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Recovery and Survival of Aerosolized *Escherichia coli* and *Enterococcus faecium* on Food-Grade Rubber, HDPE Plastic, Stainless Steel, and Waxed Cardboard

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ABSTRACT

Contamination of food contact surfaces by airborne transmission of pathogens from the environment has contributed to disease outbreaks. Therefore, this study evaluated the survival and recovery of aerosolized generic *Escherichia coli* and *Enterococcus faecium* from four food contact surfaces (food-grade silicone rubber, high-density polyethylene [HDPE] plastic, stainless steel, and waxed cardboard), after four contact times (10, 20, 40, and 60 min), two relative humidity (RH) levels (high: 80%–90%, low: 40%–50%), three distances from aerosolization source (0, 36.5, and 73 cm; *E. coli* only), and with and without airflow (*E. coli* only). ANOVA test with Tukey's HSD at $\alpha = 0.05$ was used to determine how treatment combinations influenced recovery. At high humidity, *E. coli* recovery on all materials after 40 min was ~1.0 log lower than recovery after 10 min, and further reduced by 1.0 log at 60 min. At lower humidity, *E. coli* recovery on all materials was ~1.0 log lower at 10 and 20 min compared with high humidity. Distances exerted no significance, whereas airflow presence lowered *E. coli* recovery. *E. coli* survival on all materials declined from ~5.0 log CFU/coupon at 0 h to 3.5 log CFU/coupon at 6 h, and 2.0 log CFU/coupon at 24 h post-inoculation. *E. coli* recovery was significantly lower ($p < 0.05$) on waxed cardboard. Low RH and longer contact time reduced *E. coli* recovery but not *E. faecium*. *E. faecium* recovery was consistent across treatment combinations, with changes < 0.5 log CFU/coupon. The findings are relevant for the survival of bacteria on common food contact surfaces and the potential of transmission to food products.

1 | Introduction

Past outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* have occurred in foodborne disease cases (Food and Drug Administration 2022). These two pathogens can cause severe human disease when transmitted through the food chain from animal reservoirs (Oporto et al. 2019; Rabsch et al. 2015). In addition to animal husbandry, adjacent lands are also used for manure composting and recreational or agritourism purposes. Adjacent land multipurpose usage can introduce cross-contamination far beyond their operational range. Numerous foodborne pathogen outbreaks have been attributed to adjacent land use as the potential cause. In 2006, an *E. coli* O157:H7 outbreak associated with prewashed packaged baby spinach was identified in the central coast of California, a region that is a major source of leafy greens consumed across the United States (Navarro-Gonzalez et al. 2020). Cattle (*Bos taurus*) and feral pig (*Sus scrofa*) feces from the adjacent land to the spinach fields were two of the sources for the outbreak strain (Navarro-Gonzalez et al. 2020). In 2019, three different leafy green *E. coli* O157:H7 outbreaks were most likely caused by aerosolized contamination from nearby livestock operations (Food and Drug Administration 2020). In 2020, a *Salmonella* Newport outbreak associated with red onions was responsible for 1127 reported domestic illnesses and 515 Canadian cases (Food and Drug Administration 2021a). In 2021, a *Salmonella Enteritidis* outbreak associated with peaches was responsible for 101 reported illnesses in 17 states (Food and Drug Administration 2021b). The US Food and Drug Administration (FDA) hypothesized that sheep grazing on adjacent land and other signs of animal intrusion were likely the sources of the 2020 *Salmonella* outbreak, and fugitive dust from adjacent animal operations was likely the source of the 2021 *Salmonella* outbreak (Food and Drug Administration 2020, 2021a). Berry et al. (2015) studied the effect of proximity on airborne transmission of *E. coli* O157:H7 between cattle feedlots and leafy green areas. The authors observed 120 m from cattle to leafy green fields might not be adequate to prevent transmission. Theofel et al. (2020) found *E. coli* populations to be highest in pistachio orchards adjacent (35 m) to poultry operations. Further data is needed to investigate the likelihood of foodborne pathogen survival and transfer in aerosols under different environmental conditions (relative humidity [RH] and wind) and proximity, which can be used to reduce contamination risks associated with adjacent land use and improve good agricultural practices (GAPs).

Bioaerosol control in food production environments is one of the key elements in GAP. There is always a potential for foods and food contact surfaces to be contaminated. Airborne microorganisms are often attached to aerosols that can be dispersed into the air by physical action. Different surface materials used during food production have different characteristics, which may or may not enhance the survival of aerosolized microbes when coming into contact. Wilks, Michels, and Keevil (2005) found that *E. coli* O157:H7 survived for over 28 days at both refrigeration and room temperatures on stainless steel. Kramer and Assadian (2014) observed that *E. coli* survived on inanimate surfaces from 1.5 h to 16 months, whereas Katzenberger, Rösel, and Vonberg (2021) observed that *E. coli* was inactive after 2 days on inanimate surfaces (e.g., stainless steel and PVC). Wißmann et al. (2021) compiled a thorough article, aligning all the results of *E. coli* survival time on a wide variety of surfaces: cloths (4 h to 8 weeks), plastics (24 h to 300 days), steel (14–60 days), copper alloys (60–360 min), and glass (1–14 days). Ormsby et al. (2023) showed that clinically important strains of *E. coli* can survive on plastic for at least 28 days. Margas et al. (2014) found that different *Salmonella* isolates can survive on stainless steel surfaces for at least 30 days. Wißmann et al. (2021) also compiled *Salmonella* survival time on a wide variety of surfaces in their same article: cloths (> 48 h), plastics (72 h to > 300 days), steel (> 30 days), and cardboard (1 h). This project investigated the recovery and survival of generic *E. coli* (as a surrogate for STEC) (Hu and Gurtler 2017; Woerner et al. 2018) and *Enterococcus faecium* (as a surrogate for *Salmonella*) (Hu and Gurtler 2017; Dhowlaghar and Zhu 2022) on four different surfaces at different contact times, RHs, distances from aerosolization source, and presence/absence of airflow.

2 | Materials and Methods

2.1 | Test Microorganisms

Escherichia coli (TVS353-0204, isolated from surface irrigation water) and *E. faecium* (ATCC 8459) were used for the experiments. Both strains were adapted to 80 mg/L rifampicin (Fisher Scientific, Fair Lawn, NJ) and stored at −80°C in 15% glycerol. The concentration of bacterial suspension was adjusted according to specific experiments.

2.2 | Inoculum Preparation

Before each experiment, 10 μL of frozen culture was streaked onto Tryptic Soy Agar (Difco, Becton Dickinson Co., Sparks, MD) with 80 mg/L rifampicin (TSAR) and incubated at 37°C ± 2°C for 24 h. After incubation, an isolated colony from the Petri plate was transferred with a 1-μL sterile loop into 10 mL Tryptic Soy Broth (Difco, Becton Dickinson Co., Sparks, MD) with 80 mg/L rifampicin (TSBR) and incubated at 37°C ± 2°C for 24 h. The overnight culture was subcultured once by transferring 10 μL of culture into 10 mL of fresh TSBR and incubated at 37°C ± 2°C for 24 h. The resulting bacterial suspension was used in the experiment the next day and diluted as needed.

2.3 | Food Contact Surfaces Selection and Treatment

Four different surface materials were chosen to represent those commonly used in food processing: food-grade silicone rubber, high-density polyethylene (HDPE) plastic, 304 stainless steel, and waxed cardboard (Vidacek and Bugge 2016). These materials were cut into 5 × 5 cm, making up a 25 cm^2 coupon. Food-grade rubber, HDPE plastic, and stainless steel coupons (BioSurface Technologies Corp., Bozeman, MT) were pre-cut upon purchase, whereas waxed cardboard (Uline Inc., Pleasant Prairie, WI) was cut by hand. Before inoculation, rubber, plastic, and stainless steel coupons were disinfected in 70% ethyl alcohol for 15 min, air-dried in a BSL-2 cabinet, and further treated with UVC light on both sides for 5 min each side (Lualdi et al. 2021; Rudhart et al. 2022). Waxed cardboard coupons were treated with UVC light only for 10 min each side.

2.4 | Aerosolization Chamber

All surface materials were inoculated in a 154 L polycarbonate box inside a BSL-2 cabinet at ambient temperature (22°C ± 3°C). The chamber's inner dimensions were 73 × 49 × 43 cm ($l \times w \times h$). The top side was hinged loading the box with agar plates, material coupons, and other instruments (Figure 1). The top edges of the chamber frame, where the lid met the walls, were lined with a neoprene cord to ensure a closed environment inside the box during the experiment. There were two round ports on both the box's left and right sides for a nebulizer or an air sampler to be inserted. The nebulizing port was 18.5 × 3.5 cm (height × diameter) in the middle of the chamber side. The air sampling port was 4.5 × 8 cm (height × diameter) in the middle of the opposite chamber side. A diluted quaternary detergent disinfectant solution (Bacdown Detergent Disinfectant, Decon Laboratories Inc., PA) was used to clean and disinfect the chamber after each exposure. Drierite desiccating calcium sulfate was added to reach the target RH 40%–50% inside the chamber. A thermohygrometer

FIGURE 1 | A complete setup of the aerosolization chamber inside a BSL-2 safety cabinet. A mesh nebulizer was positioned on the right-most side of the chamber (on top of the tube rack), followed by two fans (for experiments including airflow only). Petri dishes at the bottom of the chamber contained either material coupons or media, specifically with each experiment. A hygrometer was attached to the inside of the chamber to monitor RH. In low RH experiments, empty Petri dishes were filled with Drierite (not pictured) and positioned in the middle of the coupon/media rows. Distances were determined by how far the coupon/media rows were away from the aerosolization source (0 cm: Directly beneath the source, 36.5 cm: In the middle of the chamber, with the source as the starting point, 73 cm: Furthest away from the source, at opposite end of the chamber). An air sampler was positioned at the opposite end of the chamber from the aerosolization source.

(Traceable Jumbo Thermo Humidity Meter, Fisher Scientific) was used to monitor RH inside the chamber.

2.5 | Experimental Design

2.5.1 | Recovery of Aerosolized *E. coli* on Food Contact Surfaces

Four factors were included in the experiment design: four food contact surfaces (food-grade silicone rubber, HDPE plastic, stainless steel, waxed cardboard), four contact times (10, 20, 40, and 60 min), two RH levels (low: 40%–50%, high: 80%–90%), and three distances from source (0, 36.5, and 73 cm). Overall, $4 \times 4 \times 2 \times 3 = 96$ treatment combinations were performed. Four samples were collected per treatment combination, and all experiments were replicated two times ($n = 4 \times 2 = 8$). A bacterial suspension was diluted to a mean log of 6.31 ± 0.11 for aerosolization.

2.5.2 | Recovery of Aerosolized *E. coli* on Tryptic Soy Agar With Rifampicin (TSAR)

Four factors were included in the experiment design: two airflow conditions (fan on or off), four separate contact times (10, 20, 40, and 60 min), two RH (low: 40%–50%, high: 80%–90%), and three distances from source (0, 36.5, and 73 cm). Overall, $2 \times 4 \times 2 \times 3 = 48$ treatment combinations were performed. Four samples were collected per treatment combination, and all experiments were replicated two times ($n = 4 \times 2 = 8$). A bacterial suspension was diluted to a mean log of 4.36 ± 0.11 for aerosolization. Air sampling was followed at the end of each exposure duration and enumerated on TSAR plates.

2.5.3 | Survival of Spot-Inoculated *E. coli* on Food Contact Surfaces

Three factors were included in the experiment design: four food contact surfaces (food-grade silicone rubber, HDPE plastic, stainless steel, and waxed cardboard), five continuous contact times (0, 1, 6, 12, and 24 h), one RH (environment: 50%–60%). RH was not adjusted for the spot inoculation (survival) study since the desiccant became exhausted early before the end point of the experiment reached (24 h) and thus could not maintain the desired RH. Overall, $4 \times 5 \times 1 = 20$ treatment combinations were performed. Four samples were collected per treatment combination, and all experiments were replicated two times ($n = 4 \times 2 = 8$). A bacterial suspension was diluted to a mean log of 6.33 ± 0.09 for spot-inoculation.

2.5.4 | Recovery of Aerosolized *E. faecium* on Food Contact Surfaces

Three factors were included in the experiment design: four food contact surfaces (food-grade silicone rubber, HDPE plastic, stainless steel, waxed cardboard), four contact times (10, 20, 40, and 60 min), and two RH levels (low: 40%–50%, high: 80%–90%). Overall, $4 \times 4 \times 2 = 32$ treatment combinations were performed. Distance was removed as a factor in *E. faecium* experiments as minimal differences were found in our *E. coli* experiments. Four samples were collected per treatment combination, and all experiments were replicated two times ($n = 4 \times 2 = 8$). A bacterial suspension was diluted to a mean log of 6.07 ± 0.05 for aerosolization.

2.5.5 | Survival of Spot-Inoculated *E. faecium* on Food Contact Surfaces

Three factors were included in the experiment design: four food contact surfaces (food-grade silicone rubber, HDPE plastic, stainless steel, waxed cardboard), seven continuous contact times (0, 1, 6, 24, 72, 120, and 168 h), one RH (environment: 50%–60%). Four samples were collected per treatment combination, and all experiments were replicated two times ($n = 4 \times 2 = 8$). A bacterial suspension was diluted to a mean log of 6.01 ± 0.06 for spot-inoculation.

2.6 | Inoculation of Food Contact Surface Coupons

2.6.1 | Spot Inoculations of *E. coli* and *E. faecium*

For the survival experiments, 100 μL of bacterial suspension (mean concentration of 6.33 ± 0.09 log CFU/mL for *E. coli*, and mean concentration of 6.01 ± 0.06 log CFU/mL for *E. faecium*) was spot-inoculated in 15–20 droplets on each surface coupon, excluding 2 mm from the edge (Proulx et al. 2017).

2.6.2 | Aerosolization of *E. coli* and *E. faecium*

For the recovery of *E. coli* and *E. faecium* on food contact surfaces, 1 mL of an approximately 6.0 log CFU/mL bacterial suspension was aerosolized in 3 min using a mesh nebulizer. A battery-powered mesh nebulizer (model No. NE-07, Apowus, purchased from Amazon) was used for aerosolization inoculation. According to its manual, the loaded liquid could be sprayed at a rate of ≥ 0.2 mL/min, with a particle diameter of 5 μm ± 25%, which was close to the size of natural bioaerosols (Fennelly 2020; Randall et al. 2021). An extension nozzle was included with the nebulizer, which was fitted in a pre-cut hole on the aerosolization chamber wall.

For experiments where aerosolized *E. coli* was recovered directly from TSAR, the bacterial suspension was adjusted to 4.0 log CFU/mL and aerosolized using the same protocol. Additionally, two portable mini pocket fans (SKool, Kai Bros Products, purchased from Amazon) were used to create airflow inside the chamber. The fans were disinfected with 70% ethyl alcohol, air-dried inside a BSL-2 cabinet, treated under UVC light on both sides, and reused after each exposure time. A micro-anemometer (Traceable Micro-Anemometer, Fisher Scientific) was used to measure airflow speed at each designated distance in the chamber (14.48 km/h [close to source], 6.44 km/h [middle of chamber], 3.22 km/h [furthest end from source]). Wind speed was not included as a factor in this study.

2.7 | Recovery of Adherent Cells on Surface Materials

Inoculated coupons were transferred into sterile sampling bags (Fisher Scientific, Pittsburg, PA) with disinfected tweezers. Exactly 10 mL of 0.1% buffered peptone water with 0.1% Tween 80 (Fisher Scientific) was added to the bags containing the coupons, and samples received a rub–shake–rub treatment for 60 s (Lim and Harrison 2016). The subsequent liquid was serially diluted in 9 mL 0.1% peptone water tubes, plated onto TSAR plates, and incubated at 37°C ± 2°C for 24 h. For treatments that were expected to have low counts, 1 mL of liquid from bags containing the coupon was plated across four plates to achieve a 1 log CFU/coupon limit of detection (LOD). Colonies were counted and then transformed to log CFU per coupon. When colony counts fell below the LOD (< 1 log CFU/coupon), a count of 0.5 CFU was assigned. No enrichments were performed when counts were below the LOD.

2.8 | Recovery of *E. coli* From Air After Nebulization

An impaction air sampler (model Airport MD8, Sartorius, Germany) was used to collect air inside the chamber after the nebulization of the culture ended. Air (100 L) was collected in 2 min onto an 80 mm gelatin filter (Sartorius, Germany), which was then transferred directly onto a TSAR plate. After 10 min, plates were incubated at 37°C ± 2°C for 24 h.

2.9 | Statistical Analyses

Escherichia coli and *E. faecium* populations were converted to a log scale depending on each experiment: log CFU per coupon for recovery and survival on food contact surfaces, log CFU per plate for survival on TSAR (*E. coli* only), and log CFU per 100 L of air for air sampling (*E. coli* only). Data analysis was performed using R studio (version 4.2.1). Comparisons of mean recovery values in material/environmental factors for the deposition intervals and inoculation methods (aerosolization and spot) were determined using ANOVA with Tukey's HSD at $\alpha = 0.05$.

3 | Results

3.1 | Recovery of Aerosolized *E. coli* on Food Contact Surfaces at RH 80%–90%

Waxed cardboard coupons had the least recovery of aerosolized *E. coli* compared with other materials at each contact time interval. At 10 min, the mean recovery from cardboard was ~0.5 log CFU/coupon lower than for food-grade rubber, HDPE plastic, and stainless steel (Table 1). Recovery dropped significantly, starting at 20 min across all materials and all distances.

Recovery was not significantly different among distances of the materials from the aerosolization source (Table 1). Recovery was highest at 10 min after aerosolization for all materials at each distance.

3.2 | Recovery of Aerosolized *E. coli* on Food Contact Surfaces at RH 40%–50%

Overall, recovery of aerosolized *E. coli* at RH 40%–50% was 0.5–1.0 log CFU/coupon lower than recovery at RH 80%–90%. Recovery of aerosolized *E. coli* was lower on waxed cardboard coupons compared with other materials at each contact time interval (Table 2). Recovery, after 10 min, from stainless steel was higher than from other material coupons for every distance. In contrast to the drop in recovery starting after 10 min in RH 80%–90%, there was an increase in recovery at 20 min compared with 10 min in RH 40%–50%, followed by reductions at 40 and 60 min. Recovery at 73 cm was ~0.5 log lower compared with 0 and 36.5 cm from the source with all materials (Table 2).

3.3 | Recovery of Aerosolized *E. coli* on TSAR and From Air Samples at RH 80%–90%

Gravity sedimentation and air sampling provided a cumulative recovery of aerosolized *E. coli*. Recovery on TSAR was typically higher by 0.1–0.5 log CFU per plate when fans were turned off (Table 3). Recovery at 20 min increased compared with 10 min but decreased at 40 and 60 min. Recovery at 60 min was the lowest throughout all combinations. Air sampling results (Figure 2) show higher recovery at 10 and 20 min, suggesting that aerosols could stay airborne longer initially after aerosolized with high humidity and airflow. Figure 2 also showed that at 60 min, recovery from air sampling dropped significantly with airflow compared with no airflow. When RH was 80%–90%, there were significant differences in recovery at all combinations of contact time and airflow condition ($p < 0.05$).

3.4 | Recovery of Aerosolized *E. coli* on TSAR and From Air Samples at RH 40%–50%

Similar to the trends for high humidity, there were significant differences in recovery for every exposure duration when fans were either on or off. Recovery at 60 min remained the lowest throughout all combinations. Table 4 (sedimentation on TSAR) and Figure 3 (air sampling) showed higher recovery with no airflow. Differences in recovery were not significant among plate distance from the aerosolization source. Within RH 40%–50%, there were differences in recovery at all combinations of contact time and airflow condition ($p < 0.05$).

3.5 | Survival of Spot-Inoculated *E. coli* on Food Contact Surfaces at RH 50%–60%

For spot-inoculated cells, recovery on all materials did not decrease after 1 h of drying (Table 5). Within each material,

TABLE 1 | Mean log CFU (±SD) generic *Escherichia coli* recovered per coupon at different distances from aerosolization source with a chamber RH of 80%–90%.

	Log CFU/coupon			
Sample time (min)	**Rubber**	**Plastic**	**Steel**	**Cardboard**
Distance: 0 cm				
10	3.48 ± 0.17bA	3.55 ± 0.13abA	3.66 ± 0.14aA	3.06 ± 0.18cA
20	3.25 ± 0.18aB	2.93 ± 0.22bB	3.19 ± 0.33aB	2.69 ± 0.31bB
40	1.96 ± 0.23aC	2.12 ± 0.51aC	2.29 ± 0.33aC	2.22 ± 0.37aC
60	1.09 ± 0.31aD	1.02 ± 0.28aD	1.04 ± 0.29aD	0.93 ± 0.26aD
Distance: 36.5 cm				
10	3.71 ± 0.14aA	3.68 ± 0.20aA	3.59 ± 0.20aA	2.95 ± 0.24bA
20	3.32 ± 0.11aB	2.61 ± 0.19bB	3.24 ± 0.36aB	2.65 ± 0.27bB
40	2.18 ± 0.28bC	2.31 ± 0.41abC	2.50 ± 0.27aC	2.15 ± 0.32bC
60	0.85 ± 0.22aD	0.96 ± 0.29aD	0.81 ± 0.15aD	0.88 ± 0.29aD
Distance: 73 cm				
10	3.59 ± 0.13aA	3.61 ± 0.11aA	3.37 ± 0.33bA	2.89 ± 0.20cA
20	3.42 ± 0.37aA	2.71 ± 0.27bB	3.25 ± 0.49aA	2.69 ± 0.30bA
40	2.19 ± 0.33aB	2.08 ± 0.29aC	2.27 ± 0.16aB	2.16 ± 0.30aB
60	1.03 ± 0.31aC	0.83 ± 0.19abD	0.76 ± 0.12bC	0.96 ± 0.31abC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 6.31 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer.

TABLE 2 | Mean log CFU (±SD) generic *Escherichia coli* recovered per coupon at different distances from aerosolization source with a chamber RH of 40%–50%.

Sample time (min)	Log CFU/coupon			
	Rubber	Plastic	Steel	Cardboard
Distance: 0 cm				
10	2.25 ± 0.26abB	1.93 ± 0.65bcB	2.59 ± 0.18aA	1.81 ± 0.24cA
20	2.95 ± 0.19aA	2.47 ± 0.24bA	2.49 ± 0.25bA	1.93 ± 0.35cA
40	2.13 ± 0.26aB	1.78 ± 0.37bB	2.05 ± 0.16abB	1.17 ± 0.33cB
60	1.01 ± 0.36aC	0.85 ± 0.22abC	0.76 ± 0.12bC	0.72 ± 0.08bC
Distance: 36.5 cm				
10	2.27 ± 0.26bB	2.24 ± 0.25bA	2.57 ± 0.18aA	1.84 ± 0.39cA
20	2.87 ± 0.14aA	2.42 ± 0.24bA	2.45 ± 0.25bA	2.02 ± 0.33cA
40	2.13 ± 0.21aB	1.78 ± 0.19bB	1.95 ± 0.21abB	1.05 ± 0.26cB
60	1.04 ± 0.29abC	1.23 ± 0.59aC	0.89 ± 0.24bC	0.85 ± 0.15bB
Distance: 73 cm				
10	1.80 ± 0.36bB	1.69 ± 0.43bB	2.30 ± 0.17aAB	1.29 ± 0.48cB
20	2.61 ± 0.23aA	2.01 ± 0.23bA	2.38 ± 0.37aA	1.70 ± 0.25cA
40	2.37 ± 0.25aA	1.91 ± 0.35bAB	2.10 ± 0.36abB	0.98 ± 0.23cC
60	1.31 ± 0.30aC	0.97 ± 0.30bC	0.81 ± 0.19bC	0.83 ± 0.19bC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 6.31 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer.

recovery after 6 h was significantly lower than that of 0 and 1 h. Additionally, recovery after 12 and 24 h was significantly lower than that at 6 h. After 24 h, *E. coli* recovery from stainless steel was at least one log lower than from rubber, plastic, and cardboard. Recovery from waxed cardboard was similar to that from the other test surfaces, in contrast to aerosolization–inoculation where recovery was consistently lowest from this material.

3.6 | Recovery of Aerosolized *E. faecium* on Food Contact Surfaces at RH 80%–90%

Stainless steel coupons yielded the lowest recovery of aerosolized *E. faecium* compared with other materials at each contact time interval (approximately < 0.4 mean log CFU/coupon; Table 6). Recovery from all material types and time point combinations varied marginally (~0.2–0.3 log CFU/coupon). Rubber was the only material type to experience an increase in recovery (~0.2 log CFU/coupon) after 10 min.

3.7 | Recovery of Aerosolized *E. faecium* on Food Contact Surfaces at RH 40%–50%

Similar to aerosolized *E. faecium* recovery at higher humidity, recovery from stainless steel coupons was slightly lower compared with other materials at each contact time interval (approximately < mean 0.5 log CFU/coupon; Table 7). Recovery on all material types and time point combinations varied ~0.1–0.2 log CFU/coupon (Table 7). Rubber was the only material type to experience an increase in recovery (~0.2 log CFU/coupon) after 60 min. While *E. coli* recovery decreased under 40%–50% RH by ~0.5–1.0 log CFU/coupon compared with 80%–90% RH (Tables 1 and 2); lower humidity (40%–50% RH) exerted minimal effect on *E. faecium* recovery (Tables 6 and 7).

3.8 | Survival of Spot-Inoculated *E. faecium* on Food Contact Surfaces at RH 50%–60%

For spot-inoculated cells, recovery concentrations, after 7 days (168 h) were only 0.1–0.3 log lower than time zero for all test material types (Table 8). While *E. coli* survival and recovery significantly decreased by ~3 log CFU/coupon after 24 h (Table 5), *E. faecium* recovery from all materials decreased by just 0.3–0.8 log CFU/coupon, 24 h after inoculation. The populations recovered after 168 h were higher than those recovered after 24 h except for cardboard where recovery was just 0.1 log CFU/coupon lower.

4 | Discussion

The current study examined generic *E. coli* and *E. faecium* airborne recovery and survival over time on food contact surfaces under different environmental conditions. Settling time, RH, presence/absence of airflow, and material types were contributing factors for the recovery and survival of aerosolized generic

TABLE 3 | Mean log CFU (±SD) generic *Escherichia coli* recovered on TSAR per airflow condition, at different distances from aerosolization source with a chamber RH of 80%–90%.

	Log CFU/plate	
Sample time (min)	**Fan off**	**Fan on**
Distance: 0 cm		
10	2.15 ± 0.05aB	2.09 ± 0.16aA
20	2.26 ± 0.06aA	2.17 ± 0.12aA
40	2.19 ± 0.07aAB	2.11 ± 0.16aA
60	2.13 ± 0.09aB	1.76 ± 0.18bB
Distance: 36.5 cm		
10	2.19 ± 0.05aAB	1.73 ± 0.15bB
20	2.27 ± 0.11aA	1.91 ± 0.11bA
40	2.18 ± 0.02aB	1.76 ± 0.12bAB
60	1.97 ± 0.04aC	1.44 ± 0.06bC
Distance: 73 cm		
10	2.02 ± 0.07aB	1.62 ± 0.14bB
20	2.14 ± 0.07aA	1.84 ± 0.09bA
40	2.07 ± 0.06aAB	1.69 ± 0.13bAB
60	1.86 ± 0.11aC	1.32 ± 0.15bC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time for all wind conditions. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each wind condition for all contact times. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 4.36 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer.

E. coli. These factors, on the other hand, had much less influence on the recovery and survival of aerosolized *E. faecium*. Generic *E. coli* recovery from test surfaces was lower as time after nebulization increased. *E. coli* recovery across all time points and distances was generally lower with low RH. *E. coli* recovery was also reduced when fans created airflow. Contact time and RH overall did not have a considerable impact on *E. faecium* recovery.

In some cases, recovery of aerosolized *E. coli* was slightly lower when collected at further distances from their source (up to 73 cm). Sanz et al. (2021) looked at differences in bacterial recovery (Staphylococci, *Enterobacteriaceae*, and Enterococci) in the inside air and outside air of a broiler farm and found that the emission of bacteria in the outside air was much lower than that of the inside air. They also suggested that the bacteria detected in the outside air decreased with the distance to the farm. In future research, a larger aerosolization chamber may be useful to further examine the distance that aerosolized bacteria may be able to travel and remain viable. Future research may also employ cocktails of generic *E. coli* and *E. faecium* strains for a broader strain variety (Harrand et al. 2021). Even though the two strains used in this study are common surrogates for their respective pathogenic organisms of interest (*E. coli* O157:H7 and *Salmonella*), the results might only be representative of those two surrogate strains.

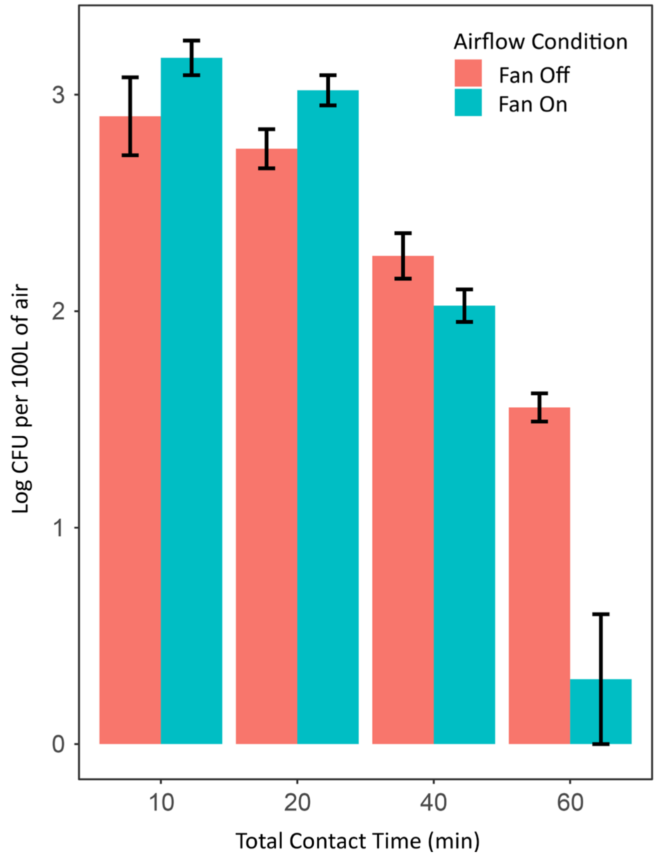

FIGURE 2 | Mean log CFU generic *Escherichia coli* (±SE) recovered with impaction air sampling per airflow condition after an aerosolization contact time of 10, 20, 40, and 60 min at different distances within the aerosolization chamber at RH 80%–90%. Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time across all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 4.36 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer.

4.1 | Nebulization Aerosolization and Impaction Sampling Influence Cell Integrity, Cell Recovery, and Depository Time

In this study, ~6.3 or 6.1 log CFU for *E. coli* or *E. faecium*, respectively, was nebulized into a closed chamber. The test bacteria settled onto a variety of food contact surface coupons (5 × 5 cm) for up to 60 min. If we consider the entire lower surface area of the chamber (73 × 49 cm), then we can extrapolate a theoretical recovery of all organisms that settled to the bottom of the chamber. Maximum recovery after 10 min and high RH was ~5.9 log CFU for both *E. coli* and *E. faecium*. Therefore, we do not believe the nebulizer caused significant injuries to the organisms or had an appreciable effect on quantitative recovery from the surfaces. A mesh nebulizer was suited for this study as it holds the most advantages among the three types of nebulizers (mesh, jet, and ultrasonic) and can mimic natural aerosols the best (Bowling et al. 2019). Cells can be damaged with aerosolization, regardless of the method. The physiological status of bacteria changes with time spent in the

TABLE 4 | Mean log CFU (±SD) generic *Escherichia coli* recovered on TSAR per airflow condition, at different distances from aerosolization source with a chamber RH of 40%–50%.

	Log CFU/plate	
Sample time (min)	**Fan off**	**Fan on**
Distance: 0 cm		
10	1.69 ± 0.15aA	1.33 ± 0.32bA
20	1.77 ± 0.07aA	1.29 ± 0.35bA
40	1.62 ± 0.11aA	1.25 ± 0.34bA
60	1.71 ± 0.11aA	0.94 ± 0.37bA
Distance: 36.5 cm		
10	1.81 ± 0.17aA	0.75 ± 0.17bAB
20	1.85 ± 0.16aA	0.83 ± 0.16bA
40	1.75 ± 0.11aA	0.80 ± 0.21bAB
60	1.78 ± 0.09aA	0.48 ± 0.35bB
Distance: 73 cm		
10	1.28 ± 0.18aA	0.94 ± 0.17bA
20	1.11 ± 0.18aA	0.78 ± 0.25bAB
40	1.19 ± 0.10aA	0.67 ± 0.26bAB
60	0.85 ± 0.18aB	0.48 ± 0.42bB

Note: Lowercase letters denoted differences in recovery ($p \le 0.05$) within each contact time for all wind conditions. Uppercase letters denoted differences in recovery ($p \le 0.05$) within each wind condition for all contact times. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 4.36 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer.

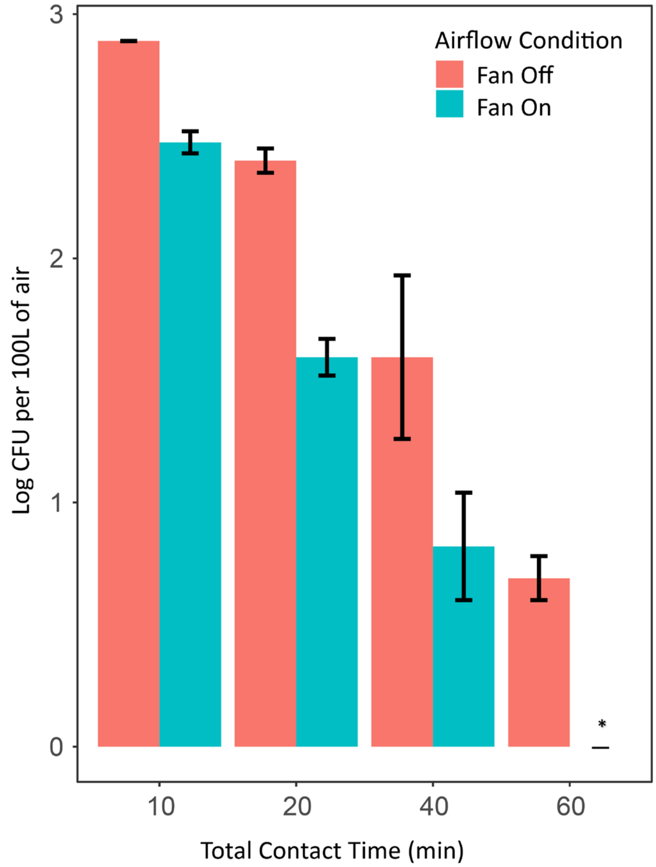

FIGURE 3 | Mean log CFU generic *Escherichia coli* (±SE) recovered with impaction air sampling per airflow condition after an aerosolization contact time of 10, 20, 40, and 60 min at different distances within the aerosolization chamber at RH 40%–50%. Lowercase letters denoted differences in recovery ($p \le 0.05$) within each contact time across all materials. Uppercase letters denoted differences in recovery ($p \le 0.05$) within each material. Both lowercase and uppercase letters only denoted differences within one distance. A mean starting concentration of 4.36 ± 0.11 log CFU/mL served as the bacterial suspension for the nebulizer. * Recovery was lower than the limit of detection.

aerosolized state (Thomas et al. 2011); however, in this study, 1.0 mL of bacterial suspension was nebulized in a fixed 3-min duration. After aerosolization, different factors will contribute to viability loss in the airborne bacteria, including desiccation (RH) and oxidative shock (Oswin et al. 2023; Smith and King 2023).

Aerosolized bacteria can exhibit a viable but nonculturable (VBNC) state (Alsved et al. 2018). Impaction air sampling could further cause cells to become VBNC by moisture withdrawal or stress from impaction force (Terzieva et al. 1996; Willeke et al. 1995). VBNC state is a major concern in food safety, as it might only lead to cell dormancy, not cell death (Scherber, Schottel, and Aksan 2009). Even though air was collected onto a gelatin filter, at a low flow rate (50 L/min), over a short period (2 min), some cells collected from air samples may have been non-culturable.

As settling times increased, a lower recovery of *E. coli* was observed. This trend might be caused by a lack of nutrients on surface coupons and on TSAR Petri dishes, along with desiccation stress caused by aerosolization. However, there are some increases in *E. coli* recovery at 20 min on contact surfaces compared with 10, 40, and 60 min (Tables 2–4). An explanation for this phenomenon might be bacterial survival mechanisms and stress responses to adapt to unfavorable environmental conditions *E. coli* combats desiccation by synthesizing trehalose, a disaccharide that prevents protein structures and membrane function from desiccation by replacing bacterial membranous water (Esbelin, Santos, and Hébraud 2018). Trehalose synthesis in *E. coli* is encoded by *otsA* and *otsB* operons, whose expression is upregulated by osmotic stress (Burgess et al. 2016). Trehalose synthesis can be problematic in processed foods, as trehalose occurs naturally in food (some mushrooms, seaweed, honey, and yeast) (Chen et al. 2022) and enhances the survivability of foodborne pathogens (Strøm and Kaasen 1993; Kandror, DeLeon, and Goldberg 2002; Algara et al. 2019). Cells in the stationary phase naturally accumulate more trehalose, and osmotic stress can further induce trehalose production (Welsh and Herbert 1999; Kandror, DeLeon, and Goldberg 2002). Additionally, *E. coli* response to osmotic stress can be studied by transcriptomics, a high-throughput application that can quantify transcriptome in cells and provide information regarding gene expression differences (Chen et al. 2022). Future studies can include transcriptomics to further discuss the VBNC state under the same conditions examined in this research. Transcriptomics can also

TABLE 5 | Mean log CFU (±SD) generic *Escherichia coli* recovered per coupon after a spot-inoculation drying time of 0, 1, 6, 12, and 24 h inside the aerosolization chamber at RH 50%–60%.

Drying time (h)	Log CFU/coupon			
	Rubber	Plastic	Steel	Cardboard
0	5.37 ± 0.12aA	5.28 ± 0.14aA	5.26 ± 0.14aA	5.26 ± 0.14aA
1	5.35 ± 0.09aA	5.13 ± 0.17bA	5.16 ± 0.23bA	5.22 ± 0.11abA
6	3.82 ± 0.27aB	3.67 ± 0.60abB	2.95 ± 0.43bcB	3.28 ± 0.35bcB
12	2.72 ± 0.41aC	2.54 ± 0.84aC	1.62 ± 0.63bC	2.41 ± 0.61aC
24	2.44 ± 0.37aC	2.40 ± 0.80aC	1.21 ± 0.71bC	2.24 ± 0.44aC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each drying time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. A mean starting concentration of 6.33 ± 0.09 log CFU/mL served as the bacterial suspension for spot inoculation.

TABLE 6 | Mean log CFU (±SD) generic *Enterococcus faecium* recovered per coupon after an aerosolization contact time of 10, 20, 40, and 60 min regardless of distance within the aerosolization chamber at RH 80%–90%.

Sample time (min)	Log CFU/coupon			
	Rubber	Plastic	Steel	Cardboard
10	3.65 ± 0.31aB	3.82 ± 0.16aA	3.67 ± 0.26aA	3.82 ± 0.19aAB
20	3.82 ± 0.16aAB	3.77 ± 0.21abAB	3.49 ± 0.24cAB	3.62 ± 0.19bcC
40	3.89 ± 0.28aA	3.82 ± 0.18abA	3.68 ± 0.14bA	3.88 ± 0.15aA
60	3.81 ± 0.22aAB	3.62 ± 0.23aB	3.34 ± 0.43bB	3.64 ± 0.28aBC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. A mean starting concentration of 6.07 ± 0.05 log CFU/mL served as the bacterial suspension for the nebulizer.

TABLE 7 | Mean log CFU (±SD) *Enterococcus faecium* recovered per coupon after an aerosolization contact time of 10, 20, 40, and 60 min regardless of distance within the aerosolization chamber at RH 40%–50%.

Sample time (min)	Log CFU/coupon			
	Rubber	Plastic	Steel	Cardboard
10	3.60 ± 0.12aB	3.37 ± 0.18bB	3.31 ± 0.18bA	3.45 ± 0.22abB
20	3.68 ± 0.16aAB	3.57 ± 0.15aA	3.37 ± 0.16bA	3.71 ± 0.15aA
40	3.74 ± 0.21aAB	3.51 ± 0.24bcAB	3.33 ± 0.15cA	3.58 ± 0.15abAB
60	3.81 ± 0.11aA	3.49 ± 0.16bAB	3.32 ± 0.16cA	3.54 ± 0.24bAB

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each contact time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. A mean starting concentration of 6.07 ± 0.05 log CFU/mL served as the bacterial suspension for the nebulizer.

be used to reveal biological pathways for *E. faecium* desiccation adaptation.

E. faecium recovery, on the other hand, varied minimally during the 60 min after nebulization (Tables 6 and 7). This might be because of the difference in cell wall components of *E. faecium* (Gram-positive) and *E. coli* (Gram-negative). Gram-positive bacteria generally have better protection and responses to unfavorable environmental conditions than Gram-negative bacteria, mainly because of the thicker peptidoglycan layer in the Gram-positive cell wall (19–33 nm thick when fully hydrated, compared with that of 2.5–6.5 nm thickness in Gram-negative) (Auer and Weibel 2017). Cell wall peptidoglycans not only contribute to cell shape but also provide cells with osmotic and mechanical stress protection (Mueller and Levin 2020). No research has been reported specifically on how aerosolization affects *E. faecium*, but Stone and Johnson (2002) studied this topic with *Bacillus subtilis* (a model organism for Gram-positive lineage) and reported that culturable concentrations from the nebulizer reservoir dropped < 0.5 log CFU from an initial concentration of 9 log when nebulized for 5 min at 20 psi.

4.2 | RH and Airflow Have Effects on Cell Recovery and Survival

Controlling RH in food processing environments is always one priority to ensure food quality and safety. Lowered humidity significantly reduces bacterial growth (Qiu et al. 2022). Undesirable

TABLE 8 | Mean log CFU (±SD) *Enterococcus faecium* recovered per coupon after a spot-inoculation drying time of 0, 1, 6, 24, 72, 120, and 168 h inside the aerosolization chamber at RH 50%–60%.

	Log CFU/coupon			
Drying time (h)	**Rubber**	**Plastic**	**Steel**	**Cardboard**
0	4.89 ± 0.13aA	4.86 ± 0.11aA	4.86 ± 0.09aA	4.87 ± 0.11aA
1	4.80 ± 0.10aA	4.48 ± 0.44bB	3.90 ± 0.37cBC	4.77 ± 0.05aA
6	4.29 ± 0.19bC	4.16 ± 0.46bC	3.76 ± 0.26cC	4.58 ± 0.12aB
24	4.33 ± 0.07bC	4.16 ± 0.29bcC	4.09 ± 0.36cB	4.62 ± 0.04aB
72	4.66 ± 0.08aB	4.56 ± 0.10abAB	4.05 ± 0.35cB	4.40 ± 0.11bC
120	4.55 ± 0.15aB	4.42 ± 0.27aBC	3.94 ± 0.24bBC	4.49 ± 0.18aBC
168	4.55 ± 0.12bB	4.56 ± 0.20bAB	4.72 ± 0.07aA	4.53 ± 0.20bBC

Note: Lowercase letters denoted differences in recovery ($p \leq 0.05$) within each drying time for all materials. Uppercase letters denoted differences in recovery ($p \leq 0.05$) within each material for all contact times. A mean starting concentration of 6.01 ± 0.06 log CFU/mL served as the bacterial suspension for spot inoculation.

RH prevents harmful bacteria from multiplying and spreading, as it enhances oxygen penetration into the bacterial cells, amplifying oxidative stress, which can potentially harm DNA and proteins (Ng, Chan, and Lai 2017). Still, foodborne pathogen survival at lower RH poses problems, as some dry time or operations might require specifically low RH. Gram-negative bacteria such as *E. coli* are more susceptible to drying, with a notable rise in cell wall roughness and stiffness when RH is ≤ 84% (Tokarskyy et al. 2018). Wathes, Howard, and Webster (1986) found that death of *E. coli* was rapid at lower than 50% RH, with half-lives of 14 and 3 min at 15°C and 30°C, respectively. Tables 1 and 2 confirmed the previous findings, showing a significant decrease in *E. coli* recovery between high RH (80%–90%) and low RH (40%–50%), from ≥ 3.0 log to ≥ 2.0 log at 10 min, but remained approximately at 1.0 log at 60 min, respectively. This result might suggest that RH alone cannot effectively control the proliferation of pathogens. Song, Kim, and Rhee (2016) discovered that RH alone can only reduce an insignificant number of bacteria, such as 0.4 and 0.5 log reduction from an initial 7.1 log of STEC at RH 40% and 60%, respectively. Tables 3 and 4 also showed differences in *E. coli* recovery between high and low RH, even with the extra moisture on TSAR compared with the test material coupons. At high RH, *E. coli* recovery was ~2.0 log at 10 min but only reduced to 1.3 log at 60 min. At low RH, the same minimal reduction was also observed in *E. coli*. Figures 1 and 2 depict *E. coli* air sampling results under high and low RH. Reduced *E. coli* recoveries, when fans were on or off, were observed 20, 40, and 60 min after nebulization under low RH compared with high RH. This might suggest that prolonged time, low RH, and airflow presence are detrimental to *E. coli* aerosols in a closed environment. *E. faecium*, on the other hand, was minimally affected by RH. *E. faecium* recovery on plastic, steel, and cardboard was 0.2–0.5 log lower in the low RH environment compared with that of high RH at every time interval (Tables 6 and 7). This result aligns with the findings of Thompson, Bennett, and Walker (2011) that different RH levels did not have a significant effect on Gram-positive bacteria (*Staphylococcus epidermidis* and *Bacillus atrophaeus*). Another study discovered that *Salmonella* could accumulate a massive amount of trehalose after exposure to desiccation (Li et al. 2023), which could be a speculation for *E. faecium* survival at low RH. A limitation of experimenting with RH in this study was the use of Drierite as a desiccant. Drierite became exhausted after a certain amount of time and could not hold more water with exhaustion. Longer settling time points and lower RH levels could not be examined as RH could not be adequately controlled with exhausted Drierite. A humidity control chamber is suggested for future studies.

Airflow control is another vital hurdle in ensuring food safety. Stagnant airflow can retain aerosols, whereas moving airflow can transmit them. Airflow monitoring in open areas, such as outdoor farms, is even more crucial as natural elements cannot be contained. Smith et al. (2023) suggested that open poultry farms that exhibit higher wind speed and more surrounding agriculture activities face more risk of introducing *Campylobacter* spp. into their flocks, as *Campylobacter* spp. was found in 250/962 (26.0%) of fecal samples. There have been numerous attempts to monitor aerosol flow dynamics, such as biosensors and active air sampling devices (Zhai et al. 2022). Tables 3 and 4 show differences in *E. coli* recovery on agar with the presence and absence of airflow. Differences in *E. coli* recovery did not exceed 1.0 log when fans were on or off, under both high and low RH. *E. coli* mean recovery from air samples (Figures 1 and 2) was usually higher with no airflow, however, at high RH (Figure 2), it was slightly higher at 10 and 20 min with airflow.

4.3 | Effect of Surface Material on Cell Recovery and Survival

Airborne pathogens can cause product cross-contamination after attachment to various food processing surfaces or processed products. As most food contact surfaces are not antibacterial by nature, sanitary practices and sanitary design can help prevent the spread of microorganisms. In this study, significant differences in microorganism recovery were observed among the product contact surface materials. Recovery was lowest on waxed cardboard at all time intervals after *E. coli* aerosolization (Tables 1 and 2). Recovery was lowest from stainless steel at all time intervals after *E. faecium* aerosolization (Tables 6 and 7).

Zheng et al. (2021) provided a detailed review of how bacterial adhesion can be influenced by different surface properties other than surface charge and wettability, such as surface roughness, topography, stiffness, or a combination of these factors. Overall, hydrophobic and negatively charged surfaces inhibit bacterial adhesion (Oh et al. 2018; Ashok et al. 2023).

Stainless steel is naturally negatively charged (Giron et al. 2018), whereas the rest are non-conductive materials and thus not charged. Stainless steel is hydrophilic (Lu et al. 2021), whereas the rest are hydrophobic (Xu et al. 2022; Emelyanenko et al. 2017; Cataldi, Profaizer, and Bayer 2019). During aerosolization inoculation for both organisms, wetness was visually observed on stainless steel, to a lesser extent on HDPE plastic and food-grade rubber, and even less on waxed cardboard. This might suggest that HDPE plastic and food-grade rubber were somewhat water resistant, but not water-repellent like waxed cardboard. This assumption corresponded to the spot inoculation experiment with both organisms, in which the droplets retained their shape best on waxed cardboard and the least on stainless steel. Low *E. coli* recovery on waxed cardboard in aerosolization experiments might be because of already damaged cells landing on a hydrophobic surface (Tables 1 and 2), but the same trend was not observed with *E. faecium* (Tables 6 and 7). The different recovery trend of stainless steel in spot inoculation compared with aerosolization inoculation (Table 5) might be because of the prolonged attachment of *E. coli* cells on the charged surface. On the contrary, *E. faecium* populations remained stable on all surfaces for 7 days after spot inoculations (Table 8), aligning with previous research that examined *E. faecium* and *Salmonella* survival on food contact surfaces (FCS) (Margas et al. 2014; Xie et al. 2024). *E. faecium* survival was observed for up to 12 weeks on stainless steel, under 34% RH (Xie et al. 2024). Their research supports the hypothesis that differences in FCS surface properties and foodborne pathogen characteristics make the prevention of FCS contamination a difficult task.

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Ethics Statement

There are neither human subjects nor animal subjects involved in this research.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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