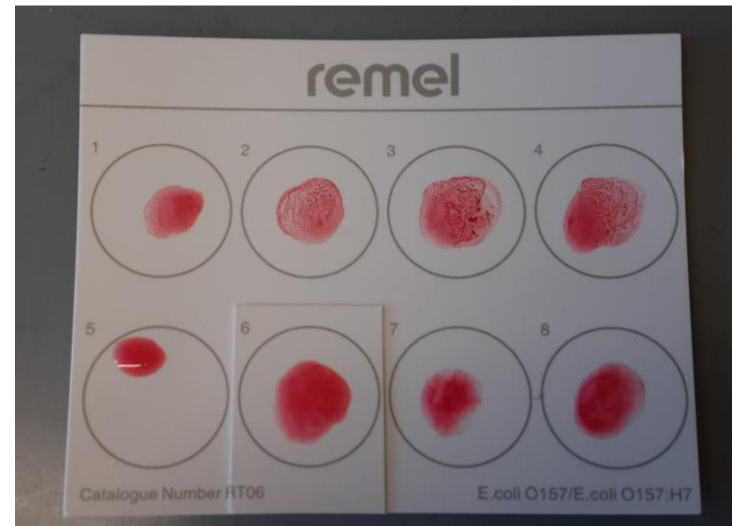
**Gram Staining of *L. monocytogens* (Gram Positive) under the Light Microscope**



## Appendix C

### *E. coli* O157:H7 Positive Clump Formation of Latex Agglutination

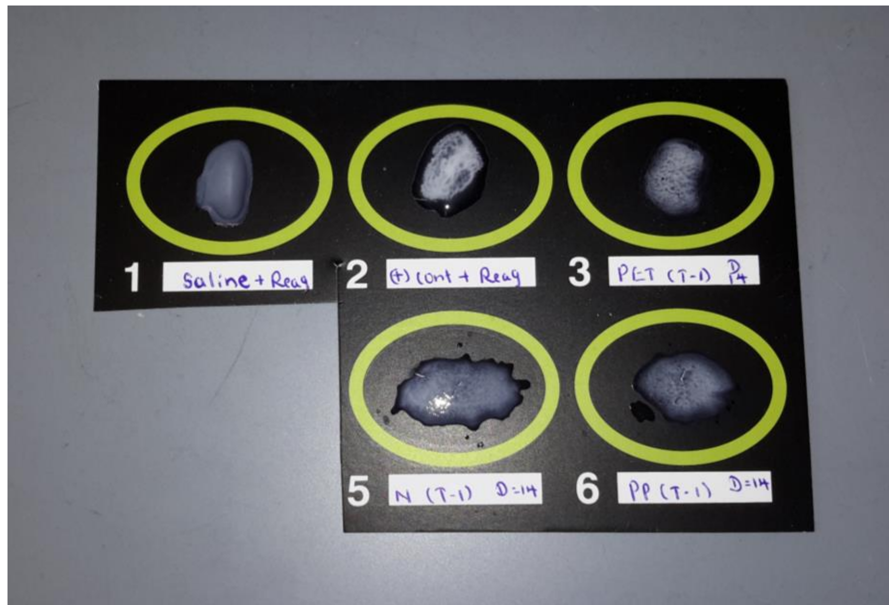



NOTE: 1, *E. coli* O157:H7 (positive control) with test latex; 2, positive sample of nylon-6 with test latex; 3, positive sample of OPET with test latex; 4, positive sample of OPP with test latex; 5, *E. coli* O106 (negative control) with test latex; 6, positive sample of nylon-6 with control latex; 7, positive sample of OPET with control latex; 8, positive sample of OPP with control latex.

# Appendix D

### *L. monocytogens* Positive Clump Formation of Latex Agglutination




NOTE: 1, (0.85%) isotonic saline with Listeria latex reagent; 2, *L. monocytogens* (positive control) with Listeria latex reagent; 3, positive sample of OPET with Listeria latex reagent; 5, positive sample of nylon-6 with Listeria latex reagent; 6, positive sample of OPP with Listeria latex reagent.

<u>**Appendix E**</u>

**Inoculated Coupons inside Petri Plates in the Humidity Control Chamber under TAPPI Conditions**