

Editorial

Foodborne Pathogen Biofilms: Development, Detection, Control, and Antimicrobial Resistance

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Bacteria can grow either as planktonic cells or as communities within biofilms. The biofilm growth mode is the dominant lifestyle of most bacterial species and 40–80% of microorganisms are associated with biofilms [1]. Biofilm is a sessile community that is irreversibly attached to a substratum or interface or to other members of the community [2]. It is surrounded by extracellular polymeric substances (EPS) that include extracellular polysaccharides, extracellular DNA, lipids, proteins, and other elements [3]. Biofilm formation is a complex but well-regulated process that can be classified into five distinct stages [4]. In the first stage, planktonic bacteria attach to a surface. *Salmonella* species, *Listeria monocytogenes*, *Campylobacter jejuni*, or *Escherichia coli* have specific structures on the surface of the bacteria, such as flagella, curli, fimbriae, and pili, which help the bacteria attach [5]. The second stage is the adhesion step, which includes an initial reversible adhesion resulting in loose adhesion and a subsequent irreversible adhesion resulting in more stable adhesion. The third stage is to secrete EPS and form microcolonies. This is followed by biofilm maturation, which produces large amounts of EPS to grow in size and build three-dimensional structures. The final stage is the stage in which the biofilm is dispersed, releasing the planktonic cells and initiating the formation of a new biofilm at another location.

Microbial cells living within biofilms are protected from various environmental stresses such as desiccation, osmotic changes, oxidative stress, metal toxicity, radiation, antibiotics, disinfectants, and the host immune system [6]. Biofilms are much less sensitive to antimicrobial agents than planktonic cells, and several mechanisms contribute to their resistance to antimicrobials [7]. The exopolysaccharide matrix prevents the entry of antimicrobial agents by reducing diffusion and acting as a primary barrier [8]. Most antimicrobial agents kill rapidly dividing cells more effectively, but slow growth of biofilms leads to resistance [9]. Changes in metabolic activity within biofilms, genetic changes of antimicrobial resistant determinants in target cells, extrusion of antimicrobial agents using efflux pumps, and the presence of persistent cells also contribute to antimicrobial resistance [10].

Foodborne pathogens, such as *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7, *C. jejuni*, *Clostridium perfringens*, *Bacillus cereus*, and *Staphylococcus aureus*, and food spoilage bacteria, such as *Pseudomonas* spp., *Lactobacillus* spp., and *Shewanella* spp., can produce biofilms and are an important food safety issue causing huge economic losses in the food industry [11,12]. The extracellular matrix of biofilms can adhere to hard surfaces on food processing equipment or to food contact surfaces and serves as a structure in sustaining these biofilms [13]. Biofilms protect spoilage and foodborne pathogens in the cleaning processes in food processing equipment, such as drying or treatment with disinfecting agents [11]. Biofilms of spoilage and foodborne pathogens that survive in the sanitizing step may contaminate food products, shortening shelf life and causing food poisoning [12]. The risk is compounded by the fact that cells in biofilms have been shown



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to have increased resistance to sanitizing agents compared to planktonic cells [14]. *L. monocytogenes* induce biofilm formation in response to low temperatures, which increase adhesion to surfaces and resistance to disinfecting treatment in many food manufacturing plants [15]. *L. monocytogenes* biofilms exposed to commercial disinfectants, including quaternary ammonium compounds, have increased resistance to disinfectants [16]. In addition, benzalkonium chloride-adapted *S. Enteritidis* biofilms can develop resistance more efficiently than planktonic counterparts [17]. Ju et al. reported an approximately 32,768-fold increase in ampicillin resistance in *S. Dublin* biofilms compared to planktonic cells [18]. González et al. found that *S. Typhimurium* biofilms were significantly more resistant to ciprofloxacin both in vitro and in vivo [19].

This Special Issue aims to discuss recent studies on biofilm formation/development, detection techniques, prevention and control measures, and antimicrobial resistance associated with foodborne pathogens. This Special Issue comprises five original research articles with contributions by 26 authors from China, Iraq, Italy, Korea, Poland, Russia, South Africa, and the USA, and two review articles by four authors from the USA.

By forming biofilms, *L. monocytogenes* can survive for long periods in food processing plants, contaminating food at various stages of production [20]. The two research articles highlight the variability in the biofilm production among *L. monocytogenes* strains collected from different sources such as food and food production environments and the prevalence of biofilm-associated genes. Wiśniewski et al. investigated biofilm formation potential and the prevalence of biofilm-forming genes (*inlB*, *luxS*, *sigB*) among *L. monocytogenes* isolated from food and food processing environments in Poland [21]. Strains isolated from food processing environments formed biofilms at a higher frequency than strains isolated from food, and *inlB*, *luxS*, and *sigB* were detected in all strains from food processing environments. Kaptchouang Tchatchouang et al. also evaluated the biofilm formation ability and the frequency of biofilm-associated genes (*flaA*, *luxS*) in *L. monocytogenes* isolated from South African food samples [22]. They found that all isolates consistently and strongly formed biofilms at 4 °C for 24, 48, and 72 h. Biofilm-forming genes *flaA* and *luxS* were detected in 72% and 56% of the isolates, respectively.

It is urgent to take effective novel strategies in preventing and eradicating biofilms to reduce the risk of microbial infection. Combining anti-biofilm agents, such as quorum sensing inhibitors, probiotics, bacteriophages, and antimicrobial peptides, with antibiotics is emerging as a promising strategy to eradicate biofilms [23]. Three research articles in this Special Issue investigated a combination treatment of antibiotics with β -lactamase inhibitors, efflux pump inhibitors, and probiotic bacteria against bacterial biofilms. Laure and Ahn studied the anti-biofilm effect of β -lactam and β -lactamase inhibitor combinations against antibiotic-sensitive and multidrug-resistant *S. Typhimurium* [24]. They discovered that a combination of a β -lactam (ampicillin, ceftriaxone) and a β -lactamase inhibitor (sulbactam) significantly inhibited biofilms of β -lactamase-producing multidrug-resistant *S. Typhimurium*. Dawan et al. assessed the effect of an efflux pump inhibitor on *S. Typhimurium* biofilm formation [25]. They discovered that combinations of antibiotics (ceftriaxone, chloramphenicol, ciprofloxacin, erythromycin, norfloxacin, tetracycline) and an efflux pump inhibitor (phenylalanine-arginine β -naphthylamide) synergistically suppressed quorum sensing and thus *S. Typhimurium* biofilm formation. AL-Dulaimi et al. investigated anti-biofilm activity of a mixture of a cationic antimicrobial peptide (polymyxin E) and probiotic bacteria, such as *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895, against *Acinetobacter* species [26]. Their results exhibited a significant inhibition of biofilm formation in *Acinetobacter* species when the cell-free supernatants of *Bacillus* strains were combined with polymyxin E, compared to the use of the antibiotic alone.

Although conventional approaches are being employed to kill the biofilms of foodborne pathogens, they are still ineffective, and more new innovative agents capable of controlling biofilms are required [27]. In particular, in the food industry, there is demand for natural compounds that can be safely added to food products to act as a biofilm remover, as

well as curtailing spoilage and preventing food contamination. Esposito and Turku bring a comprehensive review on innovative natural methods of targeting foodborne pathogens' biofilms, including bacteriocins, bacteriophages, fungi, phytochemicals, plant extracts, essential oils, gaseous and aqueous control, photocatalysis, enzymatic treatments, and ultrasound [28]. Understanding how foodborne pathogens form and survive in food processing environments is important for developing new strategies against sanitizer resistance and repeated contamination. Studies related to biofilm control of foodborne pathogens are primarily designed for single-species biofilms and ignore the fact that biofilms exist as mixed-species biofilms in most food processing environments. Dass and Wang draw attention to the potential food safety issues associated with disinfecting agents that control mixed-species biofilms in the food processing environments [29].

We are grateful to all the authors that provided valuable research findings and updated reviews. We hope that this Special Issue will be an essential resource for understanding the biofilms of foodborne pathogens and developing their mitigation strategies.

Conflicts of Interest: The authors declare no conflict of interest.

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References

1. Flemming, H.C.; Wuertz, S. Bacteria and archaea on Earth and their abundance in biofilms. *Nat. Rev. Microbiol.* **2019**, *17*, 247–260. [[CrossRef](#)]
2. O'Toole, G.; Kaplan, H.B.; Kolter, R. Biofilm formation as microbial development. *Annu. Rev. Microbiol.* **2000**, *54*, 49–79. [[CrossRef](#)] [[PubMed](#)]
3. Flemming, H.C.; Baveye, P.; Neu, T.R.; Stoodley, P.; Szewzyk, U.; Wingender, J.; Wuertz, S. Who put the film in biofilm? The migration of a term from wastewater engineering to medicine and beyond. *NPJ Biofilms Microbiomes* **2021**, *7*, 10. [[CrossRef](#)] [[PubMed](#)]
4. Sauer, K.; Camper, A.K.; Ehrlich, G.D.; Costerton, J.W.; Davies, D.G. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J. Bacteriol.* **2002**, *184*, 1140–1154. [[CrossRef](#)]
5. Kimkes, T.E.P.; Heinemann, M. How bacteria recognise and respond to surface contact. *FEMS Microbiol. Rev.* **2020**, *44*, 106–122. [[CrossRef](#)] [[PubMed](#)]
6. Jahan, F.; Chinni, S.V.; Samuggam, S.; Reddy, L.V.; Solayappan, M.; Su Yin, L. The Complex Mechanism of the *Salmonella typhi* Biofilm Formation That Facilitates Pathogenicity: A Review. *Int. J. Mol. Sci.* **2022**, *23*, 6462. [[CrossRef](#)]
7. Stewart, P.S.; Costerton, J.W. Antibiotic resistance of bacteria in biofilms. *Lancet* **2001**, *358*, 135–138. [[CrossRef](#)] [[PubMed](#)]
8. Lewis, K. Multidrug tolerance of biofilms and persister cells. *Curr. Top. Microbiol. Immunol.* **2008**, *322*, 107–131. [[CrossRef](#)]
9. Costerton, J.W.; Stewart, P.S.; Greenberg, E.P. Bacterial biofilms: A common cause of persistent infections. *Science* **1999**, *284*, 1318–1322. [[CrossRef](#)]
10. Macia, M.D.; Rojo-Molinero, E.; Oliver, A. Antimicrobial susceptibility testing in biofilm-growing bacteria. *Clin. Microbiol. Infect.* **2014**, *20*, 981–990. [[CrossRef](#)]
11. Colagiorgi, A.; Bruini, I.; Di Ciccio, P.A.; Zanardi, E.; Ghidini, S.; Ianieri, A. *Listeria monocytogenes* Biofilms in the Wonderland of Food Industry. *Pathogens* **2017**, *6*, 41. [[CrossRef](#)]
12. Hood, S.K.; Zottola, E.A. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *Int. J. Food Microbiol.* **1997**, *37*, 145–153. [[CrossRef](#)] [[PubMed](#)]
13. Galie, S.; Garcia-Gutierrez, C.; Miguelez, E.M.; Villar, C.J.; Lombo, F. Biofilms in the Food Industry: Health Aspects and Control Methods. *Front. Microbiol.* **2018**, *9*, 898. [[CrossRef](#)]
14. Hua, Z.; Korany, A.M.; El-Shinawy, S.H.; Zhu, M.J. Comparative Evaluation of Different Sanitizers Against *Listeria monocytogenes* Biofilms on Major Food-Contact Surfaces. *Front. Microbiol.* **2019**, *10*, 2462. [[CrossRef](#)]
15. Lee, B.H.; Hebraud, M.; Bernardi, T. Increased Adhesion of *Listeria monocytogenes* Strains to Abiotic Surfaces under Cold Stress. *Front. Microbiol.* **2017**, *8*, 2221. [[CrossRef](#)]
16. Siderakou, D.; Zilelidou, E.; Poimenidou, S.; Tsipra, I.; Ouranou, E.; Papadimitriou, K.; Skandamis, P. Assessing the survival and sublethal injury kinetics of *Listeria monocytogenes* under different food processing-related stresses. *Int. J. Food Microbiol.* **2021**, *346*, 109159. [[CrossRef](#)] [[PubMed](#)]
17. Mangalappalli-Illathu, A.K.; Vidovic, S.; Korber, D.R. Differential adaptive response and survival of *Salmonella enterica* serovar enteritidis planktonic and biofilm cells exposed to benzalkonium chloride. *Antimicrob. Agents Chemother.* **2008**, *52*, 3669–3680. [[CrossRef](#)]
18. Ju, X.; Li, J.; Zhu, M.; Lu, Z.; Lv, F.; Zhu, X.; Bie, X. Effect of the *luxS* gene on biofilm formation and antibiotic resistance by *Salmonella* serovar Dublin. *Food Res. Int.* **2018**, *107*, 385–393. [[CrossRef](#)]

19. Gonzalez, J.F.; Alberts, H.; Lee, J.; Doolittle, L.; Gunn, J.S. Biofilm Formation Protects Salmonella from the Antibiotic Ciprofloxacin In Vitro and In Vivo in the Mouse Model of chronic Carriage. *Sci. Rep.* **2018**, *8*, 222. [[CrossRef](#)] [[PubMed](#)]
20. Lee, B.H.; Cole, S.; Badel-Berchoux, S.; Guillier, L.; Felix, B.; Krezdorn, N.; Hebraud, M.; Bernardi, T.; Sultan, I.; Piveteau, P. Biofilm Formation of *Listeria monocytogenes* Strains Under Food Processing Environments and Pan-Genome-Wide Association Study. *Front. Microbiol.* **2019**, *10*, 2698. [[CrossRef](#)]
21. Wisniewski, P.; Zakrzewski, A.J.; Zadernowska, A.; Chajęcka-Wierzchowska, W. Antimicrobial Resistance and Virulence Characterization of *Listeria monocytogenes* Strains Isolated from Food and Food Processing Environments. *Pathogens* **2022**, *11*, 1099. [[CrossRef](#)] [[PubMed](#)]
22. Kaptchouang Tchatchouang, C.D.; Fri, J.; Montso, P.K.; Amagliani, G.; Schiavano, G.F.; Manganyi, M.C.; Baldelli, G.; Brandi, G.; Ateba, C.N. Evidence of Virulent Multi-Drug Resistant and Biofilm-Forming *Listeria* Species Isolated from Various Sources in South Africa. *Pathogens* **2022**, *11*, 843. [[CrossRef](#)]
23. Hawas, S.; Verderosa, A.D.; Totsika, M. Combination Therapies for Biofilm Inhibition and Eradication: A Comparative Review of Laboratory and Preclinical Studies. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 850030. [[CrossRef](#)]
24. Laure, N.N.; Ahn, J. Antibiofilm Activity of beta-Lactam/beta-Lactamase Inhibitor Combination against Multidrug-Resistant *Salmonella* Typhimurium. *Pathogens* **2022**, *11*, 349. [[CrossRef](#)]
25. Dawan, J.; Li, Y.; Lu, F.; He, X.; Ahn, J. Role of Efflux Pump-Mediated Antibiotic Resistance in Quorum Sensing-Regulated Biofilm Formation by *Salmonella* Typhimurium. *Pathogens* **2022**, *11*, 147. [[CrossRef](#)] [[PubMed](#)]
26. Al-Dulaimi, M.; Algburi, A.; Abdelhameed, A.; Mazanko, M.S.; Rudoy, D.V.; Ermakov, A.M.; Chikindas, M.L. Antimicrobial and Anti-Biofilm Activity of Polymyxin E Alone and in Combination with Probiotic Strains of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895 against Clinical Isolates of Selected *Acinetobacter* spp.: A Preliminary Study. *Pathogens* **2021**, *10*, 1574. [[CrossRef](#)]
27. Cacciatore, F.A.; Brandelli, A.; Malheiros, P.D.S. Combining natural antimicrobials and nanotechnology for disinfecting food surfaces and control microbial biofilm formation. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 3771–3782. [[CrossRef](#)] [[PubMed](#)]
28. Esposito, M.M.; Turku, S. The Use of Natural Methods to Control Foodborne Biofilms. *Pathogens* **2022**, *12*, 45. [[CrossRef](#)]
29. Chitlapilly Dass, S.; Wang, R. Biofilm through the Looking Glass: A Microbial Food Safety Perspective. *Pathogens* **2022**, *11*, 346. [[CrossRef](#)] [[PubMed](#)]

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