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Hygiene Status of Over-the-Row Blueberry Machine Harvesters Cleaned and Sanitized Using Various Approaches

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Abstract: Contamination of fresh blueberries via contact with an equipment surface is an important food hygiene/safety issue. In this study, four and six over-the-row blueberry machine harvesters in Georgia or Oregon were each sampled twice on two different harvest days in the 2022 harvest season. Nine sites on the top loaders (n = 8) and seven sites on the bottom loaders (n = 2) were sampled before and after cleaning/sanitation. Populations of total aerobes (TA), total yeasts and molds (YM), total coliforms (TC), and the presence of fecal coliforms (FC) and enterococci (EC) in collected samples were determined. Data collected was analyzed using the split-plot ANOVA of SAS. On average, cleaned/sanitized surfaces had about one log lower ($p \leq 0.05$) TA and YM counts than the uncleaned surfaces, while no difference in TC counts was observed. The vertical and horizontal conveyors and fruit-catch plates had significantly higher TA, YM, and TC counts than other sampled sites. FC and EC were detected in 7.8% or 14.1% of the Georgia samples and 5.6% or 10.2% of the Oregon samples. The type and concentration of sanitizers and frequency and approach of cleaning/sanitation treatments all impacted the hygiene status of berry-contact surfaces of machine harvesters.

Keywords: fresh blueberry; machine harvester; food contact surface; food hygiene; food safety; pathogen indicators

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1. Introduction

Blueberries for the fresh market are primarily harvested by hand. The hand-picking of blueberries is performed by rubbing fruit clusters or gently rolling berries off the stems [1]. Picked berries are placed in plastic picking buckets in the shade without direct sunlight exposure, and they are then transferred to larger and more durable lugs/flats covered with a tarp at the edge of the field for transportation to a facility for packing [1]. Low harvest efficiency, farm worker shortage, and high labor costs are the major constraints in maintaining the sustainability of fresh blueberry market development [2]. To improve harvesting efficiency, more and more growers are transitioning to the use of over-the-row machines for harvesting fresh market blueberries.

However, fresh blueberries can become contaminated by pathogenic or spoilage microorganisms during harvest using machine harvesters. Microbial cells on the skin of the initial batch of harvested blueberries may transfer to the berry-contact surfaces of the machine harvesters. Inappropriate harvest and handling practices may cause the dissemination of the introduced microorganisms to other niches. Some of these microorganisms

can form microcolonies or biofilms, a protective structure that can assist them to persist under adverse environmental conditions in the berry fields. As a result, additional incoming berries may be cross-contaminated by the microbial cells colonized on berry-contact surfaces on the harvest equipment if they are not appropriately cleaned and sanitized, a situation that will increase the probability for fresh market blueberries to become a vehicle for transmitting foodborne diseases.

To prevent potential food safety risks related to cross-contamination during harvest and processing, in 2016, the U.S. Food and Drug Administration issued guidance for the produce industry known as the Produce Safety Rule under the Food Safety Modernization Act [3]. The legislation emphasizes the necessity of preventive cleaning and sanitization, as well as maintenance practices for produce-contact equipment and surfaces. Although various cleaning and sanitization protocols have been implemented in blueberry and other fresh produce production establishments [4], specific guidance on the frequency of cleaning, the selection of detergent and sanitizer, and the approaches of cleaning and sanitization remains an unfilled gap. Furthermore, routine cleaning and sanitization practices are labor-intensive and time-consuming, and growers may find them challenging during the busy harvest season [5]. Thus, it is essential to gather science-based information to help growers improve their cleaning and sanitization practices.

Numerous studies have been conducted focusing on the microbial safety of fresh blueberries and the hygiene status of fresh blueberry packing lines [6–8], whereas few studies have addressed the potential food hygiene/safety issues related to harvest equipment. Holland et al. [5] determined the levels of environmental and fecal indicator microorganisms on nine selected sites on over-the-row (OTR) blueberry machine harvesters in the 2015 and 2016 harvest seasons. Several "hot spots" with heavy microbial contaminations were identified. The study, however, did not link the hygiene status of OTR harvesters to the practices that blueberry growers used to clean and sanitize them. This study aimed to fill the knowledge gap and collect information that can guide fresh market blueberry production. The specific objectives of the study were to assess the hygiene conditions of blueberry-contact surfaces on OTR machine harvesters before and after they are cleaned and sanitized by comparing the levels of microbial contamination on the two types of surfaces and correlating the hygiene conditions of the surfaces to the cleaning and sanitation practices used by blueberry growers.

2. Materials and Methods

2.1. Sample Collection

The study included four blueberry OTR mechanical harvesters in Georgia and six harvesters in Oregon. Two top loaders (G1 and G2) and two bottom loaders (G3 and G4) from two different farms (F1 and F2) were sampled in Georgia, and six top loaders (O1, O2, O3, O4, O5, and O6) from two different farms (F3 and F4) were sampled in Oregon. Each harvester was sampled twice on two different harvesting days in the 2022 harvest season. An aliquot of 25 mL Dey-Engley (D/E) neutralizing broth (Becton Dickinson, Sparks, MD, USA) was added to a sterile Whirl-Pak® bag with a sponge (3.8 × 7.6 × 1.6 cm; Nelson Jameson Inc., Marshfield, WI, USA). Moistened sponges were used to swab the surface of nine selected sites (upper and lower side walls, upper and lower beating bars, catch plates, horizontal and vertical conveyors, lugs, and filling flap) on the cleaned and sanitized top loaders, as well as the used but not yet cleaned and sanitized top loaders, and seven sites (excluding the vertical conveyer and filling flap) on the cleaned and sanitized, as well as the used but not yet cleaned and sanitized bottom loaders (Figure 1). For sites with a flat surface, an area of 100 cm^2 was swabbed, and for those with non-flat or irregular surfaces, the precise sampled areas were measured and calculated. Each sampled site was swabbed

both horizontally and vertically, with 10 strokes in each direction using a similar force to remove dry blood from a surface. Collected swab samples were stored in a portable iceless cooler (Igloo, Katy, TX, USA) at 4 °C after collection and during transportation to the laboratory for analysis.

Figure 1. Graphic illustration of sampling sites of over-the-row harvesters. Note: the top loader has sites A to I, but the bottom loader only has sites A to G. A: upper side wall; B: lower side wall; C: upper beat bars; D: lower beat bars; E: catcher plate; F: horizontal conveyor belt; G: berry lug; H: vertical conveyor belt; I: filling flap in Georgia or upper conveyor in Oregon.

2.2. Microbial Enumeration

Each sponge in the Whirl-Pak® bag was hand-massaged for 1 min to release microbial cells into the D/E broth. An aliquot of 0.1 mL sponge rinsate was surface-plated on tryptic soy agar for total aerobes (TA) and MacConkey agar for total coliforms (TC), and the inoculated plates were incubated at 37 °C for 24 h. The total yeasts and molds (YM) were plated on acidified potato dextrose agar (pH 3.5) and incubated at 25 °C for 72 h. Colonies were enumerated after the incubation and cell populations were expressed as Log CFU/cm^2. Enterococcus agar was used for selecting presumptive enterococci (EC) at 37 °C and MacConkey agar for presumptive fecal coliforms (FC) at 44.5 °C, both for 24 h. Presumptive FC were confirmed using EC broth (bio-WORLD, Dublin, OH, USA) with a Durham tube (6 × 50 mm, Kimble Chase®, Vineland, NJ, USA) at 44.5 °C for 48 h and triple sugar slants at 37 °C for 24 h. EC was confirmed by examining their salt tolerance in brain heart infusion broth amended with 6.5% sodium chloride at 37 °C for 24 h. The presence of fecal coliforms and enterococci was expressed as the percentage of positive samples in the total number of samples analyzed. The microbiological media mentioned above were purchased from Becton, Dickinson, and Company if not specified.

2.3. Data Analysis

Microbial counts were converted into logarithmic values as log CFU/cm^2 for statistical analysis. Significant differences in the average counts of TA, YM, and TC across different farms, different harvesters, different sample sites, different hygiene conditions (cleaned and sanitized vs. used), and different visits were fit in a general linear model with a split-plot arrangement, including a random blocking factor "visit", fixed whole-plot factor "individual harvester" and two subplot fixed factors "sample site" and "hygiene condition of harvester". Fisher's least significance test and the Statistical Analysis Software (version 9.4; SAS, Institute, Cary, NC, USA) were used to separate the means ($p \leq 0.05$). The percentage of samples that tested positive for FC and EC in the total number of tested swab samples from different harvesters, different visits, and different sample sites was calculated.

3. Results

3.1. Farms and Visits

Average counts of TA and TC in samples collected from F1 in Georgia were significantly ($p \leq 0.05$) higher than those from F2, while no difference ($p > 0.05$) in the mean YM counts was observed between the samples collected from the two farms (Table 1). The mean TA counts in samples collected from F3 in Oregon were significantly higher than those from F4, but the mean YM and TC counts were not significantly different (Table 1). Georgia samples collected in the first visit had significantly higher TA and TC counts but not YM counts than in the samples collected from the second visit (Table 1), while the Oregon samples only had significantly higher TC counts in samples collected from the first than the second visit (Table 2).

Table 1. Mean populations of total aerobes, total yeasts and molds, and total coliforms from samples collected from various sites of different mechanical harvesters during two visits to individual fresh blueberry farms in Georgia.

		Total Aerobes	Total Yeasts and Molds	Total Coliforms
		Log CFU/cm^2		
Farm	One (n = 72)	1.87 ^{A}	2.22 ^{A}	0.56 ^{A}
	Two (n = 56)	1.46 ^{B}	2.10 ^{A}	0.21 ^{B}
Harvester	One (n = 36)	1.73 ^{AB}	1.99 ^{A}	0.58 ^{A}
	Two (n = 36)	2.01 ^{A}	2.22 ^{A}	0.53 ^{AB}
	Three (n = 28)	1.43 ^{B}	2.22 ^{A}	0.15 ^{C}
	Four (n = 28)	1.48 ^{B}	2.22 ^{A}	0.27 ^{BC}
Site	A (n = 16)	0.54 ^{F}	0.84 ^{D}	0.006 ^{D}
	B (n = 16)	1.00 ^{EF}	1.07 ^{CD}	0.11 ^{D}
	C (n = 16)	0.92 ^{EF}	1.39 ^{CD}	0.01 ^{D}
	D (n = 16)	1.53 ^{DE}	1.65 ^{C}	0.23 ^{CD}
	E (n = 16)	2.46 ^{BC}	2.89 ^{B}	0.65 ^{BC}
	F (n = 16)	2.85 ^{AB}	3.64 ^{A}	1.06 ^{B}
	G (n = 16)	1.84 ^{CD}	2.51 ^{B}	0.19 ^{CD}
	H (n = 8)	3.36 ^{A}	3.94 ^{A}	1.66 ^{A}
	I (n = 8)	1.38 ^{DE}	2.52 ^{B}	0.28 ^{CD}
Hygiene	Cleaned (n = 64)	1.28 ^{B}	1.64 ^{B}	0.48 ^{A}
	Used (n = 64)	2.09 ^{A}	2.67 ^{A}	0.33 ^{A}
Visit	One (n = 64)	1.88 ^{A}	2.20 ^{A}	0.61 ^{A}
	Two (n = 64)	1.50 ^{B}	2.10 ^{A}	0.20 ^{B}

Different letters within the same independent variable in the same column indicate statistical differences at a 95% confidence interval.

Table 2. Mean populations of total aerobes, total yeasts and mold, and total coliforms from samples collected from various sites of different mechanical harvesters during two visits to individual fresh blueberry farms in Oregon.

		Total Aerobes	Total Yeasts and Molds	Total Coliforms
		Log CFU/cm^2		
Farm	One (n = 108)	1.23 ^{A}	1.55 ^{A}	0.11 ^{A}
	Two (n = 108)	1.07 ^{B}	1.54 ^{A}	0.13 ^{A}
Harvester	One (n = 36)	1.11 ^{BC}	1.59 ^{B}	0.12 ^{AB}
	Two (n = 36)	1.05 ^{BC}	1.43 ^{BC}	0.002 ^{B}
	Three (n = 36)	1.54 ^{A}	1.65 ^{AB}	0.21 ^{A}
	Four (n = 36)	0.98 ^{BC}	1.48 ^{BC}	0.24 ^{A}
	Five (n = 36)	0.96 ^{C}	1.22 ^{C}	0.005 ^{B}
	Six (n = 36)	1.26 ^{AB}	1.94 ^{A}	0.13 ^{AB}
Site	A (n = 24)	0.54 ^{DE}	0.76 ^{E}	0.08 ^{B}
	B (n = 24)	0.78 ^{CD}	1.14 ^{CD}	0.02 ^{B}
	C (n = 24)	0.39 ^{E}	1.03 ^{CDE}	0.01 ^{B}
	D (n = 24)	0.96 ^{C}	1.02 ^{DE}	0.08 ^{B}
	E (n = 24)	1.58 ^{B}	2.07 ^{AB}	0.10 ^{B}
	F (n = 24)	2.02 ^{A}	2.40 ^{A}	0.43 ^{A}
	G (n = 24)	1.11 ^{C}	1.83 ^{B}	0.17 ^{B}
	H (n = 24)	1.93 ^{A}	2.35 ^{A}	0.13 ^{B}
	I (n = 24)	1.01 ^{C}	1.38 ^{C}	0.06 ^{B}
Hygiene	Cleaned (n = 108)	0.63 ^{B}	0.86 ^{B}	0.10 ^{A}
	Used (n = 108)	1.67 ^{A}	2.25 ^{A}	0.14 ^{A}
Visit	One (n = 108)	1.09 ^{A}	1.54 ^{A}	0.12 ^{A}
	Two (n = 108)	1.20 ^{A}	1.56 ^{A}	0.11 ^{B}

Different letters within the same independent variable in the same column indicate statistical differences at a 95% confidence interval.

3.2. Harvesters

On average, samples from G2 had a significantly ($p \leq 0.05$) higher mean TA count than those from G3 and G4, but a similar ($p > 0.05$) TA count to samples from G1 (Table 1). The mean TA counts in samples collected from G1, G3, and G4 were not significantly different, and neither were the mean YM counts in samples collected from all four harvesters in Georgia. However, the mean TC counts in samples collected from G1 and G2 were significantly higher than the TC count from G3.

Samples from O3 had a significantly ($p \leq 0.05$) higher mean TA count than the same counts from O1, O2, O4, and O5, and those from O6 had significantly lower mean TC counts than samples from O5 (Table 2). Moreover, the YM counts in samples from O6 were significantly higher than those in samples from the other facilities except for O3. Samples from O5 had the lowest YM count, which was significantly different from the same counts in samples collected from O1, O3, and O6. The mean TC counts from O3 and O4 were similar ($p > 0.05$), both of which were significantly higher than the same counts in samples collected from the other harvesters, except for O1. Used but not yet cleaned and sanitized surfaces on the harvesters in Georgia and Oregon had significantly higher levels of mean TA and YM counts but not TC counts than the cleaned and sanitized surfaces (Tables 1 and 2).

3.3. Sample Sites

Sites H (vertical conveyer), F (horizontal conveyer), and E (catch plates) on harvesters sampled in Georgia were more heavily contaminated with the three hygiene indicator microorganisms (Table 1). The three sites had relatively higher mean TA counts, which were significantly higher ($p \leq 0.05$) than those in samples collected from other sites, except for site G. The mean YM counts from sites H and F were similar ($p > 0.05$), and so were the YM counts from sites E, G, and I, but the mean YM counts from sites H and F were significantly higher than those from the other three sites. Site H not only had the highest

TA and YM counts, but it also carried the highest TC counts. The TC count on this site was followed by sites F and E.

Similarly to the observations made in Georgia samples, sites F, H, and E on the harvesters sampled in Oregon were more heavily contaminated (Table 2). Site H and F both had the highest TA and YM counts, followed by site E. The mean TC counts on site F were significantly ($p \leq 0.05$) higher than those on the other sampled sites.

3.4. Hygiene Status

The average TA counts in samples collected from used vs. cleaned sample sites on the mechanical harvesters in Georgia were not significant ($p > 0.05$) differences, except for samples collected from sites E, F, and I (Table 3). Before cleaning, sites E, F, and H had the highest TA counts, which were insignificantly different from the TA counts from site G, and sites E, F, G, H, and I had the highest YM counts. Only samples from site H had the highest mean TA and YM counts after cleaning. However, the mean YM count in post-cleaning samples collected from site F was not significantly different from that found in samples collected from site H.

Table 3. Mean populations of total aerobes and total yeasts and molds in swab samples collected from different sites of blueberry mechanical harvesters in Georgia.

	Site A	Site B	Site C	Site D	Site E	Site F	Site G	Site H	Site I
					Log CFU/cm^2				
TA									
Cleaned	0.46 ^{dA}	0.53 ^{dA}	0.88 ^{cdA}	1.57 ^{bcA}	1.63 ^{bcB}	2.13 ^{bB}	1.00 ^{cdA}	3.50 ^{aA}	0.64 ^{cdB}
Used	0.61 ^{dA}	1.47 ^{cdA}	0.96 ^{dA}	1.50 ^{cdA}	3.29 ^{aA}	3.57 ^{aA}	2.68 ^{abA}	3.22 ^{aA}	2.12 ^{bcA}
YM									
Cleaned	0.38 ^{eY}	0.38 ^{eY}	0.99 ^{deX}	1.75 ^{cdX}	2.19 ^{bcY}	3.16 ^{abX}	1.60 ^{cdX}	3.82 ^{aX}	1.54 ^{cdY}
Used	1.30 ^{bX}	1.76 ^{bX}	1.79 ^{bX}	1.56 ^{bX}	3.58 ^{aX}	4.12 ^{aX}	3.43 ^{aX}	4.06 ^{aX}	3.51 ^{aX}

Different lowercase letters indicate significant differences in TA and YM counts in samples collected from different sample sites, and different uppercase letters show significant differences in TA or YM counts collected from samples with different hygiene statuses.

Different from the Georgia samples, most sampled sites from cleaned surfaces of machine harvesters in Oregon had significantly ($p \leq 0.05$) lower mean TA counts than the used surfaces, except for sites G and I (Table 4). The before-cleaning samples collected from all nine sites of the harvesters in Oregon had significantly higher mean YM counts than the after-cleaning samples. The before-cleaning samples from site H (insignificantly different from sites E and F) and after-cleaning samples from site F (insignificantly different from site H) had the highest mean TA counts. The before-cleaning samples from sites E (insignificantly different from sites F, H, and I) and the after-cleaning samples from sites F and H (insignificantly different from sites G) on the harvesters sampled in Oregon had the highest YM counts.

Table 4. Mean populations of total aerobes in swab samples collected from different sites of blueberry mechanical harvesters in Oregon.

	Site A	Site B	Site C	Site D	Site E	Site F	Site G	Site H	Site I
					Log CFU/cm^2				
TA									
Cleaned	0.03 ^{eB}	0.39 ^{cdeB}	0.00 ^{eB}	0.61 ^{cdB}	0.83 ^{bcB}	1.64 ^{aB}	0.67 ^{cdA}	1.27 ^{abB}	0.19 ^{deA}
Used	1.04 ^{deA}	1.18 ^{deA}	0.78 ^{eA}	1.32 ^{cdeA}	2.33 ^{abA}	2.40 ^{abA}	1.55 ^{cdA}	2.59 ^{aA}	1.84 ^{bcA}
YM									
Cleaned	0.07 ^{dY}	0.42 ^{dY}	0.62 ^{bcdY}	0.58 ^{cdY}	1.08 ^{bcY}	1.76 ^{aY}	1.21 ^{abY}	1.79 ^{aY}	0.17 ^{dY}
Used	1.44 ^{dX}	1.85 ^{cdX}	1.43 ^{dX}	1.47 ^{dX}	3.05 ^{aX}	3.05 ^{aX}	2.44 ^{bcX}	2.91 ^{abX}	2.58 ^{abA}

Different lowercase letters indicate significant differences in TA and YM counts in samples collected from different sites, and different uppercase letters show significant differences in TA or YM counts from samples with different hygiene statuses.

3.5. Presence of Total Coliforms, Fecal Coliforms, and Enterococci

Among the 128 samples collected in Georgia, 40.3% and 25.0% tested positive for TC in F1 and F2 (Table 5), respectively. The incidence of TC-positive samples was 39.1% for used surfaces and 28.1% for cleaned surfaces. Sites H and F had a relatively higher prevalence of TC, 87.5% and 81.3%, respectively.

Table 5. Number and percentage of samples positive for total coliforms, fecal coliforms, and enterococci in Georgia samples.

	Total Coliforms			Fecal Coliforms			Enterococci		
	Number of Positive	Sample No.	%Positive	Number of Positive	Sample No.	%Positive	Number of Positive	Sample No.	%Positive
Farm 1	29	72	40.3	6	72	8.3	10	72	13.9
Farm 2	14	56	25.0	4	56	7.1	8	56	14.3
Total	43	128	33.6	10	128	7.8	18	128	14.1
Used	25	64	39.1	1	64	1.6	14	64	21.9
Cleaned	18	64	28.1	9	64	14.1	4	64	6.3
Total	43	128	33.6	10	128	7.8	18	128	14.1
Site A	1	16	6.3	0	16	0.0	0	16	0.0
Site B	1	16	6.3	0	16	0.0	0	16	0.0
Site C	2	16	12.5	1	16	6.3	0	16	0.0
Site D	3	16	18.8	0	16	0.0	0	16	0.0
Site E	7	16	43.8	2	16	12.5	4	16	25.0
Site F	13	16	81.3	3	16	18.8	10	16	62.5
Site G	4	16	25.0	1	16	6.3	2	16	12.5
Site H	7	8	87.5	3	8	37.5	2	8	25.0
Site I	5	8	62.5	0	8	0.0	0	8	0.0
Total	43	128	33.6	10	128	7.8	18	128	14.1
Visit 1	27	64	42.2	2	64	3.1	2	64	3.1
Visit 2	16	64	25.0	8	64	12.5	16	64	25.0
Total	43	128	33.6	10	128	7.8	18	128	14.1

About 1.6% or 14.1% of used and cleaned surfaces from Georgia tested positive for FC (Table 6), suggesting a possible post-sanitation contamination. Site H in Georgia harvesters had the highest percentage (37.5%) of FC recovery, while no FC was detected from sites A, B, C, D, and I in Georgia harvesters. Moreover, more samples collected from visit 2 (12.5%) in Georgia tested positive for TC than those from visit 1 (3.1%), although samples from visit 2 had relatively lower counts of TA, YM, and TC compared to visit 1. About 21.9% of used surfaces and 6.3% of cleaned surfaces were EC-positive. Site F had the highest percentage of EC recovery.

Table 6. Number and percentage of positive samples for total coliforms, fecal coliforms, and enterococci in Oregon samples.

	Total Coliforms			Fecal Coliforms			Enterococci		
	Number of Positive	Sample No.	%Positive	Number of Positive	Sample No.	%Positive	Number of Positive	Sample No.	%Positive
Farm 3	17	108	15.7	9	108	8.3	15	108	13.9
Farm 4	17	108	15.7	3	108	2.8	7	108	6.5
Total	34	216	15.7	12	216	5.6	22	216	10.2
Used	23	108	21.3	7	108	6.5	20	108	18.5
Cleaned	11	108	10.2	5	108	4.6	2	108	1.9
Total	34	216	15.7	12	216	5.6	22	216	10.2
Site A	1	24	4.2	1	24	4.2	1	24	4.2
Site B	1	24	4.2	0	24	0.0	3	24	12.5
Site C	0	24	0.0	0	24	0.0	1	24	4.2
Site D	4	24	16.7	0	24	0.0	1	24	4.2
Site E	5	24	20.8	0	24	0.0	4	24	16.7
Site F	11	24	45.8	6	24	25.0	2	24	8.3
Site G	4	24	16.7	0	24	0.0	2	24	8.3
Site H	5	24	20.8	3	24	12.5	0	24	0.0
Site I	3	24	12.5	2	24	8.3	8	24	33.3
Total	34	216	15.7	12	216	5.6	22	216	10.2
Visit 1	17	108	15.7	6	108	5.6	10	108	9.3
Visit 2	17	108	15.7	6	108	5.6	12	108	11.1
Total	34	216	15.7	12	216	5.6	22	216	10.2

The incidence of TC-positive samples in F3 and F4 of Oregon was both 15.7% (Table 6). About 21.3% of used surfaces and 10.2% of cleaned surfaces tested positive for TC in Oregon. Site F had the highest incidence of TC recovery, while no TC was detected on site C.

Among the 216 samples collected in Oregon, the incidences of FC and EC occurrence were 5.6% and 10.2% (Table 6), respectively. The used surfaces in Oregon were more frequently contaminated with EC (18.5%) compared to the cleaned surfaces (1.9%), and the incidence of FC occurrence from used and cleaned surfaces was 6.5% and 4.6%, respectively. Only four sites on the harvesters sampled in Oregon tested positive for FC, and site F had the highest percentage (25.0%) of FC recovery. Site I more frequently tested positive for EC, with an incidence of 33.3%, but no EC was detected on site H.

4. Discussion

Direct detection of viable pathogen cells on fresh produce contact surfaces can be challenging [9]. Presently, there is no standard protocol for the detection of pathogenic microorganisms on food contact surfaces. Thus, like several previous works [5,8,10,11], hygiene and pathogen indicator microorganisms were used in the current study to assess the hygiene status of berry-contact surfaces and the likelihood of occurrence of foodborne pathogens on the surface of blueberry machine harvesters.

It is commonly accepted that the count of viable TA on freshly cleaned and sanitized surfaces should not exceed 2 Log CFU/cm^2 [2,11]. In the current study, the mean TA count on cleaned and sanitized surfaces of machine harvesters from the two blueberry farms in Georgia was 1.28 Log CFU/cm^2 (Table 1), whereas that from the farms in Oregon was 0.63 Log CFU/cm^2 (Table 2), which were both below the 2 Log CFU/cm^2 benchmark. Furthermore, the results in Tables 4 and 5 revealed that the participated farms did reasonably good jobs at cleaning and sanitizing the surface of machine harvesters, as evidenced by the lower ($p \leq 0.05$) mean TA populations at some cleaned/sanitized sites compared to the populations at used but not cleaned/sanitized sites. However, this does not suggest that better efforts in cleaning and sanitation are unnecessary because according to the results in Tables 4 and 5, the microbial loads on cleaned/sanitized surfaces were not always lower than those on the used but not yet cleaned/sanitized surfaces.

Holland et al. [5] investigated the microbial loads at the same nine sites of OTR top loaders as we sampled in the current study three different times a day during seven harvest days in the 2015 and 2016 seasons. Like the findings of the current study, vertical and horizontal conveyors, as well as catch plates, were found to have higher TA counts than the other sampled sites. However, the mean TA count on used but not yet cleaned/sanitized horizontal and vertical conveyors was above 5 Log CFU/cm^2, much higher than the findings of the current study. This difference may be partially ascribed to the harvest practices used by the participating growers. Furthermore, the observations could be attributed to the climate differences between the two sampling periods. Although the atmosphere temperature difference between the two sampling periods was minor (less than 1 °F), the amount of precipitation in the 2022 sampling period (0.28 cm) was relatively lower than that in 2015 and 2016 (0.56 or 0.74 cm) [12], which may influence the relative humidity of the atmosphere, and potentially the microbial levels on the surface of machine harvesters. According to the USDA National Agricultural Statistics Service [13], the average yield of blueberries in GA in 2022 was 74 million pounds, while the average yield was 77 million pounds in 2015 and 2016, although the precise amounts of fresh market blueberries produced by the participating farms in the two sampling periods are unknown. The greater yields of fresh blueberries may also affect the level of microorganisms on ma-

chine harvesters, since higher yields may denote longer operation hours and, subsequently, higher levels of microbial accumulation on harvester surfaces.

All participating farms in the current study have cleaning and sanitization protocols in place (Appendix A; Renee Holland and Sarah Doane, personal communications). Harvesters G1 and G2 of F1 in Georgia were parked in a shelter away from the field while being cleaned and sanitized. Large debris on the harvesters, like leaves, branches, and blueberries, were removed by hand. A dry cloth was used to wipe off other debris and soil. The harvesters were sanitized with a 1.5% chlorine solution, followed by rinsing with fresh well water from a water pump. Different from F1, personnel of F2 used a blower to remove visible dirt, and the remaining debris was removed using a wet cloth soaked with dish soap. A Steramine solution containing 100 ppm of quaternary ammonia compounds (QACs) was sprayed over the surface of G3 and G4. The solution was left on the harvest surface for 1 min without rinsing. Unlike G1 and G2 which were cleaned and sanitized daily, G3 and G4 were cleaned and sanitized every two or three days, but more "thorough" cleaning and sanitation (not specified) was conducted weekly.

Although the two harvesters from F1 were more frequently cleaned and sanitized than those from F2, the mean TA and TC counts in samples collected from F1 were higher ($p \leq 0.05$) than those from F2 (Table 1). The removal of debris by hand used by F1 may not be as effective a cleaning approach as the use of a blower by F2. Furthermore, the cleaning approach of F2 using a wet cloth soaked with dish soap before sanitation may have further enhanced the efficacy of the cleaning. Most importantly, the 100 ppm of QACs used by F2 might be relatively more potent than the 1.5% chlorine used by F1 for sanitizing the surfaces of machine harvesters.

Chlorine and its derivatives exert their antimicrobial activity by oxidizing biological molecules such as proteins and enzymes, thereby killing bacterial cells [14]. However, chlorine can react with organic compounds to form products like trihalomethanes, chlorate, and haloacetic acid with reduced antimicrobial effects, and it can also be destabilized by high temperatures and changes in pH [15,16]. It is important to maintain the pH of a chlorine solution at 7.2 to 7.8, as it can affect the efficacy of the sanitizer. This could have affected the results from F1. The active component of Steramine® 1-G is, nevertheless, alkyl dimethyl benzyl ammonium chloride dihydrate, a QAC [17]. QACs are commonly used disinfectants that act as cationic surfactants and lead to the lysis of bacterial cells by disrupting cell membrane interactions and enzyme activities [18]. Some previous studies found that QACs had greater bactericidal effects than chlorine. Olszewska et al. [19] evaluated the bactericidal effects of different concentrations of chlorine-based (0.018% and 0.18%) and QACs-based (0.2% and 2.0%) disinfectants on four strains of *Lactobacillus*. It was found that treatment with QACs reduced the counts of lactobacilli by 3.9 to 5.8 logs, while the treatment with chlorine only reduced the *Lactobacillus* counts by 0.3 to 1.5 logs. A previous study by Gazula et al. [4] compared the efficacy of chlorine (200 ppm), QACs (200 ppm), and two other sanitizers in removing the biofilms accumulated on six different types of materials that are commonly used in blueberry packing environments. The QAC had significantly ($p \leq 0.05$) higher efficacy in biofilm removal than the chlorine solution [4]. However, a different finding was reported by Lineback [20], who compared the antimicrobial effectiveness of chlorine with QACs against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm developed on borosilicate glass coupons. The treatment with chlorine reduced *Staphylococcus* and *Pseudomonas* counts by 8.73 and 8.51 log CFU/coupon, respectively. Treatment with QACs, nevertheless, only led to a 4.37 log reduction for *S. aureus* and a 0.82 log reduction for *P. aeruginosa*. The difference in results obtained by these studies may be attributed to different environmental conditions, such as pH, temperature, and water hardness [15]. These studies targeted different bacterial species, so it is not surprising when species or strain variation to sanitizer treatment was observed.

Both farms in Oregon had daily cleaning and sanitization routines. The two farms removed large debris before sanitization by blowers and hands. In F3, harvesters O1, O2, and O3 were parked on clean concrete ground during cleaning and sanitation. F3 used cold pressure water to rinse the harvesters first and then sprayed a 200 ppm chlorine solution over berry-contact surfaces. Harvesters O4, O5, and O6 in F4 were parked on a wash pad for cleaning and sanitation, and they were tilted after removing large objects before washing. The berry-contact surfaces on the harvesters in F4 were scrubbed with brushes after rinsing with high-pressured water, and the conveyors were then sanitized, while running, with a Persan® A solution (Enviro Tech Chemical Services, Inc., Helena, AR, USA) containing 492 ppm of active peracetic acid for about 5 min. The surfaces were then rinsed with pressure water while the machine kept running.

The average TA and TC counts in samples collected from F3 were significantly higher ($p \leq 0.05$) than those from F4 (Table 2), suggesting that the cleaning and sanitization protocol of F4 worked better than that of F3. F4 kept the machine harvester running while sanitizing and rinsing, allowing for sufficient contact between the sanitizer and microorganisms on harvest surfaces. The different sanitizers used by the two farms may also account for the difference in microbial counts on berry-contact surfaces. The active ingredients of Perasan® A are peracetic acid and hydrogen peroxide [21]. Peracetic acid has potent microbicide properties and antimicrobial capacity because it can form hydroperoxide in solutions, inactivating microorganisms on berry-contact surfaces [22]. A previous study by Krishnan et al. [23] compared the antimicrobial effects of chlorine and peracetic acid against *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* on surfaces and in groundwater. It was found that bacteria treated with 3 ppm or 5 ppm peracetic acid had lower D-values than those treated with chlorine at the same concentration, although the D-values of three strains were similar when peracetic acid and chlorine increased to 7 ppm. Furthermore, the brush-scrubbing of berry-contact surfaces used by F4 might have helped remove the microbial buildups on surfaces, which enhanced the efficacy of the cleaning, and subsequently the effectiveness of the sanitizer treatment [24].

The average TA counts of site E were 3.29 Log CFU/cm^2 before sanitation (Table 3), which were significantly higher than those from the same site after sanitation (TA of 1.63 Log CFU/cm^2). Site E comprises berry catch plates made of extruded polycarbonate [25] located at the lower portion of the harvester in a horizontal orientation. Polycarbonate is a thermoplastic material, and it has been widely used in industry due to its high resistance against impact, dimensional stability, and good transparency [26]. It had a relatively hydrophobic and rough surface compared to materials like stainless steel. Bacterial adhesion typically elevates with the increase in surface hydrophobicity [27]. It may also be affected by surface roughness [28]. The higher average bacterial count on site E before cleaning and sanitation may be ascribed to its hydrophobicity and increased surface roughness over long-term use. Furthermore, individual catch plates in the area overlap with each other, making cleaning and sanitation a great challenge. Thus, debris may easily accumulate on this site, leading to a higher level of microbial contamination.

The mean TA counts of site H were 3.22 Log CFU/cm^2 before sanitation and the count on the same site after sanitation was 3.50 Log CFU/cm^2 (Table 4). This result suggests that site H may be an area that is either inconvenient or more difficult to clean and sanitize. Site H is a vertical conveyor made of rubber textile, an elevator-bucket-like roll transferring berries to the platform at the top of the OTR harvester [29]. Thorough cleaning of this part requires disassembling the conveyor belt from the harvester. This is perhaps unlikely to be accomplished during the busy harvest season. Regardless of the reasons, more attention and better effort should be paid to maintain the hygiene status of this site.

The horizontal conveyor (site F) of machine harvesters sampled in Georgia was made of stainless steel [25], which is a better material compared to certain plastics in terms of microbial buildup and biofilm formation [30]. Nevertheless, extended long-term use without proper maintenance may make it rough and worn, a characteristic that may increase the possibility of microbial accumulation. A previous study by Frank et al. [31] compared the sanitary efficacies of QACs and chlorine on three polished and abraded surface materials, i.e., stainless steel, polycarbonate, and mineral resin. Chlorine was more effective in sanitizing mechanically polished stainless steel, which reduced cell populations to less than 1.0 log CFU/cm^2 but was less effective in sanitizing abraded electropolished stainless steel with a cell population greater than 1.0 log CFU/cm^2 after the treatment.

The average TA and YM counts on sites F, H, and E of the harvesters in Oregon were relatively higher before sanitation than those at the same sites after sanitation (Table 4). However, in comparison, the Oregon samples had relatively lower microbial counts than the Georgia samples (Table 4). The two states have different climates and grow different cultivars of blueberries. The participating facilities have different sanitation and management practices. All these factors may have contributed to the degree of organic accumulation on berry-contact surfaces of machine harvesters.

5. Conclusions

This study filled a knowledge gap and gathered information that is necessary for producing fresh market blueberries with assured microbial safety. Specifically, it revealed that vertical and horizontal conveyors, as well as fruit-catch plates, were the most heavily contaminated sites on OTR machine harvesters. The routine cleaning and sanitization practices utilized by berry growers significantly lowered the microbial levels on some berry-contact surfaces; however, certain recommendations might be necessary to improve their hygiene status. For instance, (i) remove residues and large debris and wipe berry-contact surfaces with a damp cloth to ensure sanitation efficacy; (ii) clean and sanitize fruit-catch plates thoroughly, especially the overlapping areas; (iii) increase the frequency of detaching the horizontal and vertical conveyors for thorough cleaning and sanitation during harvest season; (iv) replace the defective parts of the machine that may come into contact with berries to prevent microbial accumulation; and (v) use proper cleaners or sanitizers at appropriate concentrations for routine cleaning and sanitization.

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Appendix A

Table 1. Sanitization information of participated farms in Georgia and Oregon.

	Farm	Frequency	Location of Cleaning Area	Sanitizer	Cleaning/Sanitization Procedures	Water for Sanitization	Way of Drying	Type of Harvesters
Georgia	F1	Daily	Shelter, away from the field	1.5% chlorine	a. Removal of leaves, twigs, or branches by hand b. Wiping off residue with paper towels c. Spray chlorine solution onto the surfaces d. Rinsing with pressure water	Well water	Air dry	Top loaders
	F2	Every 2 to 3 days or thorough cleaning once a week	In the field	Steramine (QACs)/Dawn dish soap	a. Blower to remove leaves/branches b. Cloth soaked with dish soap to wipe off residue c. Rinsing with water d. Steramine solution sprayed on the surfaces and left for 1 min	Portable well water	Air dry	Bottom loaders
Oregon	F3	Daily	Designated place away from field	200 ppm chlorine	a. Wash surfaces with cold pressure water b. Spray chlorine solution on surfaces	Portable well water	Air dry	Top loaders
	F4	Daily	Designated place away from field	Perasan A @ 1 floz/gal (peracetic acid and hydrogen peroxide)	a. Removal of debris by hand or blower b. Rinsing with water c. Scrubbed surfaces with brushes d. Cover the surfaces with sanitizing foam with machine running for about 5 min e. Rinsing with cold pressure water	Well water	Air dry	Top loaders

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