Fishery Technology 59 (2022) : 49 - 55

Listeria monocytogenes Biofilms on Glass surface: Survival and Efficacy of two Sanitizing Agents for Inactivation

Sanjoy Das[1]* and K. V. Lalitha[1#]
[1]*ICAR- Central Institute of Fisheries Technology, P. O. Mastypuri, Cochin - 682 029, India*

Abstract

Listeria monocytogenes is a very important seafood-borne pathogen, which is capable of forming biofilm on different food-contact surfaces. The efficiency of two commonly used sanitizing agents of seafood-processing industry was checked for inactivation of this pathogen in biofilm formed on glass surface. Treatment with 220 ppm sodium hypochlorite for 5 min and 1000 ppm benzalkonium chloride for 30 sec could totally inactivate the *L. monocytogenes* ATCC 19115 in the biofilm. Reduction of the level of this organism to a great extent was possible even at lower concentration in case of both the sanitizers. However, the planktonic cells were found to be highly susceptible. Treatment with 130 ppm sodium hypochlorite for 5 min or 100 ppm benzalkonium chloride for 30 sec can totally inactivate planktonic cells of *L. monocytogenes*. During storage at 30ºC, the *L. monocytogenes* could be recovered from the biofilm upto 26 days of storage. The concentration of chlorine (50-100 ppm), which is commonly used for disinfection different surfaces of seafood-processing plants, can reduce the level of this pathogen to a great extent. The findings of the present study will be helpful in designing proper guidelines for prevention of transmission of *L. monocytogenes* through seafood-processing plants.

Keywords: *Listeria*; *monocytogenes*; biofilm; sodium hypochlorite; benzalkonium chloride

Received 01 September 2021; Revised 21 January 2022; Accepted 21 January 2022

**E-mail: sanjoy125@yahoo.co.in*

Present Address: Kakdwip Research Centre, ICAR- Central Institute of Brackishwater Aquaculture, Kakdwip, South 24 Parganas, West Bengal - 743 347, India

#Kuttanappilly House, Vennala P.O., Cochin - 682 028, Kerala, India

Introduction

Listeria monocytogenes is one of the leading food-borne pathogens causing various illnesses including abortion in pregnant women, septicaemia, gastroenteritis, arthritis, etc. in human beings (Ramaswamy et al., 2007). The mortality rate of disease caused by this pathogen is very high and the overall case fatality rate was found to be as high as 15.6% in European Union countries (EFSA, 2019). The ability of this organism to grow at refrigerated temperature (2-8ºC) is the reason for considering it as a severe public health hazard especially in ready-to-eat foods, which are usually stored at refrigerated temperature (Mazaheri et al., 2021). Considering the immense importance of the pathogen, United States Food and Drug Administration (USFDA) imposed 'zero tolerance' to this pathogen in ready-to-cook food items (Gravani, 1999). The infective dose of this organism has been found to be 100 cells per g of food at the time of consumption (Norrung, 2000). This organism is well known to produce biofilm on different food-contact surfaces in seafood processing plants. *Listeria* cells in biofilm are usually very much resistant to different sanitizing agents due to presence of extracellular polymeric substances that act as barrier against exposure to the sanitizing agents (Pan et al., 2006; Rodriguez-Melcon et al., 2019). Due to high resistance in nature, different commonly using sanitizing agents at their respective recommended concentration cannot completely eliminate different pathogenic bacteria including *L. monocytogenes* in the biofilm on food-contact surfaces (Møretrø et al., 2013). In the biofilm, the cells of *L. monocytogenes* remain viable for prolonged period of time and it may serve as a source of contamination to food items, which may come in contact of the contaminated surface (Mazaheri et al., 2021). The ability to produce biofilm varies among different strains and different food-contact surfaces and is also utmost public health importance. Glass surface is also considered as a suitable abiotic

surface, on which *L. monocytogenes* can form biofilm (Arizcun et al., 1998). It has been reported that different types of food soils have protective effect on *L. monocytogenes* cells during desiccation including fresh and cold-smoked salmon and tuna resulting in prolonged survival of this pathogen (Vogel et al., 2010; Takahashi et al., 2011). Thus, it is also very much required to study the survival period of this pathogen in the biofilm.

Sodium hypochlorite containing active free chlorine and benzalkonium chloride are two commonly used sanitizers in seafood processing industry (Rodriguez-Melcon et al., 2019). Sodium hypochlorite is one of the ideal disinfectants in seafood processing plants because of its strong antimicrobial activity and poor stability, due to which it doesn't leave any harmful residue on the food-contact surfaces. As a disinfectant, sodium hypochlorite has been classified as chlorine-releasing agents, which are usually the oxidizing agents and is capable of destroying cellular activities of different bacterial proteins (Bloomfield, 1996; Bansal et al., 2021). Under classification of disinfectants, benzalkonium chloride comes under quaternary ammonium compounds, which are cationic detergents. Generally, these compounds are membrane acting agents and mostly exert their action on cytoplasmic membrane of bacterial cells (McDonnell & Russell, 1999). Benzalkonium chloride residue is also permitted in food products upo 1 mg kg^{-1} level as per European Commission directives (Pereira & Tagkopoulos, 2019). Although the most common food-contact surface in seafood-processing plants are stainless steel and plastic materials, the food-contact surface may also consist of other materials including glass especially during packaging and handling (Skara & Rosnes, 2016). Till date, several studies have been carried out on biofilm formation of *L. monocytogenes* on stainless-steel surface and plastic coupon. However, the similar studies on biofilm formation of this pathogen on glass surface is comparatively scanty. Moreover, there is no reported study on survival of this pathogenic organism in the biofilm formed on glass surfaces. Keeping these facts in mind, the present study has been designed with the objectives of examining the effect of sodium hypochlorite and benzalkonium chloride on the attached cells of *L. monocytogenes* on the glass surface and also to assess the survival of this pathogen in the biofilm produced on glass surface during storage.

Materials and Methods

Listeria monocytogenes strain ATCC 19115, used in this study, was purchased from HiMedia Laboratories Pvt Ltd. The strain was preserved at -80°C in tryptic soya broth (BD Difco, USA) supplemented with 0.6% yeast extract and 25% glycerol. From this frozen stock, tryptic soya agar supplemented with 0.6% yeast extract (TSAYE) plates were inoculated and was incubated at 37°C for 48 h. The single colonies from the agar plates were picked up and was inoculated into 10 ml of tryptic soya broth with 0.6% yeast extract (TSBYE) and incubated at 37°C for around 20 h. This overnight grown culture was centrifuged at 8000 × g for 10 min at 4°C and the bacterial pellet was suspended in 10 ml of phosphate buffered saline (PBS) (NaCl 8.5 g l^{-1}; Na_2HPO_4 1.91 g l^{-1}, KH_2PO_4 0.38 g l^{-1}, pH 7.2). Bacterial population of the suspension was determined using plate count method on TSAYE. The stock suspension of bacterial cells was prepared by mixing appropriate volume of bacterial suspension and PBS to reach final cell concentration of 10^8 CFU of *L. monocytogenes* / ml.

New microscopic slides with surface area of 18.75 cm^2 (7.5 × 2.5 cm) were used in this study. The slides were boiled in distilled water for 30 min and soaked in liquid detergent for 4 h. Then the slides were cleaned by rubbing both the sides with a clean cloth and were dipped in double glass distilled water thrice to remove the remaining detergent. The slides were air-dried, wrapped in aluminium foil and were sterilized by autoclaving at 15 lbs pressure for 15 min.

The low nutrient medium containing tryptic soya broth (2 g l^{-1}) and D-glucose (8 gl^{-1}) as described by Frank & Koffi (1990) was used for growing biofilm. The glass slides were placed in 250 ml wide mouth conical flask containing 200 ml of above-mentioned diluted media and were inoculated with 0.2 ml of prepared stock solution of *L. monocytogenes* in PBS. The flasks containing glass slides were incubated at 30°C for 48 h. Two glass slides were kept in one flask. After incubation, the slides were taken out of flasks separately, washed with sterile PBS to remove the unattached cells and placed in a flask containing fresh diluted media and were incubated at 30°C for another 48 h.

Listeria monocytogenes biofilms formed on slides were exposed to two commercial sanitizing agents

commonly used in seafood processing industry such as sodium hypochlorite and benzalkonium chloride. The actual effective chlorine content of sodium hypochlorite stock solution was measured by a standard procedure of titration with sodium thiosulfate solution as mentioned in Carlos & Cintia (2013). The glass slides were washed with sterile PBS and one set of glass slides was sub-merged in 200 ml of sterile distilled water containing appropriate concentrations of sodium hypochlorite containing chlorine ranging from 10 to 400 ppm with increment of 10 ppm for 5 min and another set was submerged in 200 ml of sterile distilled water containing appropriate concentrations of benzalkonium chloride (50 to 1000 ppm with an increment of 50 ppm) for 30 sec. After exposure, the slides were removed and then placed in suitable appropriate neutralization solution (0.1% sterile sodium thiosulfate solution in case of sodium hypochlorite and neutralization solution consisting of polysorbate 80, sodium thiosulfate and lecithin as mentioned in Karayildirim & Celenk (2016) in case of benzalkonium chloride) for 1 min. The control slides were not exposed to disinfectant and were kept in 200 ml of sterile distilled water for 5 min and placed in the respective neutralization solution. The attached bacterial cells on glass slides that were exposed to sanitizers were removed using a sterile cotton swab by vigorous rubbing. The cotton swabs were placed in sterile PBS and vortexed at high speed to obtain attached bacterial cells. Different dilutions were made in PBS and were plated onto TSAYE. The plates were incubated at 37°C for 24 h and were counted. The bacterial population was expressed by Log_{10} CFU cm^{-2}.

In another set of experiment, biofilms on glass slides were exposed to sanitizers as described above and after exposure, instead of swabbing the slides with cotton, the slides were washed once in sterile PBS, transferred to respective neutralizing solution and placed in TSBYE. They were incubated at 37°C for 48 h and observed for visible growth. The visible growth was again confirmed as *L. monocytogenes* by streaking over a *Listeria* selective agar (Modified Oxford medium, BD Difco, USA) and by polymerase chain reaction (PCR) assay using primer pair LL5 /LL6 as mentioned in Herman et al. (1995) and Das et al. (2013). The study was conducted in triplicate and lowest concentration of sodium hypochlorite, which can inhibit the growth of *L. monocytogenes* in all three replicates, were noted.

Five ml from previously prepared stock solution (10^8 CFU ml^{-1}) of *L. monocytogenes* was mixed with 5 ml of appropriately diluted sodium hypochlorite solution to make varying final concentration of effective chlorine (20 to 140 ppm chlorine with 10 ppm increment). After 5 min exposure, the effect of chlorine was neutralized by 1 ml of 10% (w/v) sodium thiosulfate solution. For each chlorine concentration, three replicates were used. Following neutralization, 100 µl from this mixture was inoculated into 10 ml TSBYE and incubated at 37°C for 5 days to observe for any visible growth. The content of tubes exhibiting growth was confirmed as *L. monocytogenes* by streaking on *Listeria* selective agar and by PCR assay as mentioned previously. The lowest concentration of chlorine, in which no visible growth was observed even after 5 days in all three replicates were recorded.

In another set of experiment, the stock solution (5 ml) of *L. monocytogenes* (10^8 CFU ml^{-1}) was mixed with appropriate dilution of benzalkonium chloride to make the final concentration of 50 to 1000 ppm with an increment of 50 ppm (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 ppm) and the exposure time was 30 seconds. The neutralizing solution as described by Karayildirim & Celenk (2016) was used. Following neutralization, 100 µl from this mixture was inoculated into TSBYE and was incubated at 37°C for 5 days to observe for growth. The visible growth was confirmed as *L. monocytogenes* by streak plate method and by PCR assay as described above.

For survival study, formation of biofilm on the glass slide was carried out as mentioned previously. The glass slides containing biofilm were placed in 500 ml screw capped glass bottles and incubated at 30°C. The relative humidity level was measured twice daily. Three slides were taken out at 0, 3, 7, 11, 15, 19, 23, 27 and 30 days of incubation. The slides were washed in sterile PBS to remove the unattached cells. The unattached cells were removed by vigorous cotton swabbing and counting was done by plating on TSAYE mentioned previously.

Similarly in another set of experiment, three slides were taken out at 0, 3, 7, 11, 15, 19 days and every day post- 19 days upto 35 days of incubation (20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35 days), washed in sterile PBS and placed in TSBYE as mentioned previously. Following that, it

was incubated at 37°C overnight to observe any visible growth and the growth was confirmed as *L. monocytogenes* as mentioned previously.

Results and Discussion

Initially, the concentration of bacterial cells in biofilms on glass slide was 5.893 ± 0.06 Log_{10} CFU cm^{-2}. However, exposure to 10 ppm of effective chlorine for 5 min reduced the level of *L. monocytogenes* cells in biofilm to 1.538 ± 0.212 Log_{10} CFU cm^{-2} (Fig. 1). Further increase of concentration of chlorine resulted in reduction of concentration of bacterial cell by more than 99.99% in the biofilm to below 1.1 Log_{10} CFU cm^{-2}. But still *L. monocytogenes* could be recovered from those slides, when sodium hypochlorite treated slides were dipped in TSBYE. Treatment with 220 ppm of effective chlorine for the same duration was found to completely inactivate the cells of *L. monocytogenes* in the biofilm in all the three replicates. *L. monocytogenes* could not be recovered from those slides when dipped in TSBYE and incubated at 37°C.

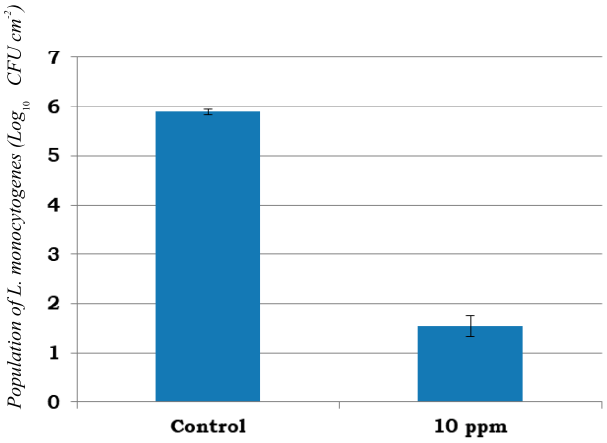

Fig. 1. Effect of chlorine on *L. monocytogenes* in biofilm on glass slide: Control (5.893 ± 0.06), 10 ppm (1.538 ± 0.212), Unit: Log_{10} CFU cm^{-2}.

Exposure to 50, 100 and 150 ppm of benzalkonium chloride could reduce the population of *L. monocytogenes* in the biofilm attached to glass surface by 1.282, 2.339 and 2.975 Log_{10} value, respectively with respect to control (5.890 ± 0.073 CFU cm^{-2}) (Fig. 2). The treatment of *L. monocytogenes* biofilms with concentration of 200 ppm and above reduced the population below 1.1 Log_{10} CFU cm^{-2}. However, the *L. monocytogenes* still could be recovered from those slides even after treatment with 950 ppm of benzalkonium chloride. But, no *L. monocytogenes* was recovered from those slides when treated with 1000 ppm of benzalkonium chloride for 30 seconds.

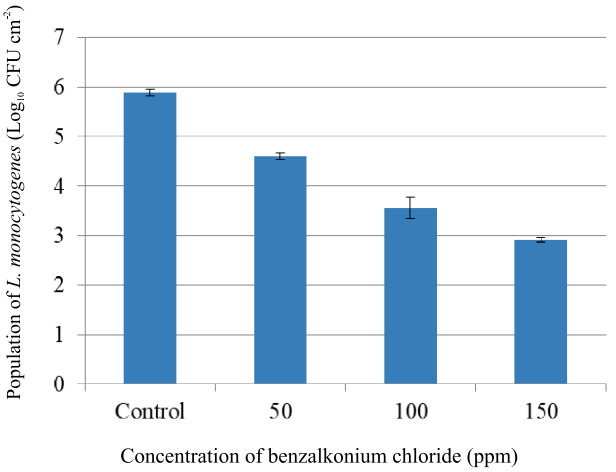

Fig. 2. Effect of benzalkonium chloride on *L. monocytogenes* ATCC 19115 in biofilm on glass slide: Control (5.890 ± 0.073), 50 ppm (4.608 ± 0.065), 100 ppm (3.551 ± 0.216) and 150 ppm (2.915 ± 0.046). Unit: Log_{10} CFU cm^{-2}.

It was observed that exposure to 130 ppm effective chlorine for 5 min could totally inactivate *L. monocytogenes* as evidenced by no visible growth in the inoculated TSB broth. The planktonic cell suspension of *L. monocytogenes* showed a high degree of sensitivity to benzalkonium chloride compared to attached cell and it was observed that just 100 ppm of this sanitizer was able to totally inactivate planktonic form of *L. monocytogenes* in just 30 seconds.

On initial day (Day 0), the concentration of *Listeria* cells in the biofilm of glass slide was estimated as 5.824 ± 0.089 Log_{10} CFU cm^{-2}. The concentration of viable cells decreased with passage of time. On Day 3, 7, 11 and 15, the counts of 4.955 ± 0.145, 3.799 ± 0.24, 2.674 ± 0.248 and 1.337 ± 0.573 Log_{10} CFU cm^{-2}, respectively (Fig. 3) were noticed. On Day 19 onwards, the count was below 1.1 Log_{10} CFU cm^{-2}. When glass coupons were dipped into TSBYE broth, viable *L. monocytogenes* could be recovered upto 26 days of incubation. However, no viable *L. monocytogenes* could be recovered in TSBYE on day 27 onwards. The relative humidity during the study varied from 78 to 95%.

Biofilm formation by different food-borne bacterial pathogens on different food-contact surfaces of

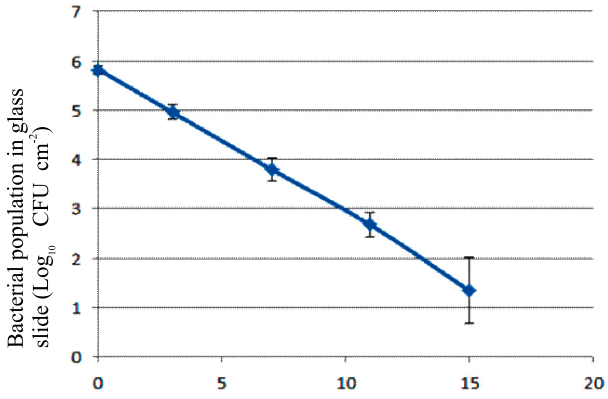

Fig. 3. Survival of *L. monocytogenes* ATCC 19115 on biofilm on glass slide: Day 0: 5.824 ± 0.089, Day 3: 4.955 ± 0.145, Day 7: 3.799 ± 0.24, Day 11: 2.674 ± 0.248, Day 15: 1.337 ± 0.673, Day 19 and 23: Below 1.1 (Unit: Log_{10} CFU cm^{-2}).

seafood processing industries is really hazardous from public health point of view as very often biofilm forms a barrier against different sanitizing agents (Pan et al., 2006). Giaouris et al. (2012) reported that biofilm formation in food-borne bacteria enhances their capacity to survive stressors that are commonly found in the food-processing environment such as refrigeration, acidity, salinity and disinfection. It has been shown that biofilms of *Listeria* are much more resistant to stress and to sanitizing agents than planktonic cells (Chavant et al., 2004). Here in this study, it is observed that exposure to 220 ppm of effective chlorine for 5 min or 1000 ppm of benzalkonium chloride for 30 sec can totally inactivate *L. monocytogenes* cells in biofilms attached to glass surface. It was also observed that exposure to low concentration of the sanitizer (20 ppm for 5 min in case of sodium hypochlorite and 200 ppm for 30 sec in case of benzalkonium chloride) could reduce the population *L. monocytogenes* in the biofilm of glass slide by more than 99.99% leading to recoverable bacterial level below 1.1 Log_{10} CFU cm^{-2}. However, *L. monocytogenes* could still be recovered from biofilms exposed to sanitizing agents by dipping the glass slides in nutrient rich media (TSBYE). It may be attributed to the injury of only certain proportion of bacterial cells in biofilms with exposure to the sub-lethal dose as both the sanitizers used in this study are known to cause injury to the bacterial cells (Scheusner et al. 1971). The organisms in biofilms are reported to enter into Viable but non-cultivable (VBNC) state (Gião & Keevil, 2014), and as a result the recovery was not there on TSAYE plates. On inert surface, the microbial biofilm formation generally takes place in different layers leading to a very complex structure (Brenner & Arnold, 2011). Swabbing with cotton swab might not have detached all the bacterial cells from the biofilm attached to the glass surface and as a result comparatively lower bacterial load was observed on TSAYE plates.

Past studies in this aspect showed that the reduction of *L. monocytogenes* in the biofilm varies from surface to surface (Mazaheri et al. 2021). It was observed that 200 ppm of chlorine could completely inactivate *L. monocytogenes* in biofilm attached to stainless steel surface with exposure time of 5 min. By using scanning electron microscopy, Bansal et al. (2021) showed that continuous exposure to sodium hypochlorite caused structural changes in biofilm produced by *L. monocytogenes* on polystyrene surface resulting in reduced attachment. In their study, it was also observed by qPCR that the expression of biofilm formation related genes was downregulated by sub-inhibitory concentration of sodium hypochlorite. However, a reduction of only 3 Log_{10} units was reported on plastic surface when exposed to same concentration of sodium hypochlorite for the same duration (Jeyasekaran et al., 2000). In the current study, it was observed that free planktonic cells of the organism were highly sensitive to both the sanitizers used in this study as compared to biofilm-adherent cells. Similar type of observation was also noticed by various past workers in their respective studies (Frank and Koffi, 1990; Jeyasekaran et al., 2000; Joseph et al., 2001).

The current study showed that the viable *L. monocytogenes* could be recovered from the biofilms on glass slide upto 26 days of incubation while stored at 30°C. Hansen & Vogel (2011) observed that in biofilm formed on stainless steel surface, the viability of *L. monocytogenes* dropped rapidly in initial 2 days and it was followed by slowing down of rate of inactivation. However, the initial level of the organism was high (8.5-8.9 Log_{10} CFU cm^{-2}) in that study on stainless steel surface at 15°C with 100% relative humidity (Hansen & Vogel 2011). In comparison to the above study, the current study showed that on the glass slide, the initial level of organism was 5.824 ± 0.989 Log_{10} CFU cm^{-2} and survival was noticed up to 26 days of incubation.

In seafood processing plants, generally food-contact surfaces are disinfected using chlorinated water

containing 50-100 ppm of chlorine. Chlorinated water with above level of chlorine may not be able to totally eliminate *L. monocytogenes* in the biofilm. However, it may reduce the level of the pathogen by more than 99.99% rendering the chance of causing disease by transmission from food-contact surface very less as the infective dose of this pathogen is around 100 g^{-1} of food at the time of consumption (Norrung, 2000). However, the stringent precautionary measures with regards to chlorine concentration for disinfection of food-contact surfaces may be adopted by the food processing industries dealing with ready-eat-food, which supports the growth of this pathogen. The current study provides a proper insight into inactivation of *L. monocytogenes* biofilms on glass slides by two commonly used sanitizers in seafood industry viz. sodium hypochlorite and benzalkonium chloride. However, the biofilm production on one surface may not correlate with biofilm formation on different surfaces. Hence the results of this study warrant further investigation involving more number of isolates on biofilm formation of *L. monocytogenes*on various abiotic surfaces with regards to seafood-processing plants to formulate guidelines for inactivation of *L. monocytogenes* in their biofilms on various surfaces in seafood-processing industry.

References

Arizcun, C., Vasseur, C. and Labadie, J.C. (1998) Effect of several decontamination procedures on *Listeria monocytogenes* growing in biofilms. J. Food Prot. 61: 731-734

Bansal, M., Dhowlaghar, N., Nannapaneni, R., Kode, D., Chang, S., Sharma, C.S., McDaniel, C and Kiess, A (2021) Decreased biofilm formation by planktonic cells of *Listeria monocytogenes* in the presence of sodium hypochlorite. Food Microbiol. 96: 103714

Bloomfield, S.F. (1996) Chlorine and iodine formulations. In: Handbook of disinfectants and antiseptics (Ascenzi, J.M. Ed.)pp 133-158, Marcel Dekker, Inc., New York, USA

Brenner, K. and Arnold, F.H. (2011) Self-organization, layered structure and aggregation enhance persistence of a synthetic biofilm consortium. PLoS one 6: e16791

Carlos, N.J. and Cintia, M.R. (2013) A new simple and practical method for establish available chlorine. Analytical Chemistry 12: 415-422

Chavant, P., Gaillard-Martine, B. and Hebraud, M. (2004) Antimicrobial effects of sanitizers against planktonic and sessile *Listeria monocytogenes* cells according to the growth phase. FEMS Microbiol. Lett. 236: 241-248

Das, S., Lalitha, K.V., Thampuran, N. and Surendran, P.K. (2013) Isolation and characterization of *Listeria monocytogenes* from tropical seafood of Kerala, India. Ann. Microbiol. 63: 1093-98

EFSA (2019) The European Union one health 2018 zoonoses report. EFSA Journal. https://doi.org/10.2903/j.efsa.2019.5926

Frank, J.F. and Koffi, R.A. (1990) Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to sanitizers and heat. J. Food Prot. 53: 550-554

Gião, M.S. and Keevil, C.W. (2014) *Listeria monocytogenes* can form biofilms in tap water and enter into the viable but non-cultivable state. Microb. Ecol. 67: 603-611

Giaouris, E. and Chorianopoulos, N. (2012) Attachment and biofilm formation by *Salmonella* in food processing environments. In: *Salmonella*: A dangerous foodborne pathogen BSM (Mahmoud, B., Ed.) pp 157-180, Intech Open Access Publisher; Rijeka, Croatia

Gravani, R, (1999) *Listeria* in Food-Processing Facilities-Status of *L. monocytogenes* in Foods In: *Listeria,* Listeriosis and Food Safety (Ryser, E,T, and Marth, E.H., Eds.). pp 657-710, Marcel Dekker, Inc. New York, USA

Hansen, L.T. and Vogel, B.F. (2011) Desiccation of adhering and biofilm *Listeria monocytogenes* on stainless steel; survival and transfer to salmon products. Int. J. Food Microbiol. 146: 88-93

Herman, L.M.F., De Block, J.H.G.E. and Moermans, R.J.B. (1995) Direct detection of *Listeria monocytogenes* in 25 milliliters of raw milk by a two step PCR with nested primers. Appl. Environ. Microbiol. 61: 817-819

Jeyasekaran, G., Karunasagar, I. and Karunasagar, I. (2000) Effect of sanitizers on *Listeria* biofilm on contact surfaces. Asian Fish. Sci. 13: 209-213

Joseph, B., Otta, S.K., Karunasagar, I. and Karunasagar, I. (2001) Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. Int. J. Food Microbiol. 64: 367-372

Karayildirim, C.K. and Celenk, V.U. (2016) Antibacterial efficiency of benzalkonium chloride base disinfectant according to European standard 13727, chemical analysis and validation studies. CBU J. Sci. 12: 39-47

Mazaheri, T., Carvantes-Huaman, B.R.H., Bermudez-Capdevila, M., Ripolles-Avila, C. and Rodriguez-Jerez, J.J. (2021) *Listeria monocytogenes* biofilms in the food industry: is the current hygiene programme sufficient to combat the persistence of the pathogen? Microorganisms. https://doi.org/10.3390/microorganisms9010181

McDonnell, G. and Russell, A.D. (1999) Antiseptics and disinfectants: activity, action and resistance. Clin. Microbiol. Rev. 12: 147-179

Møretrø, T., Langsrud, S. and Heir, E. (2013) Bacteria on meat abattoir process surfaces after sanitation: Characterisation of survivalproperties of *Listeria monocytogenes* and the commensal bacterial flora. Adv. Microbiol. 3: 255-264

Norrung, B. (2000) Microbiological criteria for *Listeria monocytogenes* in foods under special consideration risk assessment approaches. Int. J. Food Microbiol. 62: 217-221

Pan, Y., Breidt, F. Jr and Kathariou, S. (2006) Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. Appl. Environ. Microbiol. 72: 7711-7717

Pereira, B.M.P. and Tagkopoulos, I. (2019) Benzalkonium chlorides: uses, regulatory status, and microbial resistance. Appl. Environ. Microbiol. 85: e00377: 19

Ramaswamy, V., Cresence, V.M., Rejitha, V.S., Lekshmi, M.U., Dharsana, K.S., Prasad, S.P. and Vijila, H.M. (2007) *Listeria*- review of epidemiology and pathogenesis. J. Microbiol. Immunol. Infect. 40: 4-13

Rodriguez-Melcon, C., Riesco-Pelaez, F., Garcia-Fernandez, C., Alonso-Calleja, C. and Capita, R. (2019) Susceptibility of *Listeria monocytogenes* planktonic cultures and biofilms to sodium hypochlorite and benzalkonium chloride. Food Microbiol. 82: 533-540

Scheusner, D.L., Busta, F.F. and Speck, M.L. (1971) Injury of bacteria by sanitizers. Appl. Microbiol. 21: 41-45

Skara, T. and Rosnes, J.T. (2016) Emerging methods and principles in food contact surface decontamination/ prevention. In: Innovation and future trends in food manufacturing and supply chain technologies (Leadley, C.E., Ed.) Woodhead Publishing. pp 151-172

Takahashi, H., Kuramoto, S., Miya, S. and Kimura, B. (2011) Desiccation survival of *Listeria monocytogenes* and other potential foodborne pathogens on stainless steel surfaces is affected by different food soils. Food Control 22: 633-637

Vogel, B.F., Hansen, L.T., Mordhorst, H. and Gram, L. (2010) The survival of *Listeria monocytogenes* during long term desiccation is facilitated by sodium chloride and organic material. Int. J.Food Microbiol. 140: 192-200