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The effect of initial sonication on disinfectant efficacy against *Listeria monocytogenes* biofilm

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ABSTRACT

Background: *Listeria monocytogenes* is a Gram-positive, foodborne pathogen. Biofilms formed by this bacterium are a serious problem in the food industry. Bacteria in biofilms are much more resistant to cleaning and disinfection agents posing a risk of food recontamination. The aim of this study was the assessment of the influence of initial sonication on disinfectant efficacy, based on QAC, against *L. monocytogenes* biofilm on the stainless steel.

Methods: The biofilm formed on the stainless steel by the reference strain *L. monocytogenes* ATCC 19111 was sonicated for 1 and 5 minutes (500W/ 20kHz/ 100% amplitude). Then disinfection with quaternary ammonium compounds (0.5% working solution) was applied for 1 and 5 minutes and the number of bacteria recovered from the biofilm was assessed.

Results: It was found that disinfection was more efficient than sonication ($p \leq 0.05$). However, the combination of sonication and disinfection significantly improved biofilm eradication compared to the use of one of these methods separately ($p \leq 0.05$). The greatest reduction of bacteria number was achieved after 5 minutes of sonication combined with 5 minutes of disinfection ($6.42 \log \text{CFU} \times \text{cm}^{-2}$), whereas the lowest reduction was observed after 1 minute-sonication ($2.03 \log \text{CFU} \times \text{cm}^{-2}$).

Conclusions: Combination of sonication and disinfection based on quaternary ammonium compounds is an effective method allowing biofilm eradication from the food production surfaces.

Key words: *Listeria monocytogenes*, biofilm, sonication, disinfectants, quaternary ammonium compounds

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Introduction

Listeria monocytogenes is a cause of human listeriosis, dangerous especially for pregnant women and an elderly [1]. Since the pathogen is widespread in the environment and food plants it may easily contaminate food. The bacterium was isolated from the variety of food products, including vegetables, fish, meat and dairy products [2].

An important problem in the food industry is recontamination and cross-contamination of food due to biofilm formation ability by *L. monocytogenes* [2]. The pathogen is able to colonize both abiotic and biotic surfaces [3]. In biofilm, *L. monocytogenes* is more resistant to disinfectants, UV light, mechanical cleaning and disinfection [1, 5]. The biofilm structure was shown to be affected by the type of the surface. The biofilms formed

on the stainless steel were easier to eradicate compared to biofilms on the polyethylene [4]. Effective disinfection is a key factor allowing biofilm eradication and food safety. Quaternary ammonium compounds (QAC) are cationic agents that act by cell membrane disruption. QAC are widely used in hospitals, household and food industry [6]. A serious problem determining bacteria resistance in the biofilm structure is the synthesis of EPS (Extracellular Polymeric Substances) [1, 5]. This structure limits disinfectants penetration so eventually they work in a subinhibitory concentration and may generate disinfectant resistance [7]. The disinfection effectiveness through biofilm disintegration might be increased by an application of enzymatic methods or ultrasounds in a range 20–100 Hz [7, 8]. Ultrasound waves of this frequency, in the liquid environment, contribute to cavitation and change of pressure [8].

Cavitation may destruct biofilm architecture releasing single bacterial cells that are more susceptible to chemical disinfection [1].

The aim of this study was the assessment of the influence of initial sonication on disinfectant efficacy, based on QAC, against *L. monocytogenes* biofilm on the stainless steel.

Materials and methods

Materials

The study was conducted on the reference strain *L. monocytogenes* ATCC 19111. Stainless steel AISI 304 coupons of 1 cm × 2 cm and disinfectant based on QAC were used. Coupons were washed in detergent, soaked in 70% ethanol (POCH), washed with sterile water, dried and autoclaved.

The experiment was carried out in three replications.

Biofilm formation by *L. monocytogenes* strain

In a tube of bacterial suspension in Brain Heart Infusion (BHI, Becton Dickinson) (0.5 McFarland scale) a sterile stainless steel coupon was placed and was incubated for 72 hours at 37°C. The negative control were coupons placed in sterile BHI. Every 24 hours coupons were rinsed with sterile PBS and medium was changed into a fresh BHI. Then coupons were shaken in PBS at 400 rpm for 30 min., 10-fold serial dilutions were made and plated onto Columbia Agar with 5% of sheep blood (Becton Dickinson). After 24-hour incubation at 37°C, the number of bacteria per cm² was calculated.

Sonication of *L. monocytogenes* biofilm on stainless steel coupons

Coupons with biofilm were placed in a beaker containing 500 ml of sterile PBS. The height of the liquid layer above coupons was 6 cm. The sonicator probe (Sonicator VCX500, Sonics) of 19 mm diameter was placed in the beaker and the samples were sonicated for 1 and 5 minutes (500W/20kHz/100% amplitude).

Assessment of the effectiveness of disinfection based on QAC

Coupons after sonication were exposed to 0.5% QAC disinfectant for 1 and 5 minutes. Subsequently, coupons were neutralized for 2 min in a solution of Tween 80 (Sigma Aldrich) –10 g; lecithin (Sigma Aldrich) — 1 g; histidine-L (Sigma Aldrich). Finally, coupons were rinsed with a sterile PBS, shaken at 400 rpm for 30 min and serial 10-fold dilutions were

made and plated onto Columbia Agar with 5% of sheep blood. After 24-hour incubation at 37°C, the number of bacteria per cm² was calculated. The control variant were coupons with formed biofilm, immersed in PBS for 1 and 5 min, treated with QAC, but not sonicated.

Statistical analysis

Statistical analysis was made using STATISTICA 12 PL software (StatSoft). For each variant mean of 3 replicates was calculated. The statistical differences between the tested variants were evaluated using Tukey post-hoc test at the significance level $\alpha = 0.05$.

Results

The number of bacteria reisolated from biofilm was 7.11 log CFU × cm⁻². Both sonication and disinfection significantly reduced the number of bacteria, regardless of exposition time. The most efficient biofilm eradication was noted for the combination of sonication and disinfection (Fig. 1). The extension of sonication and disinfection time significantly increased the efficacy of both methods ($p \leq 0.05$). The greatest reduction of bacteria number was noted after 5-minute sonication, followed by 5-minute disinfection (6.42 log CFU × cm⁻²). In turn, the lowest reduction was observed after 1 minute of sonication (2.03 log CFU × cm⁻²). It was found that disinfection is more effective than sonication ($p \leq 0.05$). The reduction of bacteria number after 5-minute sonication and 5-minute disinfection was 2.99 log CFU × cm⁻² and 4.67 log CFU × cm⁻², respectively.

Discussion

The key element ensuring food safety of the consumer is control of cleaning and disinfection in the food-processing plants. An important problem in the food industry, hindering effective cleaning and disinfection, is a biofilm formation. This structure prevents bacteria from adverse environmental factors, e.g. disinfectants and UV light [1, 9]. In the present study sonication and disinfectant based on QAC were used to disrupt *L. monocytogenes* biofilm. It was shown that the combination of 5-minute sonication and QAC exposure resulted in the greatest reduction of the bacteria number (6.42 log CFU × cm⁻²). Bauman et al. (2009) [10] using sonication for 60s (20 kHz/ 100% amplitude/ 120 W) observed reduction of 3.8 log CFU/ml. In turn, other researchers [11–13] applying lower power of ultrasounds found the only minimal effect of sonication on biofilm

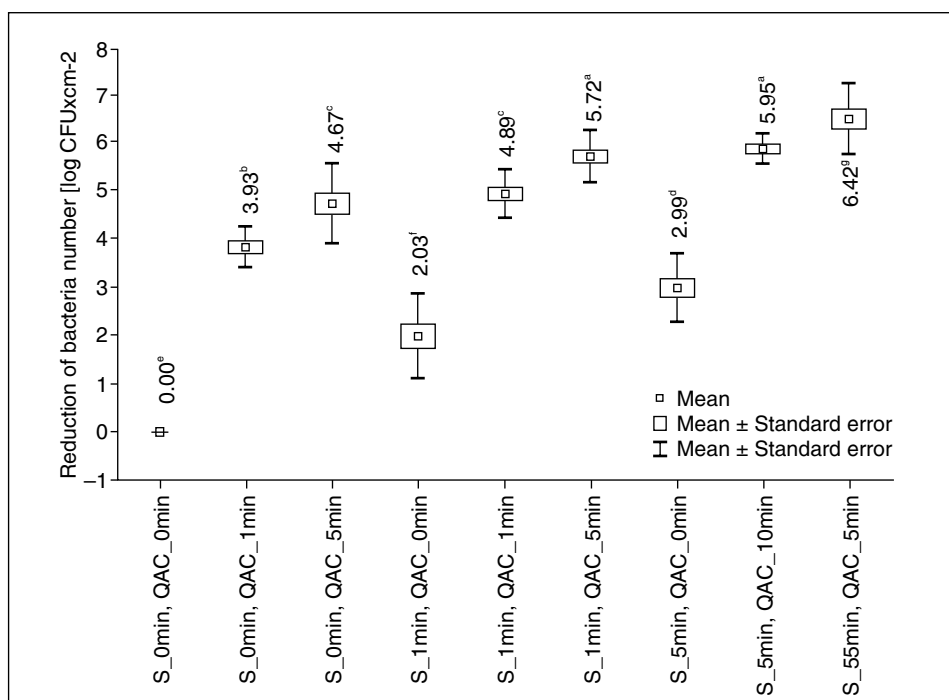


Figure 1. The reduction of bacteria number reisolated from biofilm exposed to sonication and QAC [a, b, c, ... — variables with different letters are statistically different ($p \leq 0.05$)]

eradication on the prosthesis surface. The frequency of ultrasounds also has an impact on biofilm elimination. In our study 20kHz was applied. Application of ultrasound of this frequency for 30 seconds was found to decrease bacteria number 10 times [1]. Bauman et al. (2009) [10] and Qian et al. (1996) [12] noticed that ultrasounds of 70kHz frequency better eliminate biofilm than the application of high frequency (500 kHz) ultrasounds. An important aspect is also the efficacy of the disinfectant. We showed that QAC was more effective in biofilm disruption than sonication. Torlak and Sort (2013) [14] using QAC found only 27% reduction of bacteria number on the plastic surface. However, Romanova et al. (2007) [15] stated that efficient disinfection with QAC requires at least 30 minutes.

In the present study, the greatest effectiveness in biofilm eradication was noted for the combination of sonication and QAC disinfection. This is in agreement with a study of Torlak and Sert (2013) [14] who demonstrated that regardless of time exposure the best eradication was achieved for the combination of sonication and benzalkonium chloride disinfection. Also, Berrang et al. (2008) [1] contended that sonication might improve disinfectants efficacy against bacterial biofilms. The results of mathematical modelling and conducted experiments revealed that the application of low-frequency ultrasounds boosts biomass transport

through biofilm [16–18]. Therefore, it can be assumed that an increase of biomass transport facilitates the transport of disinfectant compound to the biofilm structure [14].

Conclusions

We have demonstrated that the combination of sonication and disinfection based on QAC the most effectively eliminate biofilm from the stainless steel. Application of sonication might be an easy and cheap method to improve disinfection efficacy leading to *L. monocytogenes* biofilm eradication and food safety.

Conflict of interest

Authors declare no conflict of interest.

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