Effect of sub-lethal doses of nisin on Staphylococcus aureus toxin production and biofilm formation

Article *in* Toxicon · April 2021

DOI: 10.1016/j.toxicon.2021.03.018

4 authors, including:

Ali Shivaee
Iran University of Medical Sciences

Abbas Ali Imani Fooladi

Toxicon 197 (2021) 1–5

Contents lists available at ScienceDirect

Toxicon

journal homepage: http://www.elsevier.com/locate/toxicon

Effect of sub-lethal doses of nisin on *Staphylococcus aureus* toxin production and biofilm formation

Ali Shivaee [a], Sajad Rajabi [b], Hamed Eraghiye Farahani [c], Abbas Ali Imani Fooladi [a,*]

[a] Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] International Campus, Iran University of Medical Sciences, Tehran, Iran
[c] Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Handling Editor: Glenn King

Keywords:
Staphylococcus aureus
Nisin
Enterotoxin
Biofilm formation

ABSTRACT

Staphylococcus aureus is one of the commonest food-borne pathogens that can cause gastroenteritis owing to having several enterotoxins. Also, biofilm formation can complicate infections caused by this microorganism. Nisin is a safe food bio preservative which is usually used as an agent to prevent pathogen growth; however, it is important to identify the exact impact of nisin on the growth of *S. aureus* and to determine the suitable concentration needed for elimination of this pathogen in food. In this study, after MIC determination of nisin against *S. aureus* ATCC 29213, this strain was treated with sub-MIC (1/2) of nisin (4 μg/ml) and transcript levels of toxin-encoding (*hla*, *SEA*, *SEB*, and *SED*) and biofilm-associated (*fnb*, *ebpS*, *eno*, and *icaA*) genes were determined using Quantitative Real-time PCR at 2, 8, and 24 h post exposure. All toxin genes were down-regulated following exposure to sub-MIC of nisin, whereas biofilm-associated genes were up-regulated. The expression levels of *fnb* and *icaA* in *S. aureus* were highest after 8 h (4.5-fold and 6.8-fold increase, respectively), while the expression levels of *eno* and *ebpS* genes were highest after 2 h (3.3 and 4.5-fold increase, respectively). According to these results, although transcriptional levels of toxin genes were reduced, sub-MIC concentrations of nisin could trigger the expression of biofilm-associated genes in *S. aureus.* This can further lead to bacteriocin tolerance such that even its higher concentrations cannot kill bacterial cells after exposure to sub-lethal doses. Therefore, it is pivotal to add appropriate concentrations of nisin to food products for preservation purposes.

1. Introduction

Staphylococcus aureus is considered as one of the common bacterial pathogens that causes food poisoning outbreaks (Hennekinne et al., 2012). *S. aureus* can be widely found in meat products, as well as raw and processed foods (Wu et al., 2018). In several studies, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been found at high levels on US and European farms and in commercially-distributed meats, emerging as a potential concern for meat handlers and consumers (Bondi et al., 2014). Food poisoning by *S. aureus* is frequently caused by the ingestion of Staphylococcal enterotoxins responsible for gastroenteritis. These enterotoxins are heat-stable and resistant to proteases within the human gastrointestinal tract (Pinchuk et al., 2010). Despite several advancements in food technology, several outbreaks of staphylococcal food poisoning have been reported (Kadariya et al., 2014). Moreover, chronicity and antibiotic resistance has been reported in staphylococcal infection (Rajabi et al., 2020; Shivaee et al., 2019). The chronic and recurrent staphylococcal infections have been associated with the capability of biofilm formation in *S. aureus* (Bhattacharya et al., 2015). Therefore, it is pivotal to inhibit bacterial biofilm formation and enterotoxin production in food.

Bacteriocins are beneficial and potent alternatives to traditional preservatives that can enhance food safety. In fact, bacteriocins are antimicrobial compounds that can extend the shelf life of food by controlling the growth of foodborne pathogens, thereby being financially advantageous as they decrease food spoilage and the need for extra heating (Johnson et al., 2018).

Nisin is a bacteriocin generated by *Lactococcus lactis* subsp. *Lactis*. This compound can inhibit the growth of Gram-positive bacteria including *S. aureus* and *Listeria monocytogenes*, however, it is usually not effective on most Gram-negative bacteria, fungi, and yeasts. Nisin forms pores on bacterial membrane, thereby increasing membrane permeability and efflux of critical intracellular metabolites (Malanovic and Lohner, 2016). This agent is generally categorized as a safe compound

* Corresponding author.
E-mail address: imanifouladi.a@gmail.com (A.A. Imani Fooladi).

https://doi.org/10.1016/j.toxicon.2021.03.018
Received 7 November 2020; Received in revised form 22 March 2021; Accepted 24 March 2021
Available online 7 April 2021

that can be used as a direct food additive and is commercially available (Hampikyan, 2009). Nisin is stable under refrigerated storage of foods, demonstrates heat stability, and is degraded in the gut system. The antimicrobial activity of Nisin against bacteria in foods could be improved by the combined addition with other antimicrobial agents, such as chelators or plant essential oil (Moshtaghi et al., 2018).

Given the prevalence of staphylococcal food poisoning and the widespread use of nisin, here, we aimed at investigating the effect of this compound on *S. aureus*. Evaluation of the effect of nisin on *S. aureus* toxin production could provide valuable information about their appropriate dosing regimen as well as their potential for antimicrobial therapy. Likewise, as staphylococcal enterotoxins contribute to food poisoning, it is also necessary to understand the effect of nisin on these virulence factors.

2. Materials and methods

2.1. Culture and growth conditions

In this study, *S. aureus* ATCC 29213 was used to examine the effect of sub-MIC of nisin on *S. aureus*. The stock culture was stored at −80 °C in Brain Heart Infusion (BHI) broth (Merck, Germany) with 10% (v/v) glycerol. For further analysis, after growth on BHI broth, colonies were sub-cultured at 37 °C for 24 h for three consecutive times.

2.2. Nisin preparation

Nisin was obtained from Sigma-Aldrich (N5764, with 2.5% nisin, balanced NaCl, and denatured milk). To prepare nisin stock solution (5000 IU/g), guidelines provided by the Compendium of Food Additive Specifications (FAO, 2008) were employed. Briefly, to prepare a nisin stock solution, 128 mg of nisin was dissolved in 2 ml sterile 0.02 N HCl. For further investigations, Mueller Hinton Broth (MHB) was used for nisin dilution.

2.3. Determination of the minimum inhibitory concentration

To determine the minimum inhibitory concentration (MIC) of nisin (Sigma Aldrich) against *S. aureus* ATCC 29213, broth micro-dilution method with 96-well plates was used. After adding 100 μl of BHI broth (Merck, Germany) to each well, 100 μl of nisin solution was added to the first well and serial dilution was prepared. Then, 100 μl of the BHI broth with 10^5 CFU/ml was added to each well and the plates were incubated at 37 °C for 24 h. Finally, the MIC value was determined by direct observation based on the lowest antimicrobial dose that hindered >90% of bacterial growth. All experiments were performed in triplicates.

2.4. Transcriptional analysis of nisin-treated S. aureus

For RNA extraction, *S. aureus* ATCC 29213 was cultured in LB with sub-lethal dose of nisin. The sublethal concentration of nisin was used in the log phase. Then, after centrifugation at 5000×*g* for 5 min at 4 °C, cells were suspended in 100 μg/ml lysostaphin-containing TES buffer (Sigma-Aldrich). Samples were then incubated at 37 °C for 10 min and Qiagen RNeasy Maxi column was used for total RNA extraction, according to protocols provided by the manufacturer. For eliminating DNA contamination, RNase-free DNase I (Qiagen, Hilden, Germany) was used. To confirm the quality, integrity, and concentration of RNA, samples were run on 1% gel-electrophoresis. cDNA was synthetized using the cDNA Synthesis Kit (Thermo Scientific). Primers used in this study targeting four toxin-encoding ((*SEA*, *SEB*, *SED*, and *hla*) and four biofilm-associated (*icaA*, *fnb*, *ebpS*, and *eno*) genes are listed in Table 1. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted in triplicates using a RotorGene thermal cycler (Corbett Life Sciences, Sydney, Australia) using the SYBR Green method (Ampliqon Co, Denmark). A total of 20 ml reaction mix contained 1 ml of cDNA, 10 ml SYBR Green master mix, 7 ml nuclease-free water, and 1 ml of each primer. The thermal cycling included an initial denaturation at 95 °C for 12 min, followed by 40 cycles at 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 20 sec. 16S rRNA was used as an internal control to normalize the expression levels. The relative fold changes in expression levels were calculated based on the delta-delta Ct method.

2.5. Statistical analysis

ANOVA test was used for comparison using SPSS software v.22. P-value<0.05 was considered statistically significant.

3. Results

3.1. Effects of nisin on S. aureus growth

The MIC of nisin against *S. aureus* strains ATCC 29213 was calculated as 8 μg/ml (1/2 MIC = 4 μg/ml).

3.2. Toxin-associated gene transcriptional levels following nisin exposure

Here, we hypothesized that nisin could affect the transcriptional levels of *S. aureus* toxin genes. So, the sublethal concentration of nisin was added to the bacteria in the log phase. The suitable primers were designed by Primer 3 software. To test this hypothesis, we used Real-

Table 1
Primers in this study.

Genes	Primer sequence (5′ → 3′)		Product Size	Tm (°C)	References
SEA	F	TGCCCTAACGTTGACAACAAG	110 bp	60	In this study
	R	TGCCTAAAGCTGTTCCCTGC			
SEB	F	GCATTAACCCCTTGTTGCCA	104 bp	60	In this study
	R	CGTTAAAAACGGCGACACAGT			
SED	F	GTGTCACTCCACACGAAGGT	163 bp	60	In this study
	R	TGCAAATAGCGCCTTGCTTG			
hlA	F	TGGTTTAGCCTGGCCTTCAG	190 bp	60	In this study
	R	ATTTGCACCAATAAGGCCGC			
icaA	F	GCAGCAGTAGTTCTTGTCGC	80 bp	60	In this study
	R	CTGTCTGGGCTTGACCATGT			
Fnb	F	AGATGCGAGCGAAGGATACG	197 bp	60	In this study
	R	GTGGACGTGCACCATATTCG			
ebpS	F	TGCTTCTGCCGCTTCAAAAC	112 bp	60	In this study
	R	TACTTTGGCCATGCCACCTT			
Eno	F	AACTGCCGTAGGTGACGAAG	92 bp	60	In this study
	R	CAGCTGCTTCGATTGCTTGG			
16srRNA	F	AGAACGCGAGCGTTGTTAGA	163 bp	60	In this study
	R	CTGTCAATGACCCCATGCCT			

time PCR analysis to determine the mRNA levels of *hla*, *SED*, *SEA* and *SEB* in *S. aureus* following treatment with sub-MIC of nisin at 2, 8, and 24 h post exposure (Fig. 1). The results showed that the treatment of *S. aureus* with ½ MIC of nisin at different time intervals can influence the expression of genes coding for staphylococcal toxins. *hla*, *SED*, *SEA* and *SEB* expression reduced in a time-dependent manner. *SEA* and *SEB* are the most frequently found toxin in food poisoning caused by *S. aureus* and following exposure to sub-lethal dose of nisin (1/2 MIC), *SEA* mRNA expression showed decrease at all tested times. Another studied toxin, *SEB*, which is also found frequently in food poisoning showed downregulation. *SED* is supposedly the second most common staphylococcal toxin linked with food poisoning even at small traces. Transcription levels of *SED* also decreased. Finally, the alpha-toxin of *S. aureus*, coded by the *hla* gene, is a pore forming toxin that disrupts host cell membranes leading to osmotic swelling, rupture, and lysis. Downregulation of *hla* have been reported. According to ANOVA test, nisin treatment had a significant effect on the expression of four target genes of *S. aureus* associated with toxin production (P-value<0.05).

3.3. Biofilm-associated genes transcription levels following nisin exposure

Expression levels of four genes (*fnb*, *eno*, *ebpS*, and *icaA*) involved in biofilm formation were investigated in *S. aureus* ATCC 29213. The mRNA levels of these genes were evaluated after 2, 8, and 24 h exposure to ½ MIC of nisin. As illustrated in Fig. 2, sub-MIC of nisin generally upregulated transcripts of the studied genes. The expression level of *fnb* (fibrinogen binding protein) in *S. aureus* was highest after 8 h (4.5-fold increase), while the expression levels of *eno* (laminin-binding protein) and *ebpS* (elastin-binding protein) genes, were highest after 2 h (3.3 and 4.5-fold increase). The transcriptional levels of gene from ica operon (*icaA*) associated with biosynthesis of glucosamine polymer PIA were also monitored. The expression levels of this gene were highest after 2 and 8 h, respectively (4.7 and 6.8-fold increase). According to ANOVA results, all these genes indicated significantly higher transcriptional levels (p-value<0.05).

4. Discussion

In several countries, *S. aureus* is considered as the second or third most common bacterial pathogen leading to food poisoning outbreaks after *Salmonella* and *Clostridium perfringens* due to the ingestion of staphylococcal enterotoxins (Martinović et al., 2016). In addition, the ability of biofilm formation has been indicated for *S. aureus* which can complicate antibiotic therapy (Chen et al., 2020). For elimination or

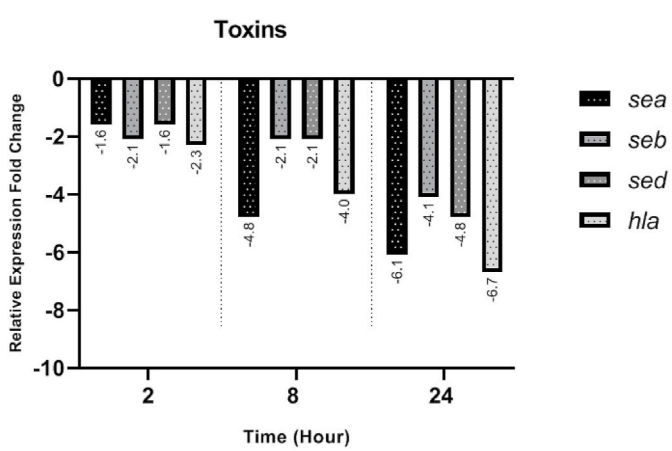

Fig. 1. Relative expression levels of toxin-encoding genes in *S. aureus* ATCC 29213 following exposure to ½ MIC of nisin. Transcriptional levels of *sea*, *seb*, *sed*, and *hla* were assessed using the quantitative RT-PCR. Relative expression was normalized with housekeeping gene 16 S rRNA.

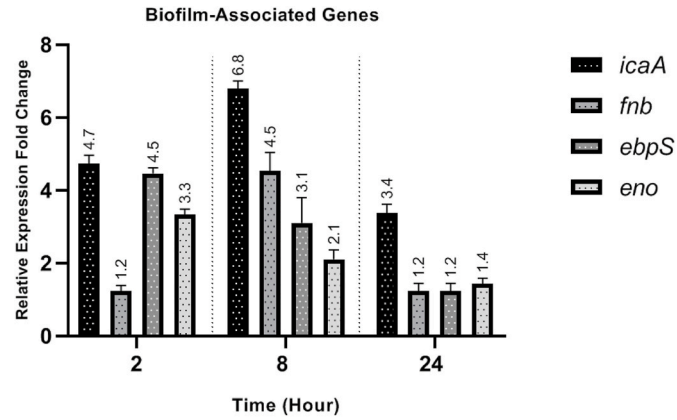

Fig. 2. Relative expression levels of biofilm-associated genes in *S. aureus* following exposure to ½ MIC of nisin. Expression levels of *icaA*, *fnb*, *ebpS*, and *eno* were evaluated by quantitative RT-PCR. Relative expression was normalized with housekeeping gene 16 S rRNA.

control of bacterial growth, natural antimicrobials have been frequently employed in the food industry. Bacteriocins is one of the most important examples of ribosomally synthesized antimicrobials that have been traditionally used as food preservatives (Chikindas et al., 2018). Nisin is the only bacteriocin that is widely used as a direct food additive and bio-preservative (Woraprayote et al., 2016). The amount of nisin used in different food industries varies and ranges from 0.25 to 37.5 μg/g (Delves-Broughton, 2012). In current study the MIC of nisin against *S. aureus* strains ATCC 29213 was calculated as 8 μg/ml. However, it is important to know the exact effect of nisin on *S. aureus* and the proper dosage required for elimination of this food pathogen. It seems that nisin can be considered for further tests regarding its potential efficacy against *S. aureus* infections. Many studies have shown that nisin is effective against *Staphylococcus* sp (Felicio et al., 2015). Few studies have analyzed the gene expression profile of *S. aureus* treated with nisin. For example, Zhao et al. have indicated that nisin treatment could reduce the expression of *isda* (cell surface protein), *sspa* (serine protease), *ribA* (riboflavin biosynthesis protein), *capC* (capsular polysaccharide synthesis enzyme Cap8C) (Zhao et al., 2016). Some other studies have been reported the impact of nisin on the hydrophobicity profile, reduction of *S. aureus* adhesion, modulation of pro-inflammatory and anti-inflammatory cytokines and therefore suppression of inflammation (De Jesus Pimentel-Filho et al., 2014; Jia et al., 2019; Zhao et al., 2020). It is also established that *S. aureus* exposure to nisin caused DNA damage, cellular membrane disruption and cell lysis (Jensen et al., 2020). The morphological changes in the structure of biofilms and reduction its dense matrix structure even confirmed by nisin (Andre et al., 2019). Nevertheless, to the best of our knowledge, no studies have investigated the effect of nisin on genes coding for staphylococcal toxin genes. Here, we hypothesized that sub-lethal doses of nisin can affect the expression levels of genes coding for *S. aureus* toxins. The results indicate that nisin, even at sub-lethal concentrations, can reduce the transcript levels of toxin genes, thereby reducing the virulence and pathogenesis of *S. aureus*. However, it is not fully understood what happens with bacteria when sub-inhibitory doses of nisin are exposed (Vasilchenko and Rogozhin, 2019). Only a handful of studies have investigated the effect of antibiotics on the transcript levels of genes coding for staphylococcal toxin. Consistent with our results, it has been indicated that sub-lethal doses of clindamycin and linezolid could significantly reduce alpha-hemolysin levels (Stevens et al., 2007). Qiu et al. also investigated the effect of thymol on *hla, SEA*, and *SEB* transcriptional levels and indicated that the sub-lethal doses of thymol could inhibit the transcription of these genes in *S. aureus* (Qiu et al., 2010).

We also monitored the effect of sub-MIC (1/2) of nisin on the transcripts levels of genes involved in biofilm formation. According to the

results, mRNA levels of *fnb*, *eno*, *ebpS*, and *icaA* increased following exposure to ½ MIC of nisin (P-value<0.05). Interestingly, *icaA* showed the highest mRNA levels after 8 h as it is involved in biofilm maturation. Also, *ebpS* and *eno* showed the highest expression levels 2 h post exposure suggesting their involvement in initial binding of *S. aureus* to surfaces while *fnb* showed the highest transcript levels 8 h post exposure, suggesting its contribution in binding of *S. aureus* in subsequent hours. These results indicate that the inappropriate doses of nisin may induce bacterial biofilm formation, and therefore, if for any reason, the efficacy of nisin is reduced within the food, bacterial cells can resume proliferation and toxin secretion. More importantly, nisin as an anti-biofilm agent, enhances ability to impair biofilm formation and reduces the density of established biofilms in *S. aureus* (Field et al., 2016).

To the best of our knowledge, studies on the effect of sub-lethal doses of nisin on the ability of biofilm formation in *S. aureus* are scarce. However, some studies have shown that sub-MICs of antibiotics can inhibit bacterial biofilm formation (Kaplan, 2011). For instance, Mirani and Jamil showed that sub-MIC concentrations of vancomycin increased *S. aureus* biofilm formation on silicon and nylon surfaces, while sub-MIC of oxacillin increased biofilm formation capabilities of *S. aureus* on glass surfaces. Increased ability of biofilm formation was about 3- to 4-fold as indicated by crystal violet binding assay (Mirani and Jamil, 2011). The exact mechanism of biofilm induction by sub-MIC doses of antibiotics in *S. aureus* is not thoroughly clear. Bisognano et al. indicated that *fnb* transcripts in *S. aureus* increased in quinolone-resistant strains following exposure to 1/4 MIC of ciprofloxacin (Bisognano et al., 1997). Subrt et al. also indicated that sub-MIC concentrations of cefalotin (1/4 MIC) led to *S. aureus* biofilm induction but did not affect expression of *agr*, which regulates *S. aureus* biofilm formation and dispersion. Sub-MIC dose of cefalotin also increased the transcriptional levels of the *S. aureus* virulence genes *lukE* (leukotoxin E) and *spa* (encoding protein A) (Subrt et al., 2011). One mechanism attributed to biofilm induction by sub-MIC doses of antibiotics is the possible involvement of the intracellular second messenger cyclic dimeric guanosine monophosphate (c-di-GMP). In fact, elevated levels of c-di-GMP have been indicated to elevate the production of exopolysaccharides and reduce bacterial motility, thereby increasing biofilm production (Aka and Haji, 2015). It is suggested that utility of nisin and nisin derivative in combination with antibiotics increase the inhibitory effect against *S. aureus* compared to the administration of antibiotics alone. In general, activities of the nisin derivative in combination with antibiotics represent a significant improvement over that of the nisin and antibiotic combination (Field et al., 2016).

Research on antibiotics has been mainly focused on their antimicrobial activity and antibiotic resistance developed by several bacterial species. Nonetheless, in the past recent years, researches are more focused on the activity of antibiotics as signals that can affect bacterial physiology. The hypothesis that low doses of different antibiotics can affect gene expression in bacterial pathogens have been brought up in most recent studies and, consistent with this study, sub-MICs of these compounds can have roles in gene expression causing phenotypic alterations. As indicated in this study, sublethal doses of nisin induced genes that altered the physiology of *S. aureus* so that it had the higher potential in biofilm formation, allowing it to survive and tolerate higher lethal concentrations. Recent data show that not only *S. aureus,* but also other bacterial pathogens can adopt different responses to sub-MIC concentrations of antibiotics and employ different approaches for survival. Consistent with our results, Knudsen et al. showed that sub-MIC concentrations of antibiotics could trigger expression of different genes in *L. monocytogenes* that can further lead to antibiotic tolerance such that even higher concentrations of antibiotics will not be able to kill bacterial cells after a period of exposure to sub-lethal doses of antibiotics (Knudsen et al., 2016). Similarly, it has been shown that *Burkholderia thailandensis* could adapt an anaerobic metabolism, thereby leading to antibiotic tolerance (Hemsley et al., 2014), while *Mycobacterium tuberculosis* activated its specific dormancy regulon which further helped bacterial cells in antibiotic survival (Baek et al., 2011).

The increasing prevalence of nisin resistance among bacteria, especially gram-positive ones, has been highlighted the importance of utility of low or inappropriate concentrations of nisin. Although the sub-lethal dose of nisin induced bacterial biofilm formation by *S. aureus* in our study, it can be active against biofilm formation at proper concentrations (Mathur et al., 2018). Therefore, it may provide an opportunity for *S. aureus* to become resistant. Godoy-Santos et al. used a direct microscopic visualization technique and showed the penetration of nisin into *S. aureus* biofilms. Their results confirmed that nisin could increase the permeability of bacteria within the biofilm structure (Godoy-Santos et al., 2018). Field et al. also found that nisin derivatives can reduce the ability of biofilm formation and decrease the density of already-established *S. aureus* biofilms. They also showed that the anti-biofilm activities of nisin derivatives increased following the addition of other antibiotics (Field et al., 2016). These results suggest that although sub-lethal doses of nisin may induce biofilm formation by *S. aureus*, using the proper dose of this antibiotic (MIC) can inhibit the formation of this structure. It also should be noted that using higher doses of this antibiotic (greater concentrations than MIC) may induce persister cell formation in this bacterium, and therefore, it is necessary to use this food additive with cautious and with proper doses. It is recommended that utility of anti-biofilm compounds in the food industry can provide significant advantages. Nevertheless, it should not be ignored that biofilm formation experiments are necessary to corroborate the results and further studies are needed to clearly dissolve this issue in the health system.

Ethical statement

Non.

Credit author statement

Ali Shivaee.(First author), Statistical analyst/Original researcher/Discussion author (25%); Sajad Rajabi. (Second author), Original researcher, Methodologist/Discussion author (20%); Hamed Eraghiye Farahani (Third Author). Methodologist/Original researcher/Discussion author (20%); Abbas Ali Imani Fooladi. (Corresponding author), Statisticalanalyst/Discussion author (35%).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aka, S.T., Haji, S.H., 2015. Sub-MIC of antibiotics induced biofilm formation of *Pseudomonas aeruginosa* in the presence of chlorhexidine. Braz. J. Microbiol. 46 (1), 149–154.

Andre, C., de Jesus Pimentel-Filho, N., de Almeida Costa, P.M., Vanetti, M.C.D., 2019. Changes in the composition and architecture of staphylococcal biofilm by nisin. Braz. J. Microbiol. 50 (4), 1083–1090.

Baek, S.-H., Li, A.H., Sassetti, C.M., 2011. Metabolic regulation of mycobacterial growth and antibiotic sensitivity. PLoS Biol. 9 (5), e1001065.

Bhattacharya, M., Wozniak, D.J., Stoodley, P., Hall-Stoodley, L., 2015. Prevention and treatment of *Staphylococcus aureus* biofilms. Expert Rev. Anti-infect. Ther. 13 (12), 1499–1516.

Bisognano, C., Vaudaux, P.E., Lew, D.P., Ng, E., Hooper, D.C., 1997. Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant *Staphylococcus aureus* exposed to subinhibitory levels of ciprofloxacin. Antimicrob. Agents Chemother. 41 (5), 906–913.

Bondi, M., Messi, P., Halami, P.M., Papadopoulou, C., De Niederhausern, S., 2014. Emerging Microbial Concerns in Food Safety and New Control Measures. Hindawi.

Chen, Q., Xie, S., Lou, X., Cheng, S., Liu, X., Zheng, W., et al., 2020. Biofilm formation and prevalence of adhesion genes among *Staphylococcus aureus* isolates from different food sources. Microbiologyopen 9 (1), e00946.

Chikindas, M.L., Weeks, R., Drider, D., Chistyakov, V.A., Dicks, L.M., 2018. Functions and emerging applications of bacteriocins. Curr. Opin. Biotechnol. 49, 23–28.

De Jesus Pimentel-Filho, N., de Freitas Martins, M.C., Nogueira, G.B., Mantovani, H.C., Vanetti, M.C.D., 2014. Bovicin HC5 and nisin reduce *Staphylococcus aureus* adhesion to polystyrene and change the hydrophobicity profile and Gibbs free energy of adhesion. Int. J. Food Microbiol. 190, 1–8.

Delves-Broughton, J., 2012. Natural Antimicrobials as Additives and Ingredients for the Preservation of Foods and Beverages. Natural Food Additives, Ingredients and Flavourings. Elsevier, pp. 127–161.

Felicio, B.A., Pinto, M.S., Oliveira, F.S., Lempk, M.W., Pires, A.C.S., Lelis, C.A., 2015. Effects of nisin on *Staphylococcus aureus* count and physicochemical properties of Minas Frescal cheese. J. Dairy Sci. 98 (7), 4364–4369.

Field, D., O'Connor, R., Cotter, P.D., Ross, R.P., Hill, C., 2016. In vitro activities of nisin and nisin derivatives alone and in combination with antibiotics against Staphylococcus biofilms. Front. Microbiol. 7, 508.

Godoy-Santos, F., Pitts, B., Stewart, P.S., Mantovani, H.C., 2018. Nisin penetrates *Staphylococcus aureus* biofilms but shows differences in killing effects against sessile and planktonic cells. BioRxiv 303636.

Hampikyan, H., 2009. Efficacy of nisin against *Staphylococcus aureus* in experimentally contaminated sucuk, a Turkish-type fermented sausage. J. Food Protect. 72 (8), 1739–1743.

Hemsley, C.M., Luo, J.X., Andreae, C.A., Butler, C.S., Soyer, O.S., Titball, R.W., 2014. Bacterial drug tolerance under clinical conditions is governed by anaerobic adaptation but not anaerobic respiration. Antimicrob. Agents Chemother. 58 (10), 5775–5783.

Hennekinne, J.-A., De Buyser, M.-L., Dragacci, S., 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 36 (4), 815–836.

Jensen, C., Li, H., Vestergaard, M., Dalsgaard, A., Frees, D., Leisner, J.J., 2020. Nisin damages the septal membrane and triggers DNA condensation in methicillin-resistant *Staphylococcus aureus*. Front. Microbiol. 11, 1007.

Jia, Z., He, M., Wang, C., Chen, A., Zhang, X., Xu, J., et al., 2019. Nisin reduces uterine inflammation in rats by modulating concentrations of pro-and anti-inflammatory cytokines. Am. J. Reprod. Immunol. 81 (5), e13096.

Johnson, E.M., Jung, D.Y.-G., Jin, D.Y.-Y., Jayabalan, D.R., Yang, D.S.H., Suh, J.W., 2018. Bacteriocins as food preservatives: challenges and emerging horizons. Crit. Rev. Food Sci. Nutr. 58 (16), 2743–2767.

Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed Res. Int. 2014.

Kaplan, J.B., 2011. Antibiotic-induced biofilm formation. Int. J. Artif. Organs 34 (9), 737–751.

Knudsen, G.M., Fromberg, A., Ng, Y., Gram, L., 2016. Sublethal concentrations of antibiotics cause shift to anaerobic metabolism in *Listeria monocytogenes* and induce phenotypes linked to antibiotic tolerance. Front. Microbiol. 7, 1091.

Malanovic, N., Lohner, K., 2016. Antimicrobial peptides targeting gram-positive bacteria. Pharmaceuticals 9 (3), 59.

Martinović, T., Andjelković, U., Gajdošik, M.Š., Rešetar, D., Josić, D., 2016. Foodborne pathogens and their toxins. Journal of Proteomics 147, 226–235.

Mathur, H., Field, D., Rea, M.C., Cotter, P.D., Hill, C., Ross, R.P., 2018. Fighting biofilms with lantibiotics and other groups of bacteriocins. npj Biofilms and Microbiomes 4 (1), 1–13.

Mirani, Z.A., Jamil, N., 2011. Effect of sub-lethal doses of vancomycin and oxacillin on biofilm formation by vancomycin intermediate resistant *Staphylococcus aureus*. J. Basic Microbiol. 51 (2), 191–195.

Moshtaghi, H., Rashidimehr, A., Shareghi, B., 2018. Antimicrobial activity of nisin and Lysozyme on foodborne pathogens *Listeria monocytogenes, Staphylococcus sureus, Salmonella typhimurium*, and *Escherichia coli* at different pH. Journal of Nutrition and Food Security 3 (4), 193–201.

Pinchuk, I.V., Beswick, E.J., Reyes, V.E., 2010. Staphylococcal enterotoxins. Toxins 2 (8), 2177–2197.

Qiu, J., Wang, D., Xiang, H., Feng, H., Jiang, Y., Xia, L., et al., 2010. Subinhibitory concentrations of thymol reduce enterotoxins A and B and α-hemolysin production in *Staphylococcus aureus* isolates. PloS One 5 (3), e9736.

Rajabi, S., Shivaee, A., Khosravi, M.A., Eshaghi, M., Shahbazi, S., Hosseini, F., 2020. Evaluation of multidrug efflux pump expression in clinical isolates of *Staphylococcus. aureus*. Gene Reports 18, 100537.

Shivaee, A., Mohammadzadeh, R., Shahbazi, S., Pardakhtchi, E., Ohadi, E., Kalani, B.S., 2019. Time-variable expression levels of mazF, atlE, sdrH, and bap genes during biofilm formation in *Staphylococcus epidermidis*. Acta Microbiol. Immunol. Hung. 66 (4), 499–508.

Stevens, D.L., Ma, Y., Salmi, D.B., McIndoo, E., Wallace, R.J., Bryant, A.E., 2007. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. 195 (2), 202–211.

Subrt, N., Mesak, L.R., Davies, J., 2011. Modulation of virulence gene expression by cell wall active antibiotics in *Staphylococcus aureus*. J. Antimicrob. Chemother. 66 (5), 979–984.

Vasilchenko, A.S., Rogozhin, E.A., 2019. Sub-inhibitory effects of antimicrobial peptides. Front. Microbiol. 10, 1160.

Woraprayote, W., Malila, Y., Sorapukdee, S., Swetwiwathana, A., Benjakul, S., Visessanguan, W., 2016. Bacteriocins from lactic acid bacteria and their applications in meat and meat products. Meat Sci. 120, 118–132.

Wu, S., Huang, J., Wu, Q., Zhang, J., Zhang, F., Yang, X., et al., 2018. *Staphylococcus aureus* isolated from retail meat and meat products in China: incidence, antibiotic resistance and genetic diversity. Front. Microbiol. 9, 2767.

Zhao, X., Meng, R., Shi, C., Liu, Z., Huang, Y., Zhao, Z., et al., 2016. Analysis of the gene expression profile of *Staphylococcus aureus* treated with nisin. Food Contr. 59, 499–506.

Zhao, M., Qu, Y., Liu, J., Mai, S., Gu, L., 2020. A universal adhesive incorporating antimicrobial peptide nisin: effects on *Streptococcus mutans* and saliva-derived multispecies biofilms. Odontology 1–10.