Alqalam Journal of Medical and Applied Sciences. 2026;9(7):1932-1945
https://doi.org/10.54361/ajmas.269714

Original article

Biofilm Formation and Phenotypic Disinfectant Tolerance of Foodborne Bacteria on Food-Contact Surfaces in Processing Environments

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Abstract
Food-contact surfaces can act as reservoirs for persistent foodborne bacteria, particularly when biofilms reduce the effectiveness of routine sanitation. This cross-sectional study investigated bacterial contamination, biofilm formation, and phenotypic disinfectant tolerance among isolates recovered from food-processing environments in Tripoli, Libya. A total of 240 food-contact surface samples were collected from meat, dairy, vegetable, and ready-to-eat food facilities. Overall, 151 samples (62.9%) were culture-positive, with the highest positivity observed in meat-processing facilities (74.3%) and raw-material handling areas (75.0%). A total of 166 bacterial isolates were recovered; *Listeria monocytogenes* was the most frequent species (19.3%), followed by *Staphylococcus aureus* (18.1%) and *Salmonella enterica* (16.9%). Strong biofilm formation was detected in 81 isolates (48.8%), particularly among *Pseudomonas aeruginosa* (68.0%) and *L. monocytogenes* (65.6%). Biofilm-associated isolates showed the highest tolerance to QAC-based disinfectants (41.0%), followed by sodium hypochlorite (20.5%), hydrogen peroxide (17.5%), and peracetic acid (10.8%). Strong biofilm formation independently predicted tolerance to at least one disinfectant (aOR = 4.87), whereas cleaning surfaces at least three times daily reduced this likelihood (aOR = 0.42). Enhanced residue removal, biofilm-focused sanitation, and targeted monitoring of high-risk surfaces are essential to reduce persistent contamination and improve food safety.
Keywords. Biofilm formation, Food-contact surfaces, Disinfectant tolerance, Quaternary ammonium compounds, Food-processing environments.

Introduction

Food safety depends not only on the microbiological quality of raw materials but also on the effectiveness of environmental hygiene throughout food processing. Contaminated food-contact surfaces can serve as immediate vehicles for the transfer of microorganisms to products during cutting, conveying, packaging, and storage. Consequently, cleaning and disinfection are recognized as essential prerequisites programmed within modern food hygiene systems, particularly in facilities handling raw meat, dairy products, fresh produce, and ready-to-eat foods [1,2]. Food-processing environments are dynamic microbial ecosystems rather than sterile spaces. Environmental microorganisms may be repeatedly introduced through raw materials, water, workers, equipment, air, and processing residues, allowing selected populations to establish persistent niches on food-contact surfaces. A recent meta-analysis identified a core surface-associated microbiome across diverse food-processing facilities, including genera such as *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Serratia*, and *Stenotrophomonas*. At the same time, commodity-specific microbial communities were also evident in meat, dairy, seafood, produce, and ready-to-eat food environments [3]. Under favorable conditions, bacteria can attach to abiotic surfaces and develop biofilms, defined as structured microbial communities embedded within a self-produced extracellular polymeric substance matrix [4,5].

Biofilm formation is a major challenge because the extracellular matrix can protect embedded cells from drying, nutrient limitation, mechanical cleaning, and chemical disinfection. The likelihood of attachment and biofilm development is influenced by microbial species, surface composition, hydrophobicity, roughness, defects, moisture, temperature, nutrient availability, and the accumulation of organic residues [4–7]. Food-contact surfaces manufactured from stainless steel, plastics, rubber, polyethylene, and other polymers may differ substantially in their ability to retain food debris and support microbial attachment. Surface wear, cracks, scratches, and inaccessible equipment components can create protected microenvironments in which cells are less exposed to cleaning agents and subsequently act as reservoirs for recurrent contamination [6,7].

Several foodborne and food-associated bacteria are particularly relevant to biofilm-associated persistence in food-processing environments. *Listeria monocytogenes* remains a major concern because of its ability to survive under refrigeration, persist on processing equipment, and contaminate ready-to-eat foods. *Salmonella enterica*, pathogenic *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* are also important foodborne hazards capable of adhering to surfaces and forming biofilms under processing-related conditions [4,5]. In addition, biofilm-competent environmental organisms such as *Pseudomonas aeruginosa* may contribute to product spoilage, surface colonization, and the development of mixed microbial communities. Recent work from meat-processing environments has confirmed substantial variation in biofilm formation among *L. monocytogenes* isolates and highlighted the limitations of conventional sanitation when sessile cells are established [8].

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Received*: 02-05-2026 -* ***Accepted****: 01-07-2026 -* ***Published****: 08-07-2026*

https://doi.org/10.54361/ajmas.269714

Routine sanitation typically combines physical removal of soil with chemical disinfection. Quaternary ammonium compounds (QACs), sodium hypochlorite, hydrogen peroxide, and peracetic acid are widely used because of their practical availability and broad antimicrobial activity. However, sanitizer performance is affected by concentration, contact time, temperature, pH, organic load, surface material, and the physiological state of microorganisms [2,9]. Studies evaluating food-contact surfaces have shown that disinfectant efficacy may differ markedly between stainless steel, plastic, rubber, polyethylene, and other materials. Organic residues and surface defects can further reduce sanitizer effectiveness by limiting contact between the active compound and biofilm-associated cells [6,10]. An important distinction should be made between disinfectant resistance and phenotypic disinfectant tolerance. In the present context, phenotypic tolerance refers to reduced killing or survival after standardized disinfectant exposure and does not necessarily indicate stable, genetically encoded resistance. Biofilm-associated tolerance may result from restricted disinfectant penetration through the matrix, reduced metabolic activity in deeper biofilm layers, altered membrane properties, stress-response pathways, efflux mechanisms, and the presence of persister-like subpopulations [5,11].

Repeated exposure to sublethal concentrations of QACs, particularly benzalkonium chloride, has also been associated with adaptive responses that enhance biofilm formation and survival in *L. monocytogenes* [12]. The complexity of microbial persistence becomes greater in mixed-species biofilms. Food-processing surfaces commonly harbor multiple bacterial species, and interactions among these organisms may increase biomass, alter extracellular matrix composition, and modify susceptibility to disinfectants. Experimental studies have shown that mixed environmental biofilms can increase the tolerance of *L. monocytogenes* to benzalkonium chloride and peracetic acid, particularly when biofilms contain members of the family *Pseudomonadaceae* [11,13]. These findings indicate that the efficacy of a disinfectant cannot be reliably inferred from tests performed only on planktonic cells or single-species laboratory biofilms. Despite increasing recognition of biofilm-associated contamination, many studies remain limited to one pathogen, one surface material, or a single disinfectant.

Integrated field-based investigations that simultaneously assess bacterial contamination levels, surface material, hygiene practices, organic residues, biofilm-forming capacity, and phenotypic tolerance to multiple disinfectants are still needed. Such evidence is particularly valuable for identifying practical risk factors that can be targeted through improved sanitation programs, equipment design, cleaning frequency, and disinfectant rotation strategies [14,15]. Therefore, this study aimed to investigate biofilm formation and phenotypic disinfectant tolerance among foodborne and food-associated bacteria recovered from food-contact surfaces in food-processing environments in Tripoli, Libya. Specifically, the study evaluated the distribution and burden of bacterial contamination across different facility types, processing areas, surface materials, and hygiene practices; characterized the biofilm-forming capacity of recovered isolates; assessed tolerance to QAC-based disinfectants, sodium hypochlorite, hydrogen peroxide, and peracetic acid; and examined factors associated with disinfectant tolerance. The findings are expected to provide evidence for strengthening sanitation strategies and reducing the risk of persistent bacterial contamination in local food-processing facilities.

Methods

Study design, setting, and laboratory location

A cross-sectional, laboratory-based environmental study was conducted from February to August 2025 in food-processing environments in Tripoli, Libya. The study investigated bacterial contamination of food-contact surfaces, the biofilm-forming capacity of recovered foodborne and food-associated bacterial isolates, and their phenotypic tolerance to selected disinfectants used in food-processing settings. Sampling was performed in meat-processing, dairy-processing, vegetable-processing, and ready-to-eat food facilities. All laboratory procedures, including sample processing, bacterial enumeration, phenotypic identification, biofilm assessment, disinfectant-challenge testing, and quality-control procedures, were conducted in the Department of Microbiology Laboratories, Libyan Center for Biotechnology, Tripoli, Libya. Participating facilities were assigned anonymous codes, and no facility names, product brands, staff identifiers, or commercially sensitive operational details were included in the study database or reported results. The study involved environmental surface samples only; no human participants, patient specimens, animal specimens, or identifiable personal data were collected.

Sample size and sampling strategy

A total of 240 food-contact surface samples were included. The sample size was selected to estimate an anticipated culture-positivity proportion of approximately 65%, with a 95% confidence level and a precision of approximately 6.5 percentage points, while allowing for clustering of samples within facilities. Samples were distributed across facility types as follows: meat-processing facilities (n = 70), dairy-processing facilities (n = 55), vegetable-processing facilities (n = 45), and ready-to-eat food-processing facilities (n = 70). A stratified purposive sampling approach was used to ensure representation of routine food-contact sites across major processing stages. The sampled sites included raw-material handling areas (n = 60), cutting or preparation areas (n = 55), washing areas (n = 45), packaging areas (n = 50), and cold-storage areas (n = 30). Eligible sites included surfaces directly involved in food preparation, processing, washing, cutting, transport, storage, or packaging. Surfaces that had been immediately disinfected before sampling, non-food-contact surfaces, inaccessible surfaces, and surfaces without a definable sampling area were excluded.

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Received: *02-05-2026 -* ***Accepted:*** *01-07-2026 -* ***Published:*** *08-07-2026*

Environmental observation and surface sampling
Before microbiological sampling, a structured observation form was completed for each sampled surface. Recorded variables included facility type, processing area, surface material, cleaning frequency, visible organic residue, and the routinely used disinfectant category. Surface material was classified as stainless steel, plastic cutting board, rubber conveyor belt, polyethylene, or glass/ceramic. Cleaning frequency was categorized as once daily, twice daily, or at least three times daily. Visible organic residue was defined as observable food debris, grease, product film, or moisture-associated deposits at the sampling site. The routine disinfectant category was recorded as a quaternary ammonium compound (QAC)-based product, sodium hypochlorite, hydrogen peroxide, or peracetic acid. Surface sampling was conducted according to the principles of ISO 18593:2018 for microbiological sampling of food-chain surfaces [16]. On flat surfaces, a sterile 10 × 10 cm template was used to delimit a 100 cm^2 sampling area. The designated area was sampled using a sterile swab or sponge moistened with Dey–Engley neutralizing broth. The sampling device was systematically moved across the surface in horizontal, vertical, and diagonal directions. For irregular, curved, or smaller surfaces, the accessible food-contact area was measured and recorded to permit expression of bacterial counts per square centimeter. Each sampling device was transferred into a sterile tube containing neutralizing diluent. Samples were transported in insulated containers at 2–8°C and processed within 4 h of collection. Sample handling and laboratory practices followed the general requirements of ISO 7218:2024 [17].

Quantification of bacterial contamination
Surface suspensions were mixed thoroughly before preparation of decimal dilutions in sterile diluent. Aerobic bacterial contamination was quantified using the Plate Count Agar method at 30°C, in accordance with ISO 4833-2:2013 [18]. Plates containing countable colonies were used for enumeration. Bacterial counts were calculated as colony-forming units per square centimeter (CFU/cm^2) and converted to log_{10} CFU/cm^2 for statistical analysis. A sample was considered culture-positive when bacterial growth was recovered from the primary culture or when a target organism was isolated following routine culture procedures.

Isolation and phenotypic identification of bacterial isolates
Primary isolation was performed using nutrient agar and selective/differential culture media, including MacConkey agar for Enterobacterales and other Gram-negative bacilli, Cetrimide agar for non-fermentative Gram-negative bacilli, Mannitol Salt Agar for staphylococci, Salmonella–Shigella agar for presumptive Salmonella isolates, and selective Listeria agar for presumptive Listeria isolates. Blood agar was used when hemolysis assessment was required. Representative colonies with distinct colonial morphology were selected from primary cultures and purified before identification. Preliminary characterization included Gram staining and routine phenotypic tests, including catalase activity, oxidase activity, motility, and spore formation, where applicable. Presumptive identification of Enterobacterales, non-fermentative Gram-negative bacilli, and staphylococci was performed using Analytical Profile Index systems (bioMérieux, France), including API 20E, API 20NE, and API Staph, respectively. API profiles were interpreted using APIWeb version 9.0. Species-level identification was accepted when the percent identification was ≥90%, and the T index was ≥0.75. Isolates not meeting these criteria were reported at the genus level only.
Presumptive Salmonella isolates recovered on Salmonella–Shigella agar were identified using Gram staining, oxidase negativity, and biochemical reactions, including triple sugar iron (TSI) agar and urease tests, followed by confirmation using an approved Salmonella confirmation procedure [19]. Presumptive Listeria isolates were identified from colonies recovered on selective Listeria agar, followed by Gram staining, catalase testing, tumbling motility at room temperature, and hemolysis assessment on blood agar. Species-level designation as Listeria monocytogenes was assigned only after confirmation using an approved Listeria confirmation procedure [20]. Presumptive Staphylococcus aureus isolates were further evaluated using growth on Mannitol Salt Agar and appropriate confirmatory phenotypic testing. Bacillus isolates were characterized using starch hydrolysis, casein hydrolysis, citrate utilization, hemolysis pattern, and other relevant phenotypic reactions.

Microtiter-plate biofilm assay
Biofilm formation was assessed using the crystal-violet microtiter-plate assay adapted from the methods described by Stepanović *et al*. and O'Toole [21,22]. Fresh standardized bacterial suspensions were inoculated into sterile flat-bottomed 96-well polystyrene microplates containing tryptic soy broth supplemented with 1% glucose. After static incubation for 24 h, non-adherent cells were removed by gentle washing. Adherent biofilm biomass was fixed, stained with 0.1% crystal violet, washed to remove excess stain, and solubilized using 95% ethanol. Absorbance was measured at 570 nm using a microplate reader. Each isolate was tested in triplicate wells in three independent experimental runs. Sterile broth was used as the negative control, while *Pseudomonas aeruginosa* ATCC 27853 was included as a positive biofilm-forming control. The optical-density cut-off value (ODc) was calculated as the mean optical density of negative-control wells plus three standard deviations. Isolates were classified as weak biofilm producers when $ODc < OD570 \leq 2 \times ODc$, moderate biofilm producers when $2 \times ODc < OD570 \leq 4 \times ODc$, and strong biofilm producers when $OD570 > 4 \times ODc$.

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***Received**: 02-05-2026 - **Accepted**: 01-07-2026 - **Published**: 08-07-2026*

https://doi.org/10.54361/ajmas.269714

Disinfectant preparation, exposure conditions, and tolerance definition

Four disinfectant categories commonly used in food-processing environments were evaluated: QAC-based disinfectant, sodium hypochlorite, hydrogen peroxide, and peracetic acid. Working disinfectant solutions were freshly prepared immediately before testing using sterile distilled water. Each preparation was verified using an appropriate colorimetric, titrimetric, or test-strip method before use. The disinfectant-challenge assay was a modified research protocol informed by the principles of EN 13697:2023 for quantitative evaluation of disinfectants on non-porous surfaces in food and industrial settings; it was not intended as a commercial product-certification test [23]. Disinfectant activity was evaluated under clean and organic-load conditions. The organic-load condition consisted of bovine serum albumin at a final concentration of 0.3% (w/v), while no additional organic material was included in the clean condition. Exposure was performed at 22 ± 2°C for 5 min. Immediately after exposure, an appropriate validated neutralizer was added to stop residual disinfectant activity (Table 1).

Table 1. Disinfectants, working concentrations, exposure conditions, and neutralization procedures

Disinfectant	Active ingredient	Working concentration	Exposure time	Temperature	Organic load condition	Neutralizer	Efficacy endpoint
QAC-based disinfectant	Benzalkonium chloride / QACs	200 ppm	5 min	22 ± 2°C	With and without organic load	Dey-Engley neutralizing broth	≥5-log10 CFU reduction
Sodium hypochlorite	Free available chlorine	200 ppm	5 min	22 ± 2°C	With and without organic load	Sodium thiosulfate-based neutralizer	≥5-log10 CFU reduction
Hydrogen peroxide	H_2O_2	3.0% v/v	5 min	22 ± 2°C	With and without organic load	Catalase-containing neutralizer	≥5-log10 CFU reduction
Peracetic acid	Peracetic acid	80 ppm	5 min	22 ± 2°C	With and without organic load	Sodium thiosulfate + catalase neutralizer	≥5-log10 CFU reduction

CFU, colony-forming units; H_2O_2, hydrogen peroxide; QAC, quaternary ammonium compound

Biofilm-associated and planktonic disinfectant challenge

To compare biofilm-associated and planktonic phenotypes, standardized biofilms were formed on sterile grade-304 stainless-steel coupons under static conditions for 48 h, with replacement of the growth medium after 24 h. Coupons were gently rinsed to remove non-adherent cells before disinfectant exposure. Planktonic suspensions from the same isolate were tested in parallel under identical disinfectant concentration, exposure time, temperature, and organic-load conditions. After neutralization, surviving cells were recovered from coupons using the approved laboratory elution procedure, while planktonic cells were processed by direct dilution and colony enumeration. The $\log_{10}$ reduction was calculated as:

$$\text{Log}_{10} \text{ reduction} = \log_{10}(N_0) - \log_{10}(N_t)$$

where N_0 represents the mean viable count of the untreated control and N_t represents the viable count after disinfectant exposure and neutralization. Phenotypic disinfectant tolerance was defined a priori as failure to achieve a ≥5-$\log_{10}$ CFU reduction under the predefined exposure condition. This definition represented reduced phenotypic susceptibility within the standardized assay and was not interpreted as evidence of genetically encoded disinfectant resistance. Each isolate was tested only once per defined disinfectant condition. No serial passaging, repeated sublethal exposure, recovery and rechallenge of surviving cells, adaptive evolution experiment, genetic manipulation, or selection procedure was performed.

Neutralizer validation and quality assurance

Neutralizer selection and validation followed the principles of ASTM E1054-22 to demonstrate neutralizer efficacy and the absence of neutralizer toxicity [24]. For each disinfectant-neutralizer pair, neutralizer toxicity controls, neutralizer efficacy controls, disinfectant-only controls, sterility controls, and growth controls were included. A neutralizer was considered acceptable when bacterial recovery was at least 90% of that obtained from the corresponding growth control and when no residual disinfectant activity was detected after neutralization. Biofilm assay reproducibility was assessed using replicate measurements within the same assay and across independent assay runs. Intra-assay and inter-assay coefficients of variation were calculated. Surface recovery efficiency was assessed using a standardized inoculum applied to sterile stainless-steel coupons and recovered using the same elution procedure applied to test coupons. Disinfectant concentrations, culture-medium performance, control outcomes, and sterility results were reviewed before acceptance of each experimental batch (Table 2).

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***Received:** 02-05-2026 - **Accepted:** 01-07-2026 - **Published:** 08-07-2026*

Table 2. Quality-control, neutralizer validation, and assay reproducibility

Validation component	Acceptance criterion	Result	Interpretation
Neutralizer toxicity control	≥90% bacterial recovery	94.8–101.6% recovery	Passed
Neutralizer efficacy control	No residual disinfectant activity after neutralization	No inhibition observed	Passed
Positive biofilm control	OD570 above the strong-biofilm threshold	OD570 = 1.12 ± 0.09	Passed
Negative biofilm control	OD570 below ODc	OD570 = 0.06 ± 0.01	Passed
Intra-assay reproducibility	CV <15%	6.8–11.4%	Passed
Inter-assay reproducibility	CV <20%	9.5–16.7%	Passed
Surface recovery efficiency	≥70% bacterial recovery	74.2–88.9%	Passed
Disinfectant concentration verification	Within ±10% of the target concentration	92.4–106.8% of target	Passed
Sterility controls	No growth	No growth detected	Passed
Growth controls	Expected bacterial recovery	Growth was observed in all controls	Passed

CV, coefficient of variation; OD570, optical density measured at 570 nm; ODc, optical-density cut-off.

Biosafety, biosecurity, and chemical-safety measures

Before commencing laboratory procedures, an agent- and procedure-specific risk assessment was reviewed under the applicable institutional laboratory safety procedures. All surface samples and recovered isolates were handled as potentially hazardous environmental materials until final disposal. No culture manipulation was performed inside food-processing facilities. Samples were transported in closed primary containers within leak-resistant secondary containment to the Department of Microbiology Laboratories, Libyan Center for Biotechnology, where all culture-based procedures were conducted by trained laboratory personnel under approved institutional standard operating procedures. Laboratory work was restricted to phenotypic examination, enumeration, identification, biofilm assessment, and one-time disinfectant-challenge testing. The study did not involve intentional release of microorganisms, genetically modified organisms, animal experimentation, enhanced-pathogen work, or any procedure intended to increase microbial virulence, transmissibility, persistence, or disinfectant tolerance. Surviving cells recovered after disinfectant exposure were used only for quantitative enumeration and were not retained, re-cultured for adaptation, transferred, distributed, or subjected to repeated disinfectant exposure. All cultures, contaminated materials, liquid waste, and disposable consumables were inactivated and discarded according to approved local laboratory procedures. Disinfectants were handled according to institutional chemical-safety procedures and the relevant manufacturer's safety data sheets. Different disinfectant products were not mixed. Preparation, neutralization, storage, use, and disposal of disinfectants were performed only by trained laboratory staff.

Statistical analysis

Data were analyzed using SPSS version 26.0. Categorical variables were presented as frequencies and percentages and compared using the chi-square test, Fisher's exact test, or Fisher–Freeman–Halton exact test, as appropriate. Continuous non-normally distributed variables were expressed as median and interquartile range (IQR) and compared using the Mann–Whitney U test, Kruskal–Wallis test, or Friedman test, with Bonferroni-adjusted post-hoc comparisons where applicable. Cochran's Q test and McNemar's test were used to compare disinfectant tolerance patterns. Associations among biofilm biomass, bacterial load, hygiene variables, and disinfectant tolerance were examined using Spearman's rank correlation coefficient. A mixed-effects logistic regression model was used to identify independent predictors of tolerance to at least one disinfectant, with facility included as a random effect. Results were reported as adjusted odds ratios (aORs) with 95% confidence intervals (CIs). Model performance was assessed using the Hosmer–Lemeshow test, Nagelkerke R^2, area under the curve, variance inflation factors, and intraclass correlation coefficient. A two-sided p-value of <0.05 was considered statistically significant.

Ethical and administrative considerations

The study involved environmental sampling of food-contact surfaces only and did not involve human participants, patient data, animal experimentation, or identifiable staff information. Therefore, formal human-subject ethical approval was not required under the applicable institutional procedures. Administrative permission was obtained from the management of participating food-processing facilities before sampling. Facility identities were coded and removed from the analytical dataset, and results were reported only in aggregated form.

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***Received:** 02-05-2026 - **Accepted:** 01-07-2026 - **Published:** 08-07-2026*

Results

Surface contamination and hygiene-related characteristics

Of the 240 food-contact surface samples collected, 151 (62.9%) were culture-positive. As presented in Table 3, bacterial recovery differed significantly by facility type (p = 0.014), with meat-processing facilities showing the highest positivity rate (74.3%), followed by dairy-processing (61.8%), ready-to-eat food (58.6%), and vegetable-processing facilities (53.3%). Culture positivity also differed across processing areas (p = 0.018). The highest proportion of positive samples was detected in raw-material handling areas (75.0%), followed by cutting/preparation areas (70.9%), washing areas (62.2%), packaging areas (50.0%), and cold-storage areas (46.7%).

Surface material was significantly associated with bacterial recovery (p = 0.031). Plastic cutting boards and rubber conveyor belts showed the highest positivity rates, at 76.4% and 72.5%, respectively, whereas glass/ceramic surfaces had the lowest rate of contamination (20.0%). Cleaning frequency was inversely associated with culture positivity (p for trend < 0.001). Surfaces cleaned once daily had a positivity rate of 80.5%, compared with 63.5% among surfaces cleaned twice daily and 38.7% among those cleaned at least three times daily. Similarly, surfaces with visible organic residue were more frequently culture-positive than visibly clean surfaces (86.5% vs. 52.4%, p < 0.001). Routine disinfectant use was also associated with bacterial recovery (p = 0.003). Facilities using QAC-based disinfectants had the highest proportion of positive samples (77.3%), whereas lower positivity rates were observed in facilities using sodium hypochlorite (59.7%), peracetic acid (51.1%), or hydrogen peroxide (48.6%).

Table 3. Characteristics of food-processing facilities, sampled surfaces, and hygiene practices

Variable	Category	Samples, n (%)	Culture-positive, n (%)	p-value
Total samples	—	240 (100.0)	151 (62.9)	—
Facility type	Meat processing	70 (29.2)	52 (74.3)	0.014
	Dairy processing	55 (22.9)	34 (61.8)	
	Vegetable processing	45 (18.8)	24 (53.3)	
	Ready-to-eat food	70 (29.2)	41 (58.6)	
Processing area	Raw material handling	60 (25.0)	45 (75.0)	0.018
	Cutting/preparation	55 (22.9)	39 (70.9)	
	Washing area	45 (18.8)	28 (62.2)	
	Packaging area	50 (20.8)	25 (50.0)	
	Cold storage	30 (12.5)	14 (46.7)	
Surface material	Stainless steel	95 (39.6)	65 (68.4)	0.031
	Plastic cutting board	55 (22.9)	42 (76.4)	
	Rubber conveyor belt	40 (16.7)	29 (72.5)	
	Polyethylene	30 (12.5)	11 (36.7)	
	Glass/ceramic	20 (8.3)	4 (20.0)	
Cleaning frequency	Once daily	82 (34.2)	66 (80.5)	<0.001
	Twice daily	96 (40.0)	61 (63.5)	
	≥3 times daily	62 (25.8)	24 (38.7)	
Visible organic residue	Present	74 (30.8)	64 (86.5)	<0.001
	Absent	166 (69.2)	87 (52.4)	
Routine disinfectant	QAC-based	88 (36.7)	68 (77.3)	0.003
	Sodium hypochlorite	72 (30.0)	43 (59.7)	
	Peracetic acid	45 (18.8)	23 (51.1)	
	Hydrogen peroxide	35 (14.6)	17 (48.6)	

Quantitative bacterial contamination levels

As shown in Table 4, median bacterial contamination levels differed significantly according to facility type (p = 0.009). Meat-processing facilities had the highest median bacterial load at 3.81 log_{10} CFU/cm^2 (IQR: 3.12–4.44), which was significantly higher than the levels recorded in vegetable-processing and ready-to-eat food facilities. Median bacterial counts also varied across processing areas (p = 0.004). Raw-material handling areas had the highest contamination level, with a median of 3.78 log_{10} CFU/cm^2 (IQR: 3.12–4.41), followed by cutting/preparation areas at 3.54 log_{10} CFU/cm^2. In contrast, packaging and cold-storage areas had the lowest median counts, at 2.74 and 2.46 log_{10} CFU/cm^2, respectively. Surface material was significantly associated with bacterial burden (p = 0.002). Rubber conveyor belts had the highest median load at 3.89 log_{10} CFU/cm^2, followed by plastic cutting boards at 3.71 log_{10} CFU/cm^2. The lowest bacterial loads were detected on glass/ceramic surfaces and polyethylene surfaces, with median counts of 2.11 and 2.48 log_{10} CFU/cm^2, respectively. The presence of visible organic residue was associated with significantly greater bacterial contamination. Surfaces with visible

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Received: *02-05-2026 -* ***Accepted:*** *01-07-2026 -* ***Published:*** *08-07-2026*

residue had a median count of 3.92 log_{10} CFU/cm^2, compared with 2.88 log_{10} CFU/cm^2 among surfaces without visible residue ($p < 0.001$).

Table 4. Quantitative bacterial contamination according to environmental variables

Variable	Category	Positive samples, n	Median log10 CFU/cm^2	IQR	p-value
Facility type	Meat processing	52	3.81	3.12–4.44	0.009
	Dairy processing	34	3.36	2.78–4.02	
	Vegetable processing	24	2.91	2.14–3.62	
	Ready-to-eat food	41	3.08	2.42–3.74	
Processing area	Raw material handling	45	3.78	3.12–4.41	0.004
	Cutting/preparation	39	3.54	2.91–4.18	
	Washing area	28	3.11	2.48–3.84	
	Packaging area	25	2.74	2.01–3.33	
	Cold storage	14	2.46	1.88–3.10	
Surface material	Rubber conveyor belt	29	3.89	3.18–4.52	0.002
	Plastic cutting board	42	3.71	3.02–4.35	
	Stainless steel	65	3.26	2.51–3.94	
	Polyethylene	11	2.48	1.97–3.08	
	Glass/ceramic	4	2.11	1.62–2.80	
Organic residue	Present	64	3.92	3.22–4.58	<0.001
	Absent	87	2.88	2.18–3.53	

Distribution of bacterial isolates and biofilm-forming capacity

A total of 166 bacterial isolates were recovered from the 151 culture-positive surface samples. As shown in (Table 5), *Listeria monocytogenes* was the most common isolate, accounting for 32/166 (19.3%) isolates, followed by *Staphylococcus aureus* (18.1%), *Salmonella enterica* (16.9%), *Escherichia coli* (15.7%), and *Pseudomonas aeruginosa* (15.1%). *Bacillus cereus* and *Klebsiella pneumoniae* represented 9.0% and 6.0% of isolates, respectively. The distribution of bacterial species differed significantly according to facility type (p = 0.041). However, species distribution did not differ significantly according to surface material (p = 0.087). Biofilm-forming capacity varied significantly among bacterial species, as illustrated in Figure 1 (p = 0.021). Overall, 81/166 isolates (48.8%) were classified as strong biofilm producers, 55/166 (33.1%) as moderate producers, and 30/166 (18.1%) as weak producers. As shown in (Figure 1), strong biofilm formation was most frequent among *P. aeruginosa* isolates, occurring in 17/25 (68.0%) isolates, followed by *L. monocytogenes*, in which 21/32 (65.6%) isolates were strong producers. Strong biofilm formation was also identified in 16/30 (53.3%) *S. aureus* isolates, 11/28 (39.3%) *S. enterica* isolates, 8/26 (30.8%) *E. coli* isolates, 4/15 (26.7%) *B. cereus* isolates, and 4/10 (40.0%) *K. pneumoniae* isolates. Median OD_{570} values differed significantly among the bacterial species (p = 0.008).

Table 5. Distribution of foodborne bacterial isolates and their biofilm-forming capacity according to species

Species	Total isolates, n (%)	Meat	Dairy	Vegetable	RTE	Stainless steel	Plastic	Rubber	Polyethylene /Glass
Listeria monocytogenes	32 (19.3)	12	8	4	8	14	7	8	3
Staphylococcus aureus	30 (18.1)	9	7	5	9	12	8	6	4
Salmonella enterica	28 (16.9)	13	3	5	7	10	9	6	3
Escherichia coli	26 (15.7)	8	4	7	7	8	8	7	3
Pseudomonas aeruginosa	25 (15.1)	5	8	3	9	11	4	8	2
Bacillus cereus	15 (9.0)	3	5	4	3	6	4	2	3
Klebsiella pneumoniae	10 (6.0)	2	3	2	3	4	2	2	2
Total	166 (100.0)	52	38	30	46	65	42	39	20

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Received: *02-05-2026 -* ***Accepted:*** *01-07-2026 -* ***Published:*** *08-07-2026*

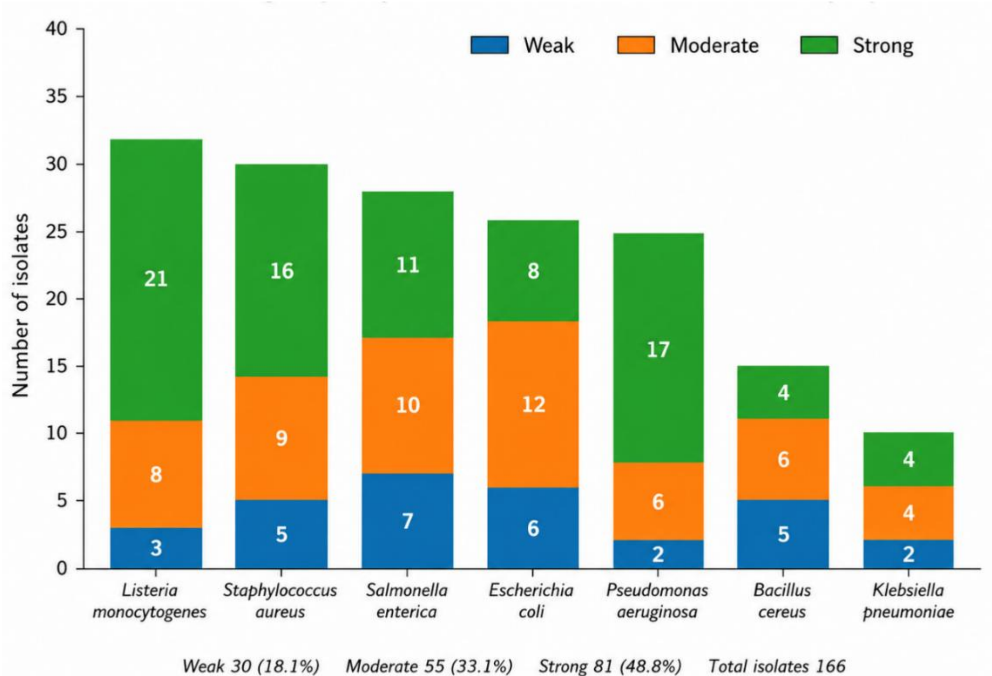
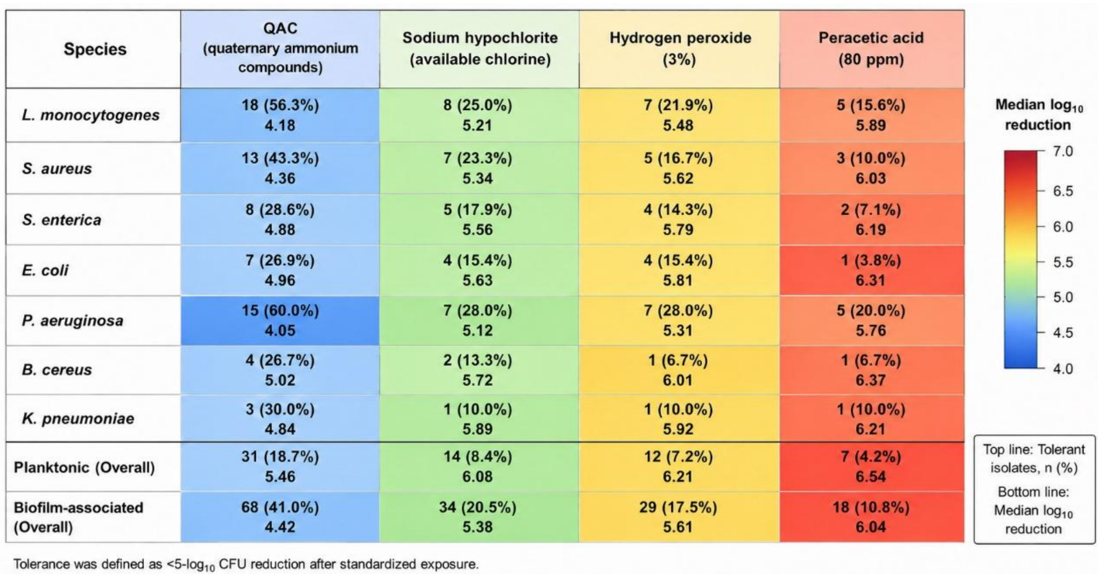


Figure 1. Biofilm-Forming Capacity of Foodborne Bacterial Isolates According to Species

Disinfectant tolerance among biofilm-associated isolates

As shown in (Figure 2), phenotypic tolerance among biofilm-associated isolates differed significantly across the tested disinfectants (Cochran's Q test, $p < 0.001$). QACs showed the highest frequency of tolerant isolates, with tolerance detected in 68/166 isolates (41.0%). This was followed by sodium hypochlorite, with 34/166 tolerant isolates (20.5%), hydrogen peroxide, with 29/166 (17.5%), and peracetic acid, with 18/166 (10.8%). The median $\log_{10}$ reduction values varied significantly among disinfectants ($p < 0.001$). QACs produced the lowest median reduction among biofilm-associated isolates (4.42 $\log_{10}$ CFU reduction), while peracetic acid produced the highest median reduction (6.04 $\log_{10}$ CFU reduction). At the species level, tolerance to one or more disinfectants differed significantly ($p = 0.012$). *L. monocytogenes* and *P. aeruginosa* demonstrated the highest tolerance frequencies, particularly against QAC-based disinfectants. QAC tolerance was observed in 18/32 (56.3%) *L. monocytogenes* isolates and 15/25 (60.0%) *P. aeruginosa* isolates. As illustrated in Figure 2, biofilm-associated isolates were significantly more tolerant than planktonic isolates to all disinfectants tested. QAC tolerance was detected in 41.0% of biofilm-associated isolates compared with 18.7% of planktonic isolates ($p < 0.001$). Likewise, tolerance to sodium hypochlorite was observed in 20.5% versus 8.4% of isolates, respectively ($p < 0.001$), while hydrogen peroxide tolerance was observed in 17.5% versus 7.2% ($p < 0.001$). Peracetic acid tolerance was also more frequent among biofilm-associated isolates than planktonic isolates (10.8% vs. 4.2%, $p = 0.003$).



Species	QAC (quaternary ammonium compounds)	Sodium hypochlorite (available chlorine)	Hydrogen peroxide (3%)	Peracetic acid (80 ppm)
L. monocytogenes	18 (56.3%) 4.18	8 (25.0%) 5.21	7 (21.9%) 5.48	5 (15.6%) 5.89
S. aureus	13 (43.3%) 4.36	7 (23.3%) 5.34	5 (16.7%) 5.62	3 (10.0%) 6.03
S. enterica	8 (28.6%) 4.88	5 (17.9%) 5.56	4 (14.3%) 5.79	2 (7.1%) 6.19
E. coli	7 (26.9%) 4.96	4 (15.4%) 5.63	4 (15.4%) 5.81	1 (3.8%) 6.31
P. aeruginosa	15 (60.0%) 4.05	7 (28.0%) 5.12	7 (28.0%) 5.31	5 (20.0%) 5.76
B. cereus	4 (26.7%) 5.02	2 (13.3%) 5.72	1 (6.7%) 6.01	1 (6.7%) 6.37
K. pneumoniae	3 (30.0%) 4.84	1 (10.0%) 5.89	1 (10.0%) 5.92	1 (10.0%) 6.21
Planktonic (Overall)	31 (18.7%) 5.46	14 (8.4%) 6.08	12 (7.2%) 6.21	7 (4.2%) 6.54
Biofilm-associated (Overall)	68 (41.0%) 4.42	34 (20.5%) 5.38	29 (17.5%) 5.61	18 (10.8%) 6.04

Tolerance was defined as <5-$\log_{10}$ CFU reduction after standardized exposure.
Data represent biofilm-associated cells unless stated otherwise.

Figure 2. Heatmap of median $\log_{10}$ reduction and tolerant isolates (%) among biofilm-associated bacterial species exposed to different disinfectants

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Received: *02-05-2026 -* ***Accepted:*** *01-07-2026 -* ***Published:*** *08-07-2026*

Correlations among biofilm biomass, contamination burden, and hygiene-related variables

The correlation matrix shown in (Figure 3) demonstrated a significant positive correlation between biofilm biomass and bacterial contamination level (ρ = 0.46, $p < 0.001$). Biofilm biomass was also positively correlated with the number of disinfectants to which isolates exhibited tolerance (ρ = 0.58, $p < 0.001$) and the presence of visible organic residue (ρ = 0.41, $p < 0.001$). As shown in (Figure 3), increasing biofilm biomass was associated with lower $\log_{10}$ reductions after exposure to QACs (ρ = −0.61, $p < 0.001$), sodium hypochlorite (ρ = −0.43, $p < 0.001$), hydrogen peroxide (ρ = −0.39, $p < 0.001$), and peracetic acid (ρ = −0.28, $p < 0.001$). Biofilm biomass was also negatively correlated with cleaning frequency (ρ = −0.38, $p < 0.001$). Bacterial contamination levels were positively correlated with visible organic residue (ρ = 0.47, $p < 0.001$) and the number of tolerated disinfectants (ρ = 0.34, $p < 0.01$). Conversely, bacterial load was negatively correlated with cleaning frequency (ρ = −0.44, $p < 0.001$).

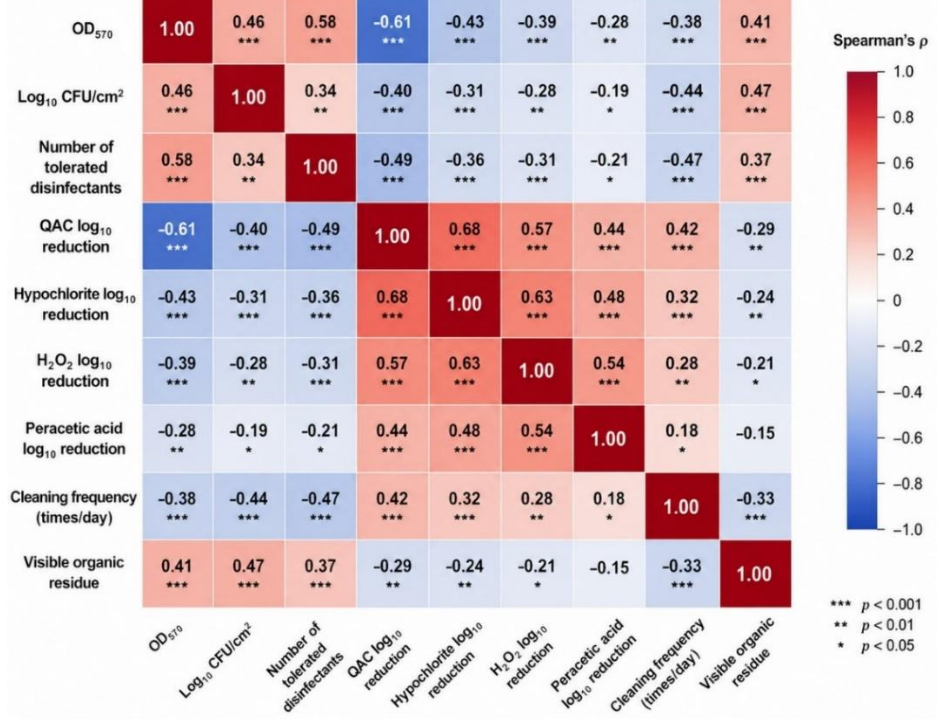

Figure3. Spearman correlation heatmap showing associations among biofilm biomass, bacterial contamination, disinfectant tolerance, organic residue, and cleaning frequency

Predictors of disinfectant tolerance

As illustrated in (Table 6), strong biofilm formation was the strongest independent predictor of tolerance to at least one disinfectant (adjusted odds ratio [aOR] = 4.87, 95% CI: 2.34–10.13; $p < 0.001$). Moderate biofilm formation was also associated with significantly increased odds of disinfectant tolerance (aOR = 2.41, 95% CI: 1.02–5.71; p = 0.045). Among bacterial species, *Pseudomonas aeruginosa* (aOR = 3.28, 95% CI: 1.21–8.89; p = 0.019) and *Listeria monocytogenes* (aOR = 2.94, 95% CI: 1.13–7.66; p = 0.027) were independently associated with a greater likelihood of disinfectant tolerance. Routine use of QAC-based disinfectants was likewise associated with increased odds of tolerance (aOR = 3.76, 95% CI: 1.79–7.92; $p < 0.001$). The presence of visible organic residue was another significant predictor, increasing the odds of tolerance by 2.68-fold (95% CI: 1.31–5.48; p = 0.007). Conversely, cleaning surfaces at least three times daily was associated with a significantly reduced likelihood of disinfectant tolerance (aOR = 0.42, 95% CI: 0.19–0.91; p = 0.028). Rubber/plastic surfaces showed a non-significant positive association with tolerance (aOR = 1.71, 95% CI: 0.84–3.47; p = 0.139).

Table 6. Mixed-effects logistic regression for predictors of disinfectant tolerance

Predictor	Adjusted OR	95% CI	p-value
Strong biofilm formation	4.87	2.34–10.13	<0.001
Moderate biofilm formation	2.41	1.02–5.71	0.045
P. aeruginosa	3.28	1.21–8.89	0.019
L. monocytogenes	2.94	1.13–7.66	0.027
Routine QAC use	3.76	1.79–7.92	<0.001
Visible organic residue	2.68	1.31–5.48	0.007
Cleaning ≥3 times/day	0.42	0.19–0.91	0.028
Rubber/plastic surface	1.71	0.84–3.47	0.139
High bacterial load >10^3 CFU/cm^2	2.53	1.18–5.41	0.017

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***Received:** 02-05-2026 - **Accepted:** 01-07-2026 - **Published:** 08-07-2026*

Discussion

Food-contact surfaces in processing environments can act as persistent reservoirs for foodborne and food-associated bacteria, particularly when organic residues, surface defects, and inadequate sanitation facilitate microbial attachment. The present study examined the relationships among environmental contamination, biofilm formation, and phenotypic tolerance to commonly used disinfectants in food-processing facilities. The findings are discussed in relation to hygiene practices, surface characteristics, bacterial persistence, and implications for improving sanitation management.

Surface contamination and hygiene-related determinants

Food-contact surfaces constitute critical points for the transfer of microorganisms throughout food-processing operations. Their contamination reflects the continuous introduction of bacteria through raw materials, water, workers, equipment, aerosols, and residual food material. Accordingly, food-processing facilities should be viewed as dynamic microbial ecosystems in which selected microorganisms can colonize recurrent niches when sanitation is incomplete or environmental conditions favor attachment and persistence [3,25]. The observed variation in contamination according to facility type and processing area is biologically plausible. Raw-material handling and preparation areas are repeatedly exposed to microorganisms originating from unprocessed foods and therefore experience a greater contamination pressure than downstream areas. In contrast, packaging and cold-storage areas may have lower microbial exposure when hygienic zoning, equipment cleaning, and traffic control are consistently maintained. Nevertheless, contamination in downstream areas remains relevant because even low numbers of persistent microorganisms may contaminate finished products, particularly in ready-to-eat food operations [3,4]. The association between organic residues and microbial recovery is particularly important. Food debris, grease, protein films, and retained moisture can provide nutrients for microbial survival and create a physical barrier that limits contact between disinfectants and attached cells. Organic matter may also reduce the activity of oxidizing disinfectants and facilitate the retention of cells within small surface irregularities. Therefore, the effectiveness of a sanitation program depends first on adequate cleaning and soil removal, followed by the correct application of a disinfectant [4,5].

Surface material, equipment condition, and microbial attachment

The influence of surface material on bacterial contamination should not be interpreted solely according to whether a surface is stainless steel, plastic, rubber, polyethylene, or glass. Surface roughness, aging, cracks, scratches, hydrophobicity, and accessibility during cleaning are equally important determinants of bacterial attachment. Damaged plastic cutting boards and rubber conveyor belts can retain food particles and moisture within surface irregularities, creating protected microenvironments that are difficult to reach during routine sanitation [6]. Recent research has shown that *Salmonella* biofilm attachment can differ markedly among food-contact materials. Ivers *et al*. demonstrated that material type and biofilm age influenced attachment and hydrophobicity, with stronger attachment observed on selected polymeric and porous materials than on smoother surfaces [26]. Similarly, Counihan *et al*. reported that biofilm formation by different *Salmonella* serotypes varied according to both temperature and food-contact material, confirming that bacterial persistence is influenced by the interaction between strain characteristics and the physical properties of the surface [27]. This observation is consistent with previous reports describing the persistence of *Listeria* and *Pseudomonas* on food-processing equipment and their contribution to long-term environmental contamination [28–30]. These findings suggest that repeated contamination of a specific location may result from both procedural and structural problems. Improving sanitation alone may not be sufficient when equipment surfaces are visibly damaged, poorly designed, or difficult to dismantle. Preventive maintenance should therefore include routine inspection of cutting boards, conveyor belts, joints, gaskets, drains, and other difficult-to-clean components. Replacement or resurfacing of worn materials may be necessary when microbiological control cannot be achieved consistently [6].

Biofilm formation and species-specific persistence

Biofilm formation provides a biologically plausible explanation for the environmental persistence of foodborne and food-associated bacteria. Within biofilms, microorganisms are embedded in an extracellular polymeric matrix that can reduce the effects of dehydration, nutrient limitation, mechanical cleaning, and disinfectant exposure. Biofilm-associated tolerance is multifactorial and may result from restricted disinfectant penetration, altered metabolic activity, stress-response activation, membrane changes, efflux activity, and persister-like subpopulations [5,11]. The relevance of *Listeria monocytogenes* is especially important because this organism can survive under refrigerated conditions and establish long-term reservoirs in food-processing environments. Studies from meat-processing facilities have confirmed the capacity of *L. monocytogenes* to persist on equipment and form biofilms that are difficult to control once established [8]. Recent environmental monitoring in ready-to-eat meat plants also identified *Listeria* together with highly QAC-tolerant *Pseudomonas* isolates, emphasizing the potential coexistence of foodborne pathogens and persistent environmental bacteria within the same sanitation niches [28]. The occurrence of *Listeria* in mixed environmental communities should also be considered. Pracser *et al*. demonstrated that *Listeria* may occur within biofilm-associated microbial communities in frozen-vegetable processing environments, where environmental composition and interspecies interactions may influence its persistence [29]. These findings indicate that control programs should not focus only on a single target

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***Received:** 02-05-2026 - **Accepted:** 01-07-2026 - **Published:** 08-07-2026*

https://doi.org/10.54361/ajmas.269714

pathogen but should consider the broader microbial ecology of food-processing surfaces. The frequent recovery of *Pseudomonas aeruginosa* and related organisms is also relevant. Members of the genus *Pseudomonas* are well adapted to moist environments, can produce substantial biofilms, and may contribute to food spoilage and microbial persistence. Recent evidence indicates that *Pseudomonas* populations in meat-processing environments may influence the survival of foodborne pathogens by participating in mixed biofilms and increasing tolerance to antimicrobial agents [30].

Mixed-species biofilms and their implications

Food-processing surfaces are rarely colonized by a single bacterial species. Mixed-species biofilms may differ substantially from single-species laboratory biofilms in biomass, matrix composition, nutrient utilization, and disinfectant susceptibility. The interaction among pathogens, spoilage organisms, and environmental bacteria can either inhibit or protect individual members of the community [11,13]. Koti *et al*. showed that the efficacy of disinfectants against mixed biofilms containing Shiga toxin-producing *Escherichia coli*, lactic acid bacteria, and spoilage organisms was influenced by both microbial composition and environmental conditions [31]. This is important because standard disinfectant validation often relies on planktonic cells or single laboratory strains, which may not reflect the behavior of mature multispecies biofilms in operational food-processing environments. The association between cleaning frequency and lower contamination should also be interpreted cautiously. Frequent sanitation may reduce microbial accumulation, but its effectiveness depends on cleaning quality, mechanical action, detergent use, water quality, equipment accessibility, and verification procedures. Wang *et al*. reported that intensive sanitation can modify environmental biofilm communities, yet persistent bacterial niches may remain when structural or ecological conditions continue to support survival [32]. Therefore, sanitation frequency should be combined with microbiological verification rather than treated as an independent indicator of hygiene effectiveness.

Biofilm-associated phenotypic disinfectant tolerance

The greater survival of biofilm-associated cells compared with planktonic cells is consistent with established biofilm biology. In suspension, disinfectants can directly contact individual cells. In contrast, cells embedded within a mature biofilm may be protected by extracellular matrix components and may exhibit reduced metabolic activity or stress-adapted phenotypes. These conditions can lower the killing effect of disinfectants without necessarily indicating permanent genetic resistance [5,11]. Phenotypic disinfectant tolerance should therefore be distinguished from genetically stable disinfectant resistance. Reduced killing following standardized exposure indicates survival under the tested conditions, but it does not alone prove the presence of resistance genes or heritable mutations. Molecular confirmation would require additional investigations, such as screening for QAC-associated genes, efflux pump determinants, stress-response pathways, or genomic adaptations. Repeated exposure to sublethal concentrations of disinfectants can nevertheless favor the survival of tolerant subpopulations. Alonso *et al*. reviewed multiple mechanisms that may contribute to reduced sanitizer susceptibility in food-associated bacteria, including altered membrane permeability, efflux activity, stress adaptation, and biofilm formation [33]. This underlines the importance of correct disinfectant dilution, adequate contact time, removal of organic material, and preparation of fresh working solutions.

Interpretation of disinfectant-specific patterns

The apparent differences among QACs, sodium hypochlorite, hydrogen peroxide, and peracetic acid should not be interpreted as a universal ranking of disinfectant efficacy. Their activity is influenced by concentration, contact time, temperature, pH, organic load, biofilm age, surface material, and bacterial species. Therefore, a disinfectant that performs well in one facility or laboratory model may show reduced activity against mature biofilms or heavily contaminated equipment in another setting [9,10]. The reduced activity of QAC-based products against some biofilm-associated isolates is especially relevant because QACs are widely used in food-processing facilities. Their practical performance may decline when biofilms are mature, when organic residues remain after cleaning, or when microorganisms are repeatedly exposed to sublethal concentrations. Adaptation to benzalkonium chloride has been associated with increased biofilm formation and enhanced survival in *L. monocytogenes* [12]. A systematic review of *L. monocytogenes* responses to sanitizers found that disinfectant performance varies substantially according to the active compound, exposure conditions, and test system used [34]. In addition, a recent meta-analysis of sanitizer efficacy against biofilms of *Listeria*, *Salmonella*, and Shiga toxin-producing *E. coli* demonstrated that disinfectant type and bacterial species are major determinants of log reduction, with peracetic-acid-based treatments generally showing strong activity against laboratory biofilms [35]. These findings support the need for site-specific validation of sanitation procedures. Facilities should not rely solely on manufacturer instructions but should verify disinfectant concentration, contact time, solution freshness, and performance on high-risk food-contact surfaces. Flores *et al*. further showed that common food-industry sanitizers may differ in their effectiveness depending on the bacterial target and experimental conditions, reinforcing the importance of tailored sanitation programs rather than reliance on a single universal product [36].

Implications for sanitation management

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Received*: 02-05-2026 -* ***Accepted****: 01-07-2026 -* ***Published****: 08-07-2026*

The findings support a risk-based sanitation strategy focused on high-risk processing areas, damaged surfaces, visible residues, and sites with recurrent microbiological positivity. Cleaning procedures should prioritize the physical removal of food debris before disinfectant application. Mechanical scrubbing, periodic dismantling of equipment, and cleaning of joints, gaskets, belts, and inaccessible components are particularly important for disrupting established biofilms. Environmental monitoring should also extend beyond routine end-product testing. Surface sampling, trend analysis, ATP monitoring, and targeted microbiological cultures can help identify recurrent contamination niches before they become persistent reservoirs. Monitoring should include major foodborne pathogens as well as environmental organisms that may contribute to biofilm formation or indirectly protect pathogens within mixed communities [30,32]. Novel interventions may provide additional options when conventional sanitation repeatedly fails. Strategies such as enzymatic cleaners, bacteriophages, essential oils, cold plasma, ultrasonication, electrolyzed water, and antimicrobial surface technologies are increasingly being investigated for biofilm control [14,15]. Sadeghzadeh *et al*. highlighted the potential of physical, biological, and natural approaches for controlling *P. aeruginosa* biofilms in food environments, particularly where conventional chemical sanitation is insufficient [37]. These approaches should complement, not replace, effective routine cleaning and disinfectant validation. Their practical value should be assessed under real facility conditions, including organic residues, mixed microbial communities, surface aging, and operational temperature.

Dry surfaces, hidden biofilms, and future directions

Although wet processing areas are traditionally regarded as the main locations for biofilm development, dry food-contact surfaces can also support persistent microbial communities. Dry-processing environments may permit the formation of biofilms that are difficult to detect visually and may persist despite routine cleaning. Dhaliwal *et al*. emphasized the importance of environmental monitoring and validated disinfection approaches in dry food industries, where traditional wet cleaning may not always be appropriate [38]. Recent work on *Salmonella* dry-surface biofilms has shown that these structures differ from conventional wet biofilms in morphology, cellular physiology, and sanitization response [39]. This indicates that future food-safety research should consider both wet and dry biofilm models, particularly in facilities handling low-moisture foods or equipment that is intermittently exposed to moisture.

Conclusion

This study highlights that bacterial contamination of food-contact surfaces is strongly influenced by hygiene practices, organic residues, surface condition, and biofilm formation. Biofilm-producing isolates, particularly *Listeria monocytogenes* and *Pseudomonas aeruginosa*, showed increased tolerance to disinfectants, especially QAC-based products. Therefore, effective control requires more than routine disinfection, including thorough cleaning before disinfection, correct disinfectant use, maintenance of damaged surfaces, and regular microbiological monitoring of high-risk areas.

Strengths, limitations, and future direction

A major strength of this study is its comprehensive approach, combining environmental sampling, bacterial identification, biofilm assessment, disinfectant testing, and multivariable analysis. However, the cross-sectional design cannot confirm causal relationships, and the absence of molecular testing limits understanding of the mechanisms of disinfectant tolerance. Laboratory biofilm models may also not fully represent real industrial biofilms. Future research should use longitudinal and molecular approaches, including strain typing, whole-genome sequencing, mixed-species biofilm models, and intervention studies to evaluate improved sanitation strategies.

Acknowledgments

The authors acknowledge the Department of Microbiology at the Libyan Center for Biotechnology, Tripoli, Libya, for providing the laboratory facilities used to conduct the microbiological analyses in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

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Received*: 02-05-2026 -* ***Accepted****: 01-07-2026 -* ***Published****: 08-07-2026*