Attachment of *Salmonella* serovars and *Listeria monocytogenes* to stainless steel and plastic conveyor belts

G. A. Veluz,* S. Pitchiah,† and C. Z. Alvarado*[1]

*Department of Poultry Science, Texas A&M University, College Station, 77843;
†Department of Animal and Food Science, Texas Tech University, Lubbock 79409

ABSTRACT In poultry industry, cross-contamination due to processing equipment and contact surfaces is very common. This study examined the extent of bacterial attachment to 6 different types and design of conveyor belts: stainless steel-single loop, stainless steel-balance weave, polyurethane with mono-polyester fabric, acetal, polypropylene mesh top, and polypropylene. Clean conveyor belts were immersed separately in either a cocktail of *Salmonella* serovars (*Salmonella* Typhimurium and *Salmonella* Enteritidis) or *Listeria monocytogenes* strains (Scott A, Brie 1, ATCC 6744) for 1 h at room temperature. Soiled conveyor chips were dipped in poultry rinses contaminated with *Salmonella* or *Listeria* cocktail and incubated at 10°C for 48 h. The polyurethane with mono-polyester fabric conveyor belt and chip exhibited a higher ($P < 0.05$) mean number of attached *Salmonella* serovars (clean: 1.6 to 3.6 cfu/cm^2; soiled: 0.8 to 2.4 cfu/cm^2) and *L. monocytogenes* (clean: 4.0 to 4.3 cfu/cm^2; soiled: 0.3 to 2.1 cfu/cm^2) in both clean and soiled conditions. The stainless steel conveyor belt attached a lower ($P < 0.05$) number of *Salmonella* serovars (clean: 0 to 2.6 cfu/cm^2; soiled: 0.4 to 1.3 cfu/cm^2) and *L. monocytogenes* (clean: 0.4 to 2.9 cfu/cm^2; soiled: 0 to 0.7 cfu/cm^2) than the polymeric materials, indicating weaker adhesion properties. Plastic conveyor belts exhibited stronger bacterial adhesion compared with stainless steel. The result suggests the importance of selecting the design and finishes of conveyor belt materials that are most resistant to bacterial attachment.

Key words: bacterial attachment, conveyor belt, stainless steel, plastic

2012 Poultry Science 91:2004–2010
http://dx.doi.org/10.3382/ps.2011-01689

INTRODUCTION

In poultry industry, reduction of bacterial contamination during production is a major concern among processors (Arnold and Silvers, 2000). Process automation and mechanical equipment has created the presentation of new surface areas repeatedly in contact with carcasses and greater opportunities for bacterial attachment and cross-contamination (McEldowney and Fletcher, 1988). Modern food processing equipment manufactured from different materials, such as stainless steel, glass, plastics, rubber, and polytetrafluoroethylene, can attach bacteria (Brooks and Flint, 2008). These attached microorganisms may increase in number and detach on their own or by physical movement of product through the production line and cause cross-contamination.

Several studies have demonstrated bacterial attachment to poultry processing equipment (Lundén et al., 2003; Arnold, 2007; Rivas et al., 2007; Stocki et al., 2007; Chia et al., 2009) and food contact surfaces (Kusumaningrum et al., 2002; Trachoo, 2007; Nguyen et al., 2010). In particular, the adherence of *Listeria monocytogenes* in stainless steel, rubbers, and polymers has been demonstrated (Hood and Zottolla, 1997; Smoot and Pierson, 1998; Beresford et al., 2001; Midelet and Carpentier, 2002; Tolvanén et al., 2007, 2009). Attachment of *Salmonella* serovars to food contact surfaces were also reported (Hood and Zottolla, 1997; Oliveira et al., 2006; Chia et al., 2008; Chia et al., 2009; Rodrigues et al., 2009). Likewise, mixed microbial population from poultry rinse can adhere to poultry processing equipment surfaces (Arnold and Silvers, 2000; Arnold and Bailey, 2000). Bacterial attachment to material surfaces is a complicated process. Its mechanism is dictated by several factors, such as surface conditioning, surface charge, surface roughness, growth medium, and hydrophobicity of the contact surface and bacterial cells (Boulané-Petermann et al., 1997; Bayoudh et al., 2006; Palmer et al., 2007; Tresse et al., 2007; Goulter et al., 2009, Nguyen et al., 2011). In general, the physicochemical properties, surface charge and hydrophobicity, of both bacteria cells and substratum surface are the main influencing factors in bacterial adhesion (Bayoudh et al., 2006; Chia et al., 2008), but cellular structure like flagella (Tresse et al., 2006) and

Received June 21, 2011.
Accepted May 2, 2012.
[1]Corresponding author: calvarado@poultry.tamu.edu

curli (Patel et al., 2011) can also influence bacterial adhesion. Hydrophobic bacterial cells were reported to have a higher adhesion rate than hydrophilic cells and have better adhesion to hydrophobic surfaces than hydrophilic surfaces (Bayoudh et al., 2006). Chia et al. (2009) also concluded in their study that surface materials with more positive interfacial free energies have a higher number of adhering bacteria. However, some authors (Oliveira et al., 2006) found that *Salmonella* adhesion is strongly strain dependent.

In the processing environment, the complex structure of processing machines makes cleaning difficult. Routine sanitation procedures may be unsuccessful in eradicating *L. monocytogenes* (Lundén et al., 2003; Tolvanén et al., 2009), and pathogenic organisms can remain viable even on a dry surface environment (Kusumaningrum et al., 2002). Conveyor belts are difficult or impossible to dismantle for cleaning, thus, effective cleaning methods should be available for daily use in food processing plant, and otherwise, the use of materials resistant to bacterial attachment is very important. There is now a considerable scientific interest in the fabrication of food surfaces that can prevent bacteria from coming into contact and adhering to the surface (Crick et al., 2011). The use of antimicrobial belts was even recommended to reduce cross-contamination of final products by pathogenic bacteria (Gundelley et al., 2007).

Previous studies have provided valuable information about bacterial attachment. This current research examined the attachment of mixed strains of *Salmonella* or *Listeria* to commercial stainless steel and plastic conveyor belts, in clean and soiled conditions. Two types of stainless steel (single loop and balance weave) and 4 polymer materials (polyurethane, acetal, polypropylene, and polypropylene-meshtop) were tested in the experiment. Results of the study can be a source of vital information for equipment manufacturers and food processors in choosing materials and finishes that are most resistant to bacterial attachment and eventually biofilm formation.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Three streptomycin-resistant (1 ng/mL) strains of *L. monocytogenes* (Scott A, Brie 1, and ATCC 6744) and 2 streptomycin-resistant (1 ng/mL) *Salmonella* serovars (*Salmonella* Typhimurium and *Salmonella* Enteritidis) were used in the study. Individual strains of bacteria were maintained in tryptic soy broth (**TSB**; Hardy Diagnostics, Sta. Maria, CA) with 10% glycerol and stored at −80°C. Working cultures were kept on tryptic soy agar (**TSA**) slants and stored at 4°C. Cells from these slants were grown separately in TSB (containing 1 ng/mL of streptomycin) and incubated at 37°C. Three consecutive transferences were done to get overnight (12–24 h) grown cells. A cocktail of *Salmonella* serovars was made by mixing equal volumes (500 mL) of the 2 overnight-grown strains (1:1). Similarly, a cocktail of *L. monocytogenes* was prepared by mixing equal volumes of the 3 overnight-grown strains (1:1:1 ratio). The cell density of the cocktail culture was determined by serial dilution and plating on TSA plates. Cell density of the prepared cocktail was approximately 5 log cfu/mL, a concentration enough to monitor any significant log reduction in bacterial count.

Bacterial Attachment to Clean Conveyor Belts

Preparation of Conveyor Belt Surfaces. Different types of commercially available conveyor belts made of polyurethane with mono-polyester fabric (**PUMP**), acetal (**ACE**), polypropylene-meshtop (**PPM**), polypropylene (**PP**), stainless steel-single loop (**SSSL**), and stainless steel-balance weave (**SSBW**) were used in the study. Each conveyor belt was cut into sections (70 × 15 cm), cleaned with distilled water, and sprayed with 200 ppm of quaternary ammonium detergent solution and left overnight to air dry. Prior to use, sterile distilled water was sprayed for 1 min to remove residual quaternary ammonium detergent.

Inoculation and Enumeration of Bacteria. Inoculation of the conveyor belts prepared previously was done by individually immersing the test materials in a stainless steel tub containing the *Salmonella* or *Listeria* cocktail. Bacterial attachment was allowed for 1 h at room temperature. The conveyor belt materials were removed and placed on a clean table lined with sterilized aluminum foil. Enumeration of attached bacteria was determined at 0, 1, 2, 4, 6, 8, 12, 24, and 48 h using the swab method. A Whirlpak (Salida, CA) swab was moistened in 25 mL of buffered peptone water and used to sample the area. A 100-cm^2 stencil template was used to determine the swab area. At each time point, a different belt section was swabbed. The Whirlpak swabs were mixed in a stomacher 400 circulator (Seward, Inc.) at 230 rpm for 1 min. Enumeration of bacteria was done using an Autoplater (Spiral Biotech 4000, Advanced Instruments Inc., MA) machine and TSA plates with streptomycin (1 ng/mL) to determine *Listeria* and *Salmonella* counts. Plates were incubated at 37°C for up to 48 h. Colonies were counted using Q-count software and procedures (Spiral Biotech, Advanced Instruments Inc.), and data were gathered.

Bacterial Attachment to Soiled Conveyor Chips

Preparation of Conveyor Chips. Similar types and materials of conveyor belts from previous experiment were used. The test surfaces, referred to as chips, were prepared by cutting them into different dimensions to include the interlocking part of the conveyor belts (Table 1). The chips were washed briefly in 1% microclean-

Table 1. Materials, design, and specifications of conveyor belt chips used in the study

Materials/Design	% Open mesh	Dimension (cm)
Polyurethane with mono-polyester fabric	0	5.0 × 4.0
Acetal	3.2	5.0 × 5.5
Polypropylene-meshtop	24	4.5 × 5.0
Polypropylene	48	6.5 × 5.0
Stainless steel-single loop	80	8.7 × 2.7
Stainless steel-balance weave	70	6.0 × 6.0

ing solution, rinsed with distilled water, and sonicated for 30 min as described by Arnold and Silvers (2000).

Poultry Rinse and Bacterial Inoculation. Two kinds of poultry rinse from raw and cooked poultry products were used in the study. All poultry products were obtained from a retail store and refrigerated before preparation. The chicken breast fillets and chicken loaves were inoculated with *Listeria* and *Salmonella* cocktail, respectively, and allowed to stand for 1 h. Each poultry product was placed in a stomacher bag containing 300 mL of phosphate-buffered saline (**PBS**, pH 7.2) with Tween 80 (pH 7.4, 0.01 *M*, Sigma Aldrich, St. Louis, MO) and rinsed by shaking 10 times. The inoculated poultry rinse was transferred to sterile beakers and served as inoculum. The conveyor chips previously prepared were separately immersed in the poultry rinse and incubated at 10°C to mimic the industry setting. The bacterial enumeration was done at 1, 6, 12, 24, and 48 h.

Enumeration of Bacteria. At each sampling time, the soiled conveyor chips were removed and placed individually into Petri dishes containing 5.0 mL of PBS with Tween 80 (0.1%) and manually shaken in a clockwise and counterclockwise direction, 10 times each, to remove unattached bacteria. To get attached cells of *Listeria* and *Salmonella* off from the test surface, the test chips were placed in a sterile beaker containing 5.0 g of glass beads with 25 mL of PBS with Tween 80 (0.1%), covered with parafilm, and vortexed (Fisher Scientific, Touch mixer, model 231) for 30 s. Glass beads were used to facilitate dislodging of attached bacteria cells. Enumeration of bacteria was done using an Autoplater (Spiral Biotech 4000, Advanced Instruments Inc.) and TSA plates with streptomycin (1 ng/mL) to determine *Listeria* and *Salmonella* counts. Plates were incubated at 37°C for 48 h. Colonies were counted using Q-count (Spiral Biotech, Advanced Instruments Inc.) software and results were recorded.

Statistical Analysis

The experiments were performed in triplicates, and the data were analyzed by a one-way ANOVA for treatments that showed any interaction between conveyor belt and time. Means were separated using Duncan's multiple range test of the Statistical Analysis System software (SAS Institute Inc., Cary, NC). A significance level of $P = 0.05$ was employed.

RESULTS

Attachment of Salmonella and Listeria to Clean Conveyor Belts

Salmonella. Adhesion of *Salmonella* serovars (*Salmonella* Typhimurium and *Salmonella* Enteritidis) to the 6 different conveyor belts made of different plastic materials and stainless steel was examined. Initial (0 h) mean number of *Salmonella* attached was not different ($P > 0.05$) among the 6 conveyor belts (Table 2). After 1 h, a higher ($P < 0.05$) number of *Salmonella* cells attached to SSBW conveyor belt (3.5 cfu/cm^2) was noted in contrast to SSSL conveyor belt (2.3 cfu/cm^2), but the attached bacteria on SSSL material was not different ($P > 0.05$) from the other 4 polymeric materials (PUMP, ACE, PPM, and PP). Adhered *Salmonella* cells to the surface were generally found to be higher ($P < 0.05$) on the PUMP conveyor belt from 2 h (3.4 cfu/cm^2) to 48 h (1.6 cfu/cm^2), compared with other materials (Table 2).

Among the 4 plastic materials, bacterial adhesion was higher ($P < 0.05$) on PUMP conveyor belt (3.4 to 1.6 cfu/cm^2) from 2 h to 48 h in comparison to ACE (2.2 to 0 cfu/cm^2), PPM (1.8 to 0 cfu/cm^2), and PP (2.2 to 0 cfu/cm^2) materials. Between the 2 stainless steel materials, a higher ($P < 0.05$) number of *Salmonella* cells was enumerated on SSBW than SSSL conveyor belt at 1 h (3.5 vs. 2.3 cfu/cm^2), 2 h (3.6 vs. 1.7 cfu/cm^2), and 6 h (2.2 vs. 0.7 cfu/cm^2) but not in 4 h (2.6 vs. 1.4 cfu/cm^2), 8 h (1.6 vs. 1.0 cfu/cm^2), 12 h (1.2 vs. 0.5 cfu/cm^2), 24 h (1.9 vs. 0.3 cfu/cm^2), or 48 h (0.9 vs. 0 cfu/cm^2). Between the plastic and stainless steel materials, a difference ($P < 0.05$) in number of adhered bacterial cells was only observed between SSSL (2.3 to 0 cfu/cm^2) and PUMP conveyor belts (3.2 to 1.6 cfu/cm^2) from 1 h to 48 h. The mean number of cells attached to SSSL, SSBW, ACE, PPM, and PP were generally not different ($P > 0.05$) from 2 h to 48 h (Table 2).

A decreasing number of bacterial cells were also observed for all test materials from 0 to 48 h. At the onset of 24 h, higher ($P < 0.05$) numbers of *Salmonella* were still found attached to PUMP (2.9 cfu/cm^2) and SSBW (1.2 cfu/cm^2) conveyor belts. Similarly, the PUMP conveyor belt showed a higher ($P < 0.05$) mean number of *Salmonella* cells attached after 48 h (1.6 cfu/cm^2) whereas the rest of the test materials have very minimal or undetected microbial counts (Table 2).

Table 2. Mean log attachment of streptomycin-resistant *Salmonella* serovars to different types of conveyor belts over 48 h

	Mean (log cfu/cm^2) and SD[2]								
Belt type[1]	0 h	1 h	2 h	4 h	6 h	8 h	12 h	24 h	48 h
PUMP	$3.5^{a} \pm 0.8$	$3.2^{ab} \pm 0.6$	$3.4^{a} \pm 0.5$	$3.6^{a} \pm 0.5$	$3.5^{a} \pm 0.3$	$3.5^{a} \pm 0.5$	$3.3^{a} \pm 0.1$	$2.9^{a} \pm 0.9$	$1.6^{a} \pm 0.6$
ACE	$2.7^{a} \pm 0.2$	$2.4^{ab} \pm 0.34$	$2.2^{b} \pm 0.1$	$1.9^{b} \pm 0.0$	$1.3^{bc} \pm 0.1$	$1.1^{b} \pm 0.1$	$0.5^{b} \pm 0.3$	0^{b}	0^{b}
PPM	$2.5^{a} \pm 0.1$	$2.4^{ab} \pm 0.1$	$1.8^{b} \pm 0.6$	$1.5^{b} \pm 0.4$	$1.4^{bc} \pm 0.5$	$1.2^{b} \pm 0.1$	$0.4^{b} \pm 0.3$	$0.3^{b} \pm 0.1$	0^{b}
PP	$2.6^{a} \pm 0.2$	$2.7^{ab} \pm 0.2$	$2.2^{b} \pm 0.2$	$2.0^{b} \pm 0.1$	$1.4^{bc} \pm 0.3$	$1.2^{b} \pm 0.0$	$0.7^{b} \pm 0.3$	0^{b}	0^{b}
SSSL	$2.6^{a} \pm 0.1$	$2.3^{b} \pm 0.4$	$1.7^{b} \pm 0.2$	$1.4^{b} \pm 0.4$	$0.7^{c} \pm 0.2$	$1.0^{b} \pm 0.2$	$0.5^{b} \pm 0.3$	$0.3^{b} \pm 0.1$	0^{b}
SSBW	$3.7^{a} \pm 0.1$	$3.5^{a} \pm 0.1$	$3.6^{a} \pm 0.4$	$2.6^{b} \pm 0.3$	$2.2^{b} \pm 0.2$	$1.6^{b} \pm 0.6$	$1.2^{b} \pm 0.7$	$1.9^{ab} \pm 1.1$	$0.9^{b} \pm 0.9$

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]PUMP, polyurethane with mono-polyester fabric; ACE, acetal; PPM, polypropylene-meshtop; PP = polypropylene; SSSL, stainless steel-single loop; SSBW, stainless steel-balance weave.

[2]Results are presented as the means of triplicate measurements followed by standard deviation.

Listeria. Adhesion of *L. monocytogenes* cells (Scott A, Brie 1, and ATCC 6744) to the 6 different types of conveyor belts was monitored from 0 to 48 h (Table 3). Among the 6 materials tested, higher ($P < 0.05$) mean numbers of *Listeria* cells were found attached, ranging from 4.0 to 4.3 cfu/cm^2, to the PUMP conveyor belt compared with other polymer materials (ACE, PPM, and PP) and 2 stainless steel belts (SSSL and SSBW). No difference ($P > 0.05$) on the number of attached bacteria was, however, noted among the 3 polymers ACE, PPM, and PP. Between the 2 stainless steel conveyor belts, higher ($P < 0.05$) mean numbers of *Listeria* cells were found attached to SSBW at 8 h (2.6 cfu/cm^2), 12 h (2.5 cfu/cm^2), 24 h (2.2 cfu/cm^2), and 48 h (2.2 cfu/cm^2) in contrast to SSSL. No difference ($P > 0.05$) in the mean number of attached bacteria was noted between SSBW and the 3 polymeric materials ACE, PPM, and PP over the 48-h period (Table 3). Weaker bacterial adhesion was exhibited by SSSL conveyor belt as shown by the lower ($P < 0.05$) mean number (Table 3) of *Listeria* cells attached at 24 h (1.3 cfu/cm^2) and 48 h (0.4 cfu/cm^2).

Attachment of Salmonella and Listeria to Soiled Conveyor Chips

Salmonella. Bacterial adhesion to 6 conveyor chips soiled with *Salmonella*-inoculated poultry rinse was monitored to determine the effects of food matrix on bacterial attachment. Over the 48-h period there was an increasing number of *Salmonella* cells found attached to all types of soiled conveyor chips (Table 4). Between the soiled plastic and stainless steel conveyor chips, initial (1 h) mean number of attached *Salmonella* cells was lower ($P < 0.05$) on SSSL (0.4 cfu/cm^2) and SSBW (0.4 cfu/cm^2) than the plastic materials PUMP (0.8 cfu/cm^2), ACE (1.0 cfu/cm^2), PPM (1.0 cfu/cm^2), and PP (0.7 cfu/cm^2). A difference ($P < 0.05$) in the mean number of attached *Salmonella* was also observed between the 2 polymers (PUMP and PPM) and the 2 stainless steel (SSSL and SSBW) conveyor chips at 6 h (1.3 and 1.1 vs. 0.5 and 0.5 cfu/cm^2) and 12 h (1.1 and 1.2 vs. 0.5 and 0.5 cfu/cm^2). Likewise, differences ($P < 0.05$) in the number of *Salmonella* cells attached were noted between the PPM and the 2 stainless chips at 12 h (1.2 vs. 0.5 and 0.5 cfu/cm^2), ACE and the 2 stainless chips at 24 h (1.9 vs. 1.2 and 1.2 cfu/cm^2), and between the 2 polymeric materials (ACE and PPM) and SSSL chips at 48 h (2.6 and 2.4 vs. 1.3 cfu/cm^2). No difference ($P > 0.05$), however, on the mean number of *Salmonella* cells was seen attached among the 4 soiled polymer chips (PUMP, ACE, PPM, and PP) over the 48-h period. Between the 2 soiled stainless steel conveyor chips (SSSL and SSBW), no difference ($P > 0.05$) was noted at all sampling times (Table 4).

Listeria. In general, a higher ($P < 0.05$) mean number of *Listeria* cells was found attached to soiled plastic materials (PUMP, ACE, PPM, and PP) than stainless steel materials (SSSL and SSBW) over the 48-h period. Bacterial counts were minimal during the first 12 h

Table 3. Mean log attachment of streptomycin-resistant *Listeria monocytogenes* to different types of conveyor belts over 48 h

	Mean (log cfu/cm^2) and SD[2]								
Belt type[1]	0 h	1 h	2 h	4 h	6 h	8 h	12 h	24 h	48 h
PUMP	$4.2^{a} \pm 0.1$	$4.3^{a} \pm 0.0$	$4.3^{a} \pm 0.0$	$4.3^{a} \pm 0.0$	$4.0^{a} \pm 0.0$	$4.1^{a} \pm 0.0$	$4.0^{a} \pm 0.1$	$4.1^{a} \pm 0.1$	$4.2^{a} \pm 0.1$
ACE	$3.0^{b} \pm 0.1$	$3.0^{b} \pm 0.0$	$2.8^{b} \pm 0.1$	$2.5^{b} \pm 0.1$	$2.4^{b} \pm 0.0$	$2.4^{b} \pm 0.0$	$2.3^{bc} \pm 0.0$	$2.1^{b} \pm 0.4$	$1.8^{b} \pm 0.2$
PPM	$2.9^{b} \pm 0.1$	$2.7^{bc} \pm 0.1$	$2.4^{b} \pm 0.1$	$2.4^{b} \pm 0.2$	$2.4^{b} \pm 0.2$	$2.2^{bc} \pm 0.3$	$2.4^{bc} \pm 0.0$	$2.2^{b} \pm 0.1$	$1.8^{b} \pm 0.2$
PP	$2.7^{b} \pm 0.2$	$2.5^{bc} \pm 0.2$	$2.0^{b} \pm 0.6$	$2.3^{b} \pm 0.3$	$2.2^{b} \pm 0.4$	$2.6^{b} \pm 0.1$	$2.4^{b} \pm 0.3$	$2.7^{b} \pm 0.1$	$2.6^{b} \pm 0.2$
SSSL	$2.9^{b} \pm 0.3$	$2.3^{c} \pm 0.3$	$2.2^{b} \pm 0.6$	$2.2^{b} \pm 0.3$	$2.1^{b} \pm 0.4$	$1.1^{c} \pm 0.4$	$1.4^{c} \pm 0.6$	$1.3^{c} \pm 0.2$	$0.4^{c} \pm 0.4$
SSBW	$3.0^{b} \pm 0.0$	$2.8^{b} \pm 0.0$	$2.5^{b} \pm 0.3$	$2.8^{b} \pm 0.0$	$2.6^{b} \pm 0.0$	$2.6^{b} \pm 0.1$	$2.5^{b} \pm 0.1$	$2.2^{b} \pm 0.3$	$2.2^{b} \pm 0.3$

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]PUMP, polyurethane with mono-polyester fabric; ACE, acetal; PPM, polypropylene-meshtop; PP = polypropylene; SSSL, stainless steel-single loop; SSBW, stainless steel-balance weave.

[2]Results are presented as the means of triplicate measurements followed by standard deviation.

Table 4. Mean log attachment of streptomycin-resistant *Salmonella* serovars to different types of conveyor chips soiled with poultry rinse over 48 h

	Mean (log cfu/cm^2) and SD[2]				
Belt type[1]	1 h	6 h	12 h	24 h	48 h
PUMP	$0.8^{a} \pm 0.1$	$1.3^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$1.5^{ab} \pm 0.1$	$2.4^{ab} \pm 0.3$
ACE	$1.0^{a} \pm 0.0$	$0.9^{ab} \pm 0.4$	$0.9^{ab} \pm 0.2$	$1.9^{a} \pm 0.3$	$2.6^{a} \pm 0.3$
PPM	$1.0^{a} \pm 0.1$	$1.1^{a} \pm 0.1$	$1.2^{a} \pm 0.1$	$1.8^{ab} \pm 0.2$	$2.4^{a} \pm 0.5$
PP	$0.7^{a} \pm 0.1$	$0.8^{ab} \pm 0.2$	$1.1^{a} \pm 0.1$	$1.4^{ab} \pm 0.0$	$2.2^{ab} \pm 0.2$
SSSL	$0.4^{b} \pm 0.1$	$0.5^{b} \pm 0.1$	$0.5^{b} \pm 0.2$	$1.2^{b} \pm 0.3$	$1.3^{b} \pm 0.2$
SSBW	$0.4^{b} \pm 0.1$	$0.5^{b} \pm 0.0$	$0.5^{b} \pm 0.1$	$1.2^{b} \pm 0.0$	$1.6^{ab} \pm 0.1$

[a,b]Means within a column with different superscripts differ ($P < 0.05$).

[1]PUMP, polyurethane with mono-polyester fabric; ACE, acetal; PPM, polypropylene-meshtop; PP = polypropylene; SSSL, stainless steel-single loop; SSBW, stainless steel-balance weave.

[2]Results are presented as the means of triplicate measurements followed by standard deviation.

(Table 5). No differences ($P > 0.05$) were observed at 1, 6, and 12 h sampling points for all the soiled chips. Variation in *Listeria* counts was, however, seen at the onset of 24 h. The PUMP conveyor chip exhibited a higher ($P < 0.05$) number of adhered cells (1.9 cfu/cm^2) compared with other test materials, especially after 24 h of incubation time. Similarly, a difference ($P < 0.05$) was observed between the 2 polymers (ACE and PPM) and the 2 stainless steel soiled chips. After 48 h, no difference ($P > 0.05$) was observed on the mean number of *Listeria* cells attached to PUMP and the other 3 polymer chips (ACE, PPM, and PP), but the number (2.1 cfu/cm^2) was higher ($P < 0.05$) in contrast to SSSL and SSBW soiled chips (0.4 and 0.7 cfu/cm^2). Between the 2 stainless steel materials, no difference ($P > 0.05$) was seen in all sampling points (Table 5).

DISCUSSION

This study demonstrated the extent of pathogen attachment to clean and soiled conveyor belts made from different materials. Bacterial attachment is a complicated 2-step process that involves electrostatic forces, van der Waals forces, and hydrophobic interactions during a nonspecific initial step followed by the irreversible and specific attachment of cells to the surface with accompanying production of exopolysaccharides (such as cellulose for *Salmonella*) and or ligand that complex with the surface (Ledeboer and Jones, 2005; Bayoudh et al., 2006; Palmer et al., 2007). In general, polymeric materials used in our study have a stronger affinity for *Listeria* and *Salmonella* cells. Conveyor belts manufactured from polyurethane with monopolyester fabric (PUMP) attached a higher number of *L. monocytogenes* and *Salmonella* serovars than stainless steel conveyor belts (SSSL and SSBW) in both clean and soiled conditions. The PUMP conveyor belt exhibited a higher number of attached *Salmonella* and *Listeria* than the other 3 polymers (ACE, PPM, and PP) in clean surface conditions (Tables 2 and 3), but in soiled chips, *Salmonella* attachment did not differ among the 4 polymeric materials (Table 4). Attachment of *Listeria* was, however, variable among the 4 soiled plastic conveyor belts (Table 5).

The differences in bacterial adhesion among the 6 test materials most likely involved a multitude of factors, as reported by many authors, including the hydrophobicity and surface charge of the bacteria (Hyde et al., 1997; Sinde and Carballo, 2000; Bayoudh et al., 2006; Oliveira et al., 2006; Chia et al., 2008), the surface conditioning, mass transport, surface roughness, and surface microtopography (Palmer et al., 2007) and cellular structure such as flagella (Tresse et al., 2006). Hydrophobicity of the test organisms and the conveyor belts were not examined in our study, however, glass and stainless steel are generally considered hydrophilic

Table 5. Mean log attachment of streptomycin-resistant *Listeria monocytogenes* to different types of conveyor chips soiled with poultry rinse over 48 h

	Mean (log cfu/cm^2) and SD[2]				
Belt type[1]	1 h	6 h	12 h	24 h	48 h
PUMP	$0.3^{a} \pm 0.1$	$0.5^{a} \pm 0.0$	$0.8^{a} \pm 0.3$	$1.9^{a} \pm 0.1$	$2.1^{a} \pm 0.5$
ACE	0^{a}	$0.6^{a} \pm 0.3$	$0.8^{a} \pm 0.1$	$1.2^{b} \pm 0.2$	$1.8^{a} \pm 0.6$
PPM	0^{a}	$0.4^{a} \pm 0.1$	$0.1^{b} \pm 0.2$	$1.2^{b} \pm 0.4$	$2.0^{a} \pm 0.5$
PP	0^{a}	$0.1^{a} \pm 0.2$	$0.1^{b} \pm 0.2$	$0.6^{bc} \pm 0.1$	$1.3^{ab} \pm 0.2$
SSSL	0^{a}	0^{a}	0^{b}	$0.2^{c} \pm 0.2$	$0.7^{b} \pm 0.4$
SSBW	0^{a}	$0.3^{a} \pm 0.3$	0^{b}	$0.2^{c} \pm 0.1$	$0.4^{b} \pm 0.1$

[a–c]Means within a column with different superscripts differ ($P < 0.05$).

[1]PUMP, polyurethane with mono-polyester fabric; ACE, acetal; PPM, polypropylene-meshtop; PP = polypropylene; SSSL, stainless steel-single loop; SSBW, stainless steel-balance weave.

[2]Results are presented as the means of triplicate measurements followed by standard deviation.

materials, whereas rubber and plastic are hydrophobic materials. *Salmonella* and *L. monocytogenes* were reported to attach in higher numbers to more hydrophobic materials such as rubber and plastic (Sinde and Carballo, 2000). As confirmed in our findings, a higher mean number of *Salmonella* and *L. monocytogenes* were found attached to polymeric materials than stainless steel materials. Midelet and Carpentier (2002) also reported similar results for *L. monocytogenes* having weaker attachment strength to stainless steel compared with plastic materials.

Cellular surface hydrophobicity is another factor to consider. Chia et al. (2009) strongly suggested that adhesion of *Salmonella* is strain-dependent, whereas Tresse et al. (2007) found that intrinsic ability of *L. monocytogenes* to adhere to inert surface such as polystyrene and stainless steel surface is stronger than the physicochemical properties of the surface materials. Most *Salmonella* cell surfaces are hydrophobic (Ukuku and Fett, 2006). Cell surface hydrophobicity was highly correlated with the attachment strength of *Salmonella*, *E. coli*, and *L. monocytogenes* to the cantaloupe rind (Ukuku and Fett, 2002), but this may not be the same for abiotic surfaces like the various conveyor belt materials used in our study. Because mixed strains of either *Salmonella* or *L. monocytogenes* were used, the interaction between the strains and how it affects their hydrophobicity before attachment is hard to conclude. In commercial poultry processing line, a complex array of different bacteria, including pathogenic strains, is likely to be present and bacterial attachment becomes more complicated. Our findings only confirmed that *Salmonella* serovars and *L. monocytogenes* have variable ability to adhere to conveyor belts made from plastic and stainless steel materials. In a separate study (data not shown) a significant *Listeria* biofilm formation was observed on the 4 polymeric materials compared with the 2 stainless steel, confirming the previous findings that *Listeria* had stronger affinity to plastic conveyor belts. Similar to many studies (Hood and Zottolla, 1997; Smoot and Pierson, 1998; Beresford et al., 2001; Midelet and Carpentier, 2002; Oliveira et al., 2006; Tolvanén et al., 2007; Chia et al., 2009; Rodrigues et al., 2009), our findings showed the adhesion ability of *Salmonella* and *Listeria* to different surface materials at different degrees depending on the surface materials, its hydrophobicity, and surface roughness or finishes.

Surface conditioning and the availability of the nutrients were also suspected to play important roles in the initial attachment process, as evident in the increasing number of bacterial count over the 48-h period (Table 5). A different trend on bacterial attachment was seen between the clean and soiled conditions. Higher bacterial counts were reported for *Salmonella* and *Listeria* in clean conveyor belts (Tables 3 and 4) than the soiled conveyor chips (Tables 4 and 5). Presence of organic molecules may alter the physicochemical properties of the surface with accompanying changes in surface free energy, hydrophobicity, and electrostatic charges, resulting in reduced bacterial attachment due to competition for binding sites on the surface (Palmer et al., 2007). In a polymer-based surface, bacterial adherence is a function of both surface finish and surface chemistry (Hyde et al., 1997) and those bacterial surface properties also affect cell attachment (Adetunji and Isola, 2011). When a surface becomes more hydrophobic due to surface conditioning, there is a decrease in microbial attachment (Zeraik and Nitschke, 2010). The presence of food matrix affects bacterial attachment. From our results, the population of *Salmonella* serovars decreased gradually over a period of 48 h (Table 2) due to unavailability of nutrients and onset of death phase, but the opposite trend was observed for soiled conveyor chips (Table 4) wherein bacterial population was increasing. A similar trend in the number of attached *L. monocytogenes* was observed in both clean and soiled conveyor chips. However, high log numbers of *Listeria* cells were still detected even after 48 h in most conveyor belts (Table 3) possibly because they are the persistent *Listeria* strains that can survive for a longer period of time and is subject for further study. The low and almost undetectable initial bacterial counts (Table 5) can be attributed to the reduced attachment due to competition for binding sites but the increasing trend is most likely the effect of available nutrients necessary for bacterial growth.

In our study, the antimicrobial properties of the polymers were not exactly known, but some polymers may have inherent antimicrobial properties based on their original structure (Munoz-Bonilla and Fernadez-Garcia, 2011). The difference in bacterial attachment can be partly explained by the hardness of the material. The surface hardness of stainless steel is greater than the surface hardness of polymer materials, and similar to previous reports (Midelet and Carpentier, 2002; Tolvanén et al., 2007), our finding revealed that the attachment of *L. monocytogenes* to stainless steel is weaker than it is to plastic materials. Conveyor belts consist of multiple parts and joints, and these factors were also suspected to affect the washing step and the bacterial enumeration. The stainless conveyor belts have minimal surface area for bacteria to attach whereas the plastic conveyor belts have more interlocking sections and more surface areas for bacteria to attach.

Several studies have been reported to explain bacterial attachment to abiotic surfaces and the different factors affecting the adhesion process. *Salmonella* and *Listeria* can attached to a wide range of contact surfaces and the consequential contamination and cross-contamination of products still remains. Stainless steel showed weaker adhesion for bacteria and can be considered as the better type of conveyor belt to minimize cross-contamination. Similarly, cleaning is more efficient on hard surfaces such as stainless steel than soft materials such as plastic (Tolvanén et al., 2007). For equipment manufacturers, the design stage and selection of materials should be considered, and for food processors, selection of appropriate materials to prevent unwanted

incidence of contamination and cross-contamination in the production is always a better option.

REFERENCES

Adetunji, V. O., and T. O. Isola. 2011. Adhesion of *E. coli* and *E. coli* O157:H7 isolates from a typical tropical abattoir on wood, steel and glass surfaces. Res J Microbiol 6:669–677.

Arnold, J. W. 2007. Bacterial contamination on rubber picker fingers before, during, and after processing. Poult. Sci. 86:2671–2675. http://dx.doi.org/10.3382/ps.2007-00187.

Arnold, J. W., and G. Bailey. 2000. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. Poult. Sci. 79:1839–1845.

Arnold, J. W., and S. Silvers. 2000. Comparison of poultry processing equipment surfaces for susceptibility to bacterial attachment and biofilm formation. Poult. Sci. 79:1215–1221.

Bayoudh, S., A. Othmane, F. Bettaieb, A. Bakhrouf, H. Ben Ouada, and L. Ponsonnet. 2006. Quantification of the adhesion free energy between bacteria and hydrophobic and hydrophilic substrata. Mater. Sci. Eng. C 26:300–305.

Beresford, M. R., P. W. Andrew, and G. Shama. 2001. *Listeria monocytogenes* adheres to many materials found in food-processing environments. J. Appl. Microbiol. 90:1000–1005.

Boulané-Petermann, L. 1996. Processes of bioadhesion on stainless steel surfaces and cleanability: A review with special reference to the food industry. Biofouling 10:275–300.

Brooks, J. D., and S. H. Flint. 2008. Biofilms in the food industry: Problems and potential solutions. Int. J. Food Sci. Technol. 43:2163–2176.

Chia, T. W., N. Fegan, T. A. McMeekin, and G. A. Dykes. 2008. *Salmonella* Sofia differs from other poultry-associated *Salmonella* serovars with respect to cell surface hydrophobicity. J. Food Prot. 71:2421–2428.

Chia, T. W. R., R. M. Goulter, T. McMeekin, G. A. Dykes, and N. Fegan. 2009. Attachment of different *Salmonella* serovars to materials commonly used in a poultry processing plant. Food Microbiol. 26:853–859.

Crick, C. R., S. Ismail, J. Pratten, and I. P. Parkin. 2011. An investigation into bacterial attachment to an elastomeric superhydrophobic surface prepared via aerosol deposition. Thin Solid Films 519:3722–3727.

Goulter, R. M., I. R. Gentle, and G. A. Dykes. 2009. Issues in determining factors in influencing bacterial attachment: a review using the attachment of *Escherchia coli* to abiotic surfaces as an example. Lett. Appl. Microbiol. 49:1–7.

Gundelley, R., G. W. Youm, and Y. M. Kwon. 2007. Survival of bacterial pathogens on antimicrobial conveyor belts. J Rapid Methods Autom Microbiol 15:259–266.

Hood, S. K., and E. A. Zottolla. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int. J. Food Microbiol. 37:145–153.

Hyde, F. W., M. Alberg, and K. Smith. 1997. Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal. J. Ind. Microbiol. Biotechnol. 19:142–149.

Kusumaningrum, H. D., G. Riboldi, W. C. Hazeleger, and R. R. Beumer. 2002. Survival of foodborne pathogen on stainless steel surfaces and cross-contamination to foods. Int. J. Food Microbiol. 85:227–236.

Ledeboer, N. A., and B. D. Jones. 2005. Exopolysacharide sugars contribute to biofilm formation by *Salmonella enterica* serovar Typhimurium on HEp-2 cells and chicken epithelium. J. Bacteriol. 187:3214–3226.

Lundén, J. M., T. J. Autio, A.-M. Sjoberg, and H. J. Korkeala. 2003. Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. J. Food Prot. 66:2062–2069.

McEldowney, S., and M. Fletcher. 1988. Bacterial desorption from food container and food processing surfaces. Microb. Ecol. 15:229–237.

Midelet, G., and B. Carpentier. 2002. Transfer of microorganisms, including *Listeria monocytogenes*, from various materials to beef. Appl. Environ. Microbiol. 68:4015–4024.

Munoz-Bonilla, A., and M. Fernadez-Garcia. 2011. Polymeric materials with antimicrobial activity. Prog. Polym. Sci. 37:281–339.

Nguyen, V. T., M. S. Turner, and G. A. Dykes. 2010. Effect of temperature and contact time on *Campylobacter jejuni* attachment to, and probability of detachment from, stainless steel. J. Food Prot. 73:832–838.

Nguyen, V. T., M. S. Turner, and G. A. Dykes. 2011. Influence of cell surface hydrophobicity on attachment of *Campylobacter* to abiotic surfaces. Food Microbiol. 28:942–950.

Oliveira, K., T. Oliveira, P. Teixeira, J. Azeredo, M. Henriques, and R. Oliveira. 2006. Comparison of the adhesion ability of different *Salmonella* Enteritidis serotypes to materials used in kitchens. J. Food Prot. 69:2352–2356.

Palmer, J., S. Flint, and J. Brooks. 2007. Bacterial cell attachment, the beginning of a biofilm. J. Ind. Microbiol. Biotechnol. 34:577–588.

Patel, J., M. Sharma, and S. Ravishakar. 2011. Effect of curli expression and hydrophobicity of *Escherichia coli* 0157:H7 on attachment to fresh produce surfaces. J. Appl. Microbiol. 110:737–745.

Rivas, L., N. Fegan, and G. A. Dykes. 2007. Attachment of Shiga toxigenic *Escherichia coli* to stainless steel. Int. J. Food Microbiol. 115:89–94.

Rodrigues, L. B., L. R. dos Santos, N. N. Rizzo, V. Z. Tagliari, A. P. de Oliveira, G. Trenhago, S. C. Rodegheri, R. M. Taglieti, E. L. Dickel, and V. P. do Nascimento. 2009. Hydrophobicity and biofilm formation on polystyrene by *Salmonella* Heidelberg isolated from a poultry slaughterhouse. Acta Scientiae Veterinariae. 37:225–230.

Sinde, E., and J. Carballo. 2000. Attachment of *salmonella* spp. And *Listeria monocytogenes* to stainless steel, rubber and polytetrfluorethylene: The influence of free energy and the effect of commercial sanitizers. Food Microbiol. 17:439–447.

Smoot, L. M., and M. D. Pierson. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. J. Food Prot. 61:1293–1298.

Stocki, S. L., C. B. Annett, C. D. Sibley, M. McLaws, S. L. Checkley, N. Singh, M. G. Surette, and A. P. White. 2007. Persistence of *Salmonella* on egg conveyor belts is dependent on the belt type but not on the rdar morphotype. Poult. Sci. 86:2375–2383. http://dx.doi.org/10.3382/ps.2007-00121.

Tolvanén, R., J. Lunden, A. Horman, and H. Korkeala. 2009. Pilot-scale continuous ultrasonic cleaning equipment reduces *Listeria monocytogenes* levels on conveyor belts. J. Food Prot. 72:408–411.

Tolvanén, R., J. Lunden, H. Korkeala, and G. Wirtanen. 2007. Ultrasonic cleaning of conveyor belt materials using *Listeria monocytogenes* as model organism. J. Food Prot. 70:758–761.

Trachoo, N. 2007. Attachment of *Listeria monocytogenes, Escherichia coli O157:H7* and *Pseudomonas aeruginosa* on food soiled plastic surfaces. Pak. J. Biol. Sci. 10:1918–1921.

Tresse, O., V. Lebret, T. Benezech, and C. Faille. 2006. Comparative evaluation of adhesion, surface properties, and surface protein composition of *Listeria monocytogenes* strains after cultivation at constant pH of 5 and 7. J. Appl. Microbiol. 101:53–62.

Tresse, O., K. Shannon, A. Pinon, P. Malle, M. Vialete, and G. Midelet-Bourdin. 2007. Variable adhesion of *Listeria monocytogenes* isolates from food-processing facilities and clinical cases to inert surfaces. J. Food Prot. 70:1569–1578.

Ukuku, D. O., and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. J. Food Prot. 65:1093–1099.

Ukuku, D. O., and W. F. Fett. 2006. Effects of cell surface and hydrophobicity on attachment of 16 *Salmonella* serovars to cantaloupe rind and decontamination with sanitizers. J. Food Prot. 69:1835–1843.

Zeraik, A. E., and M. Nitschke. 2010. Biosurfactant as agent to reduce adhesion of pathogenic bacteria to polystyrene surfaces: Effects of temperature and hydrophobicity. 2010. Curr. Microbiol. 61:554–559.